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METAL COMPLEXES FOR DEFENSE AGAINST CYANIDE INTOXICATION

FINAL REPORT

Yee W. Chan and Robert B. Wilson Jr.

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Metal complexes having high affinities for cyanide binding can be used as antidotes for cyanide intoxication. A number of water-soluble phthalocyanine complexes have been synthesized and tested for this purpose. Compounds tested contain a variety of metal ions (Co^{3+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, Ru^{3+}, Pd^{2+}, Mn^{4+} and V^{4+}) and functional groups (SO_3^-, NH_3^+ and CO_2^-). The rate of cyanide binding to these complexes was studied by spectroscopic methods and by the use of an ion-selective electrode. The rate of cyanide binding depends on the metal and its oxidation state. The following order of decreasing rate is observed: $Co^{2+} > Fe^{3+} > Fe^{2+}$ $Co^{3+} > Pd^{2+}$ Ni^{2+} $Ru^{3+} > Cu^{2+} > Mn^{4+}$, V^{4+}. The rate constant of cobalt(II) tetracarboxyphthalocyanine is $3.4 \times 10^3\ M^{-1}\ s^{-1}$, which is about twenty times the reported rate of binding of cyanide to silated cytochrome oxidase.

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SUMMARY

Cyanide is an asphxiant that prevents tissue use of oxygen by inhibiting the cellular respiratory enzyme--cytochrome oxidase. Current treatment for cyanide intoxication in the United States is the administration of sodium nitrite followed by sodium thiosulfate. The sodium nitrite oxidizes hemoglobin to methemoglobin, which is more effective for binding cyanide in competition with the enzyme. The sodium thiosulfate provides a source of sulfur atoms for the enzymatic conversion of cyanide to thiocyanate, which is excreted. Rather than this two-stage treatment, which primarily depends on cyanide binding to methemoglobin, an improved treatment or pretreatment of cyanide poisoning is to use a metal complex that has high affinity for cyanide. This complex will compete with cytochrome oxidase in binding cyanide and will form a stable cyanide complex that is subsequently excreted.

This research effort was directed toward the design and synthesis of metal complexes that are efficacious against cyanide intoxication because of their rapid and efficient binding of cyanide. We have prepared a series of phthalocyanine complexes that cover a range of metals and functional groups. Phthalocyanine complexes were chosen because they are very stable and are structurally similar to the porphyrin complexes that form the active site of cytochrome oxidase. A wide variety of phthalocyanine metal complexes can be synthesized to allow comparisons of the cyanide affinity of different metals. Our results indicate that cobalt is the best choice of metal, based on the rate of binding. The cobalt(II) complex of tetracarboxyphthalocyanine binds cyanide at a rate of 3.9×10^3 M^{-1} s^{-1}, which is about twenty times faster than the isolated cytochrome oxidase. Other metals--such as iron, palladium, ruthenium, copper, and nickel--bind cyanide at a rate that is faster or competitive with the rate of binding by the isolated enzyme. The oxidation state of the metal has a significant effect on the rate of cyanide binding, but the effect is dependent on

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the metal. For example, cobalt(II) binds cyanide faster than cobalt(III), but the iron analogs behave in the opposite manner.

Electronic effects due to functional group substitution also affect the rate of cyanide binding. In tests of the tetrasulfonate and tetracarboxy derivatives, we found that the tetrasulfonate derivatives of iron phthalocyanine bind cyanide at a higher rate. However, the carboxylate derivatives demonstrate more rapid binding in the cobalt complexes.

The development of metal complexes as antidotes for cyanide intoxication is quite promising as demonstrated by these *in vitro* results. However, key questions such as toxicity and *in vivo* efficacy await the results of the *in vivo* studies, which will guide future synthetic efforts. Twelve compounds syntheisized in this study have been submitted to WRAIR for *in vivo* testing.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

I INTRODUCTION

Cyanide is a potent and rapidly acting asphxiant[1] that prevents tissue use of oxygen by inhibiting the cellular respiratory enzyme cytochrome oxidase.[2,3] The classical antidotal combination of sodium nitrite and sodium thiosulfate has been used against cyanide toxicity for moe than 40 years in the United States.[4] Europeans use a different antidote, Kelocyanor, which is cobalt-ethylenediamine tetraacetate (EDTA),[4] but Kelocyanor does not have FDA approval for use in the United States.

Cytochrome oxidase is the oxygen-activating enzyme of cellular respiration. In most organisms the enzyme is located in the inner mitochondrial membrane. Cytochrome oxidase enables the cell that contains it to use molecular oxygen to oxidize foodstuffs by catalyzing electron transfer from cytochrome c to O_2.[5] Cytochrome oxidase has four redox centers: cytochromes a and a_3 and a pair of copper atoms (Cu_A and Cu_B).[6]

The mechanism of cyanide toxicity might be compared with that of other asphyxiants, particularly carbon monoxide. Carbon monoxide forms a complex with hemoglobin, called carboxy hemoglobin, with a binding constant about 200 times greater than for the corresponding oxygen complex. A fatal dosage occurs when enough carbon monoxide is present to disrupt the oxygen transport to the tissue. However, cyanide is toxic at much lower concentrations. According to ligand field theory, the strong bonding between the Fe(II) of hemoglobin and CO is the result of a σ-bond formed by the donation of a lone-pair from the carbon to an empty d-orbital on the metal, plus a π-bond, which is formed by the back-donation of a filled metal d-orbital to the empty antibonding molecular orbital of CO.[7]

Cyanide, however, has a low affinity for Fe(II) hemoproteins.[8] This is probably due to a repulsion between the negatively charged cyanide ion and the high charge density at the iron arising from

electron donation from the prophyrin and the fifth axial ligand, the imidazole side chain of a protein histidine residue. Cyanide is not as efficient as CO at π back-bonding to relieve the charge density at the metal.[9] Thus, cyanide is toxic, not because of affinity to Fe(II), but because it has a very high affinity for Fe(III) hemoprotein where the iron now bears a net positive charge. The theory is that cyanide binds to the oxidized Fe(III) form of the cytochrome a or a_3 of cytochrome oxidase to inhibit cellular respiration.[10]

The current therapy is to use an oxidizing agent (sodium nitrite) that oxidizes the Fe(II) of hemoglobin to Fe(III) of methemoglobin, which effectively competes with cytochrome oxidase for the binding of cyanide ion. This treatment is followed by treatment with sodium thiosulfate, which provides sulfur so that the enzyme rhodanese can detoxify cyanide to thiocyanate.[11]

A distinctly different therapy is used by the Europeans, who fail to see the logic of creating one disease (methemoglobinemia) to treat another. They administer massive doses of a cobalt salt [cobalt (II) EDTA] to bind the cyanide in the form of $Co(CN)_6^{3-}$, which is very stable and is excreted.[12]

Both therapies work by binding the cyanide in competition with the cytochrome oxidase rather than reactivating the inhibited enzyme. Thus, treatment must be started quickly to prevent a fatal level of inhibition, within 30 minutes if administered orally in sheep.[13]

Note fact that the isolated oxidized enzyme does not react rapidly with cyanide, and the slow reaction that does occur does not lead to an EPR signal, which is expected for a low spin Fe(II) cyanide complex.[14] The proposed explanation is that the cyanide binds to form a binuclear spin-coupled complex involving cytochrome a_3 and Cu_B, both of which are spin 1/2. In the presence of a reducing agent or in intact mitochondria, cyanide reacts with cytochrome oxidase very rapidly to give the low spin Fe(III) cyanide complex of cytochrome a_3. The equilibrium constants for reaction of cyanide with isolated cytochrome a have been measured with surprising results:[15]

$$\text{Fe(III) cytochrome a} + {}^{-}\text{CN} \overset{K_1}{\rightleftharpoons} \text{Fe(III)(CN)cytochrome } \underline{a} \quad (1)$$

$$\text{Fe(II) cytochrome a} + {}^{-}\text{CN} \overset{K_2}{\rightleftharpoons} \text{Fe(II)(CN)cytochrome } \underline{a} \quad (2)$$

$K_1 = 1.087 \times 10^4$ and $K_2 = 1.351 \times 10^3$

Reaction (1) was reported to be very slow and took a long time to reach equilibria, whereas reaction (2) reached equilibria ". . .so rapidly that it could not be followed by the method employed,"[15] less than 1 second. Thus the reaction of $^{-}$CN with Fe(III) hemoproteins is thermodynamically favored, whereas the reaction with Fe(Il) is kinetically favored. However, the interaction of -CN with cytochrome oxidase is not simply governed by these two reactions. The inhibition constant of cytochrome oxidase, which is a measure of how tightly bound cyanide is to the enzyme, has been measured to be 10^{-6} M or roughly two orders of magnitude more tightly bound than in isolated Fe(III) cytochrome _a_.[15]

Our examination of the literature indicates that the mechanism of cyanide binding to the enzyme is not clearly understood. The detailed effects of the therapies are also not well understood. For example, the sodium nitrite may be effective as much because it maintains cytochrome oxidase in the oxidized Fe(III) form and thus slows down the rate of the nearly irreversible binding of the cyanide as because it oxidizes hemoglobin to methemoglobin. If so, an oxidizing agent more selective for cytochrome oxidase might be designed that would be effective for delaying the binding of cyanide to cytochrome oxidase, but would not cause methemoglobinemia.

Our approach to the development of new antidotes for cyanide intoxications with improved efficacy is based on metal complexes that have high binding constants for cyanide. We have synthesized a variety of phthalocyanines complexes that cover a range of metals [Co(II), Co(III), Fe(II), Fe(III), Ni(II), Cu(II), Ru(III), Pd(II), MN(IV), and

V(IV)] and functional groups ($-SO_3^-$, $-NH_3^+$, and $-CO_2^-$). As a preliminary *in vitro* for measuring screen cyanide binding rates, we have developed a reliable technique for the synthesized complexes. Based on our results, we have selected twelve complexes that have high cyanide binding constants and have sent these to U.S. Army Medical Research and Development Command (USAMRDC) for *in vivo* tests. The preparation of these complexes and the cyanide binding measurements are discussed in this report.

II BACKGROUND

In this section we briefly review the literature of cytochrome oxidase structure and function, cyanide inhibition of cytochrome oxidase enzyme, current therapies for cyanide intoxication, and the use of metal complexes as antidotes with potential application for cyanide intoxication.

Cytochrome Oxidase Structure

A great deal of controversy exists in the literature regarding the structure of the cytochrome oxidase enzyme (ferrocytochrome C: oxidoreductase, EC 1.9.3.1), which undoubtedly will have to wait for single-crystal x-ray analysis for definitive resolution. Single-crystal x-ray analysis of this enzyme has not been reported although a successful crystallization of its cytochrome C complex has been described.[16] Thus the structure of this very complex enzyme must be inferred from other data.

Several reviews on cytochrome oxidase structure and function have recently appeared.[5,6,17,18] The isolated enzyme contains protein, lipid, hemeiron, and protein bound copper in varying ratios depending on the procedure for isolation. However, we need not be concerned with the detailed chemical composition and will focus instead on the active site associated with the metal centers. Cytochrome oxidase has a number of subunits. Although the exact number is still in doubt, a minimum for catalytic activity is two haem groups and two copper ions.

Extraction of the noncovalently bound haem from the protein gives only haem a (Figure 1). However, the two haems of the enzyme are in very different environments in the enzyme and are therefore called haems a and a_3. The difference between haems a and a_3 is that a_3 is usually high spin and reacts with various incoming ligands, whereas haem a is low spin and does not react with ligands. The reaction of haem a_3 with incoming ligands means that the spectroscopic features (e.g., ESR, UV-

VIS) assigned to haem a_3 are perturbed by the ligand. Currently some ambiguity still exists in these assignments.

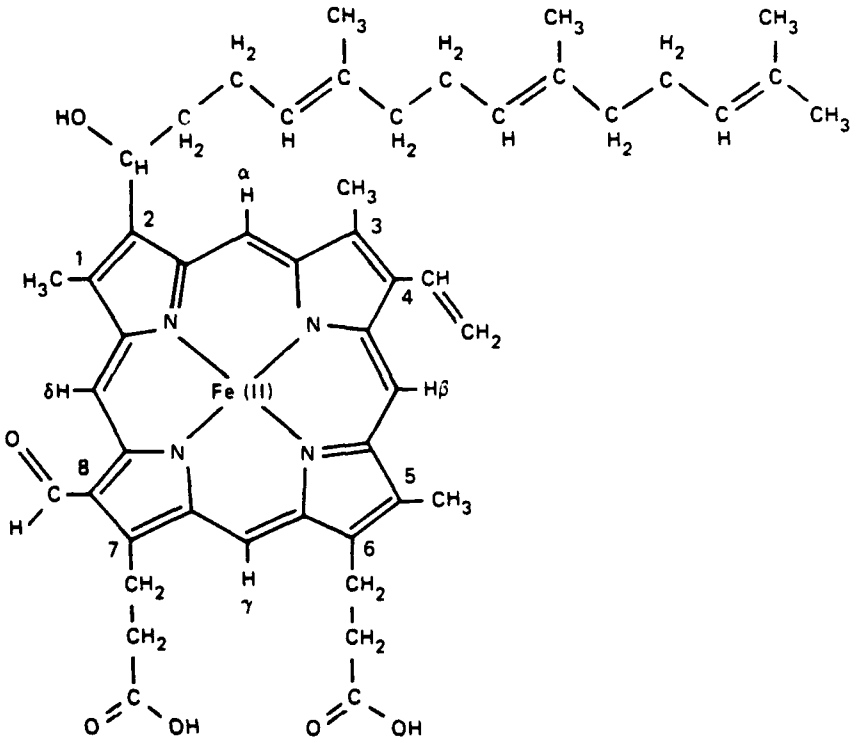


FIGURE 1 HAEM a

The two copper atoms of the enzyme are also in very different environments. One copper (Cu_A) is detectable by electron spin resonance (ESR) spectroscopy and appears to be in rapid redox equilibrium with haem a. (However they do not appear to be in physical proximity because they are not spin-spin coupled.) The second copper (Cu_B) is undetectable by ESR and is in close functional and physical contact with haem a_3. However, the detailed coordination of the two coppers is unknown.

The oxidation state of the metal centers of cytochrome oxidase are not clearly defined. It is clear that conversion between fully oxidized and fully reduced forms of the enzyme is a four-electron process.[19] Falk et al.[20] studied the oxidation state of the enzyme by bulk magnetic susceptibility measurements. Their results are still open to some

interpretation, but they generally fit the scheme of one high spin and one low spin Fe(II) haem and two Cu(I) ions in the fully reduced cytochrome oxidase. The fully oxidized enzyme has magnetic susceptibility well below what would be expected for the one-electron oxidation of each of the metal centers. The explanation is that one Cu(II) is antiferromagnetically coupled to the high spin Fe(III) haem. The ESR spectrum can be interpreted to fit this scheme. The ESR spectrum of the oxidized enzyme shows one Cu(II) and one low spin Fe(III). Thus the high spin Fe(III) and one Cu(II) are ESR silent due to strong coupling. The ESR spectrum of the active Cu(II) has been analyzed to show that a low spin Fe(III) is apparently about 7 Å away (thus leading to the strong redox couple).[21]

The strong metal-metal interaction in cytochrome oxidase is thought to be critical to its function. Cohen has recently reviewed the evidence for strong interaction.[22] The haem a_3 and Cu_B have a strong metal-metal interaction; they are antiferromagnetically spin coupled and redox coupled. The magnitude of the coupling is effected by cyanide coordination, giving an antiferromagnetic coupling constant of over 200 cm^{-1} in the free enzyme and 40 cm^{-1} for the cyanide bound enzyme. Cu_A and haem a are in rapid redox equilibrium, but they are not spin coupled. No apparent interaction exists between Cu_A and haem a_3 or Cu_B, and similarly, no direct interaction is observed between Cu_B and haem a. However, the haems display some interaction. They are clearly in slow redox equilibrium because an electron is transferred between them. However, the exact nature of the interaction is open to debate.[6]

The manner in which the oxidase enzyme resides in the mitochondria membrane has also been investigated. Henderson et al.[23] showed that the enzyme is placed asymmetrically in the membrane; it has a total length of 100 Å with 40 Å protruding into the matrix. Figure 2 shows a hypothetical model of cytochrome oxidase, including its orientation to the membrane.[5]

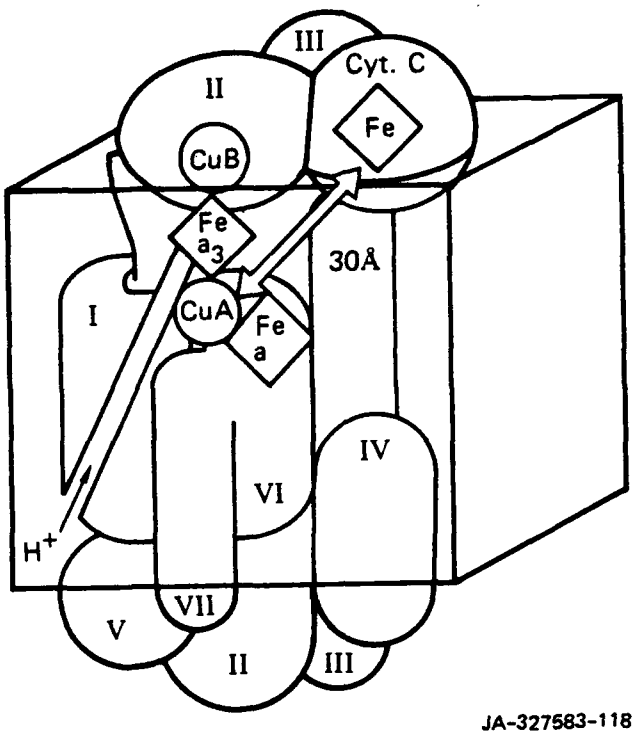


FIGURE 2 HYPOTHETICAL MODEL OF CYTOCHRONE OXIDES

Cytochrome Oxidase Functions

Cytochrome oxidase is the terminal enzyme in the mitochrondrial electron transport chain. It has at least four functions: reducing dioxygen to water, oxidizing Fe(II) cytochrome c, participating in the production of ATP (adnosive triphosphate), and acting as a proton pump. The details of the mechanism for each of these processes are still unclear, but this is an active area of research and some postulates can be presented.[24]

The proposed mechanism for O_2 reduction by cytochrome oxidase is shown in Figure 3, as adapted from Reference 6. Oxygen reacts rapidly

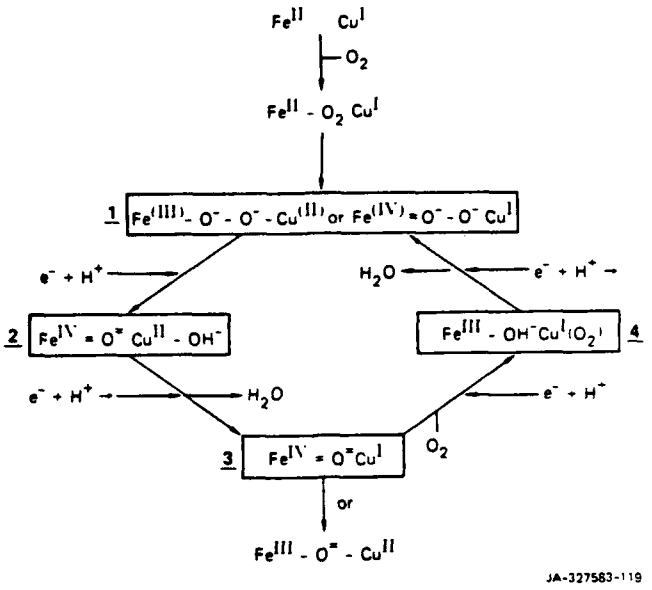


FIGURE 3 PROPOSED MECHANISM FOR O_2 REDUCTION

with the reduced form of the enzyme apparently at the haem a_3 and Cu_B centers to give first an absorption complex. This is followed rapidly by electron transfer, most likely in a two-electron process to give a peroxy complex 1.

The oxidation state of the metals in this peroxy complex is still open to debate because it is ESR silent and the ultraviolet-visible (UV-VIS) spectrum is unusual for Fe(III) haem. 1 reacts with two electrons and two protons to give 3 and one molecule of water. The reaction from 1 to 3 could occur as a two-electron process or in two one-electron steps as shown. Because the structure of 3 is as yet undetermined, both forms are shown. 3 then reacts with O_2, two electrons, and two protons to give back 1 and complete the catalytic cycle.

The details of the conversion of the fully oxidized 3 to 1 are not understood. Perhaps 3 reacts with two protons and two electrons to give water and the fully reduced Fe(II)Cu(I) form, which then reacts with O_2 to give 1. It is also possible that this center may never be fully reduced in a catalytic cycle and thus may proceed through an intermediate such as 4.

The mechanism of reduction of cytochrome oxidase by cytochrome c is reasonably well understood. The Fe(II) cytochrome c rapidly binds to the enzyme and transfers its electron to Fe(III) haem a. The enzyme in either the fully oxidized or partially reduced (2 e^-) form accepts only one electron in this fast step. However, the electron is in equilibrium between the haem a and Cu_A centers. The slow step is transfer of that electron to the a_3 Cu_B center. The overall reaction scheme is shown in reactions (3) through (5), where C is cytochrome c.

$$C^{2+} + a^{3+} \underset{}{\overset{K_1}{\rightleftharpoons}} C^{2+} - a^{3+} \qquad (3)$$

$$\begin{array}{l} C^{2+} - a^{3+} \xrightarrow{k_1} C^{3+} - a^{2+}(Cu_A^{2+}) \xrightarrow{k_2} C^{3+} - A^{3+}(Cu^{2+}) + \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad (Cu_B^{2+}\ ,\ a_3^{2+}) \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \Big\uparrow\Big\downarrow K_3 \\ \qquad\qquad\qquad\qquad C^{3+} - A^{3+}(Cu_A^{+}) \xrightarrow{k_2} (Cu_B^{+},\ a_3^{3+}) \end{array} \qquad (4)$$

$$C^{3+} - A^{3+} \underset{}{\overset{K_4}{\rightleftharpoons}} C^{3+} + a^{3+} \qquad (5)$$

In this scheme k_2 is the rate by which the electron is transferred from haem a to the haem a_3/Cu_B center and is the slow step. The last step is the transfer of the electron to oxygen as described above.

Cytochrome oxidase participates in the production of ATP by providing the energy needed to produce it. This energy is stored as a proton gradient across the membrane. The details of this pump are not well known. However, we do know that $1e^-$ and $1H^+$ are transferred from the matrix side to the outside and that this transfer is associated with haem a. In addition, a second H^+ is taken from the matrix side and used to form water. Thus, overall, a $2H^+$ differential per electron is generated.

Cyanide Binding to Cytochrome Oxidase

Cyanide reacts with isolated oxidized cytochrome oxidase with an apparent second-order rate constant of 2 M^{-1} s^{-1},[25] as measured by a UV-VIS spectral change; this behavior is consistent with a high spin to low spin transition in Fe(III) haem a_3. This haem a_3 is originally high spin and ESR silent, apparently because it is coupled to Cu_B. The cyanide binding to this iron apparently does not uncouple these two spin systems. The measured high spin to low spin transition is independent of cyanide and leads to the proposal that some binding of the cyanide occurs before any interaction with the haem a_3, as shown in reaction (6).

$$(a^{3+}C_A{}^{2+}a_3{}^{3+}C_B{}^{2+}) + HCN \longrightarrow [(a^{3+}C_A{}^{2+}a_3{}^{3+}C_B{}^{2+})(HCN)] \rightleftharpoons a^{3+}C_A{}^{2+}C_B{}^{2+}(a_3{}^{3+}HCN) \qquad (6)$$

The details of this initial binding to the enzyme are not understood, but it is clear that it does not involve either haem. The magnetic susceptibility studies of Tweedle et al.[26] indicate that the final oxidized cyanide-enzyme complex is binuclear, possibly with a bridging cyanide ligand. The dissociation constant of this species is roughly 2×10^{-6} M. The haem a_3 and Cu_B that are associated with cyanide binding are no longer easily reducible; however, the haem a and Cu_A are still reducible.

The reaction of cyanide with the fully reduced enzyme has a second-order rate constant of 2×10^2 M^{-1} s^{-1} and a dissociation constant of 1×10^{-4} M. Thus although the fully reduced enzyme reacts much faster with cyanide, the equilibrium lies further toward free cyanide and may be an important factor in reactivation studies.

The kinetics and equilibria of the two-electron reduced enzyme have been measured only from the inhibition data for the enzyme; from these data, a second-order rate constant for cyanide reaction with the enzyme has been calculated to be 5×10^3 M^{-1} s^{-1}; the dissociation constant for

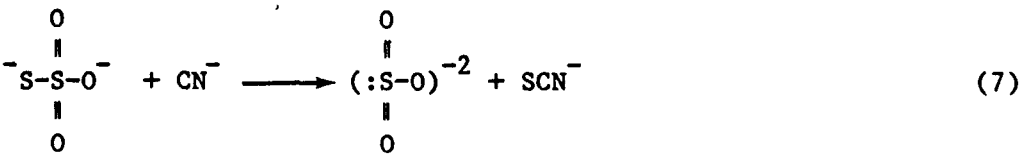
the inhibited enzyme is 1 x 10^{-7} M. These data are calculated from the inhibition data based on the assumption that all inhibition activity in the active enzyme is due to the reaction of cyanide with this partially reduced state.

This assumption is based only on the much higher rate of inhibition than the reaction with either the oxidized or reduced form. Many other explanations may be possible. One plausible explanation is the existence of two forms of the fully oxidized enzyme, one a "resting" state and the other the oxidized state of the catalytic cycle. Clearly, the details of cyanide inhibition of cytochrome oxidase warrant further investigation.

Therapies

The basic strategy for cyanide antidotes is to rapidly bind the cyanide in a form that will not allow it to bind to the cytochrome oxidase and then follow this with a treatment that will convert the cyanide to a nontoxic form for excretion. The current therapies do not regenerate inhibited cytochrome oxidase at an appreciable rate.

The principle nontoxic form by which cyanide is excreted is as the thiocyanate. Cyanide is converted to the thiocyanate by the enzyme rhodanese, which catalyzes the transfer of sulfur from thiosulfate according to reaction (7).



(7)

The thiosulfate is provided in massive quantities as part of the antidotal preparation. The rhodanese enzyme can be isolated as the active form with the transferable sulfur atoms already attached, and it may be an improvement to the current therapy to include some rhodanese in the preparation to speed up this conversion. The primary difficulty with this treatment is that the conversion is rather slow and the cyanide must be bound in some other form before conversion to thiocyanate.[11]

In the current therapy, this initial binding is to methemoglobin to give cyanomethemoglobin. Methemoglobin is produced from hemoglobin by oxidation with sodium nitrite according to reaction (8).

$$Fe^{2+}(hem) + NaNO_2 \longrightarrow Fe^{3+}(hem) + {}^{-}CN \longrightarrow NCFe^{3+}(hem) \qquad (8)$$

The major problems with this therapy are that only a limited amount of methemoglobin can be produced before oxygen transport is critically reduced and that the methemoglobin produced is only able to bind one cyanide per molecule.

An alternative is to introduce a chemical that will strongly bind large quantities of cyanide. Cobalt compounds have long been known to be effective in this function and cobalt (II) chloride and dicobalt (II) EDTA have been used.[13,27] Using these cobalt complexes in combination with thiosulfate has some beneficial effect, but primarily when these salts are used, the cyanide is excreted as cobalt (III) hexacyano trianion.[12] These cobalt complexes have the disadvantages that they are somewhat toxic and must be administered in limited doses and that the cobalt must be oxidized to cobalt (III) in situ (although it is not established that this in situ oxidation is truly a disadvantage).

Hydrocobalamin (Vitamin $B_{12}a$) is a cobalt (III) complex that strongly binds cyanide to give cyanocobalamin (Vitamin B_{12}). Hydroxocobalamin is an effective antidote for cyanide, particularly in conjunction with sodium thiosulfate,[28-29] but it has two disadvantages: (1) it has limited solubility and (2) it binds only one cyanide per molecule.[30]

In a previous contract with USAMRDC, we have demonstrated the synthesis of water-soluble cobalt (III) complexes.[31] After testing these cobalt complexes for reaction with cyanide, we find they react rapidly.[32] One of these complexes has shown efficacy as a pretreatment in vivo.[33]

Metal Complex Antidotes

Metal complexes can serve as antidotes for specific toxins in

several ways. The two most common are to catalyze a detoxifying chemical reaction or to coordinate the toxin in a metal complex that will not allow it to function in its toxic manner. Cyanide is detoxified by the following two chemical reactions:

$$[S{=}S{=}O]^{2-} + CN \longrightarrow {}^{-}SCN + [S{=}O]^{2-} \qquad (9)$$

$${}^{-}CN + 1/2\ O_2 \longrightarrow {}^{-}OCN \xrightarrow{O_2} CO_2 \qquad (10)$$

and both of these may be metal catalyzed.

Cyanide is a very good ligand,[34] which facilitates detoxification by complexation. The mechanism of metal complex antidotes is shown in equation (11).

$$M + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} K = \frac{[MS]}{[M]\cdot[S]}\ , \qquad (11)$$

where S is the toxic substance (i.e., cyanide). The parameters that govern the efficacy of the metal M are k_1 and K. The formation constant k_1 is a measure of the speed with which the metal binds the ligand, and K is the binding constant, a measure of how tightly bound the ligand is. Unless K is high enough to make very little ligand available at equilibrium, the speed at which equilibrium is reached is of little importance. However, in the case of cyanide, not only is the cyanide tightly bound to the enzyme, but also equilibrium is reached rapidly. Thus an effective cyanide antidote must have very high K and k_1 values. For effective excretion of the intact metal cyanide complex, it is important that k_{-1} be small.

Basolo and Pearson, in their classic text on inorganic reactions, discuss the reaction between cyanide and metal ions.[35] The order of

rate (k_1) of achieving equilibrium was:

$$V^{3+} > Mn^{+3} > Cr^{2+} > V^{2+} \sim Mn^{2+} >> Cr^{3+} > Fe^{2+} > Fe^{3+} \sim Co^{3+} \qquad (12)$$

Thus Fe^{3+} and Co^{3+} are the slowest of the metal ions in achieving equilibrium. The binding constants for cyanide with a large number of metals are available.[36,37] Binding constants for the most stable metal ion-cyanide complexes are given below (as log K) in Table I.

Table I

STABLE METAL ION-CYANIDE COMPLEXES

Ion	Log K	Equilibria
Fe^{2+}	35.4	$Fe(CN)_6^{4-} \rightleftharpoons Fe^{2+} + 6CN^-$
Fe^{3+}	43.6	$Fe(CN)_6^{3-} \rightleftharpoons Fe^{3+} + 6CN^-$
Pd^{2+}	45.3	$Pd(CN)_5^{3-} \rightleftharpoons Pd^{2+} + 5CN^-$
Mn^{2+}	34.5	$Mn(CN)_6^{4-} \rightleftharpoons Mn^{2+} + 6CN^-$
Co^{3+}	64.0	$Co(CN)_6^{3-} \rightleftharpoons Co^{3+} + 6CN^-$
Co^{2+}	19.1	$Co(CN)_6^{4-} \rightleftharpoons Co^{2+} + 6CN^-$
NI^{2+}	22.2	$NI(CN)_4^{2-} \rightleftharpoons NI^{2+} + 6CN^-$
Cu^{2+}	26.7	$Cu(CN)_4^{2-} \rightleftharpoons Cu^{2+} + 4CN^-$

Thus, as cyanide antidotes, complexes of V, Mn, or Cr would be preferred on the basis of k_1, and complexes such as Fe^{3+}, Pd^{2+}, and Co^{3+} would be preferred on the basis of K.

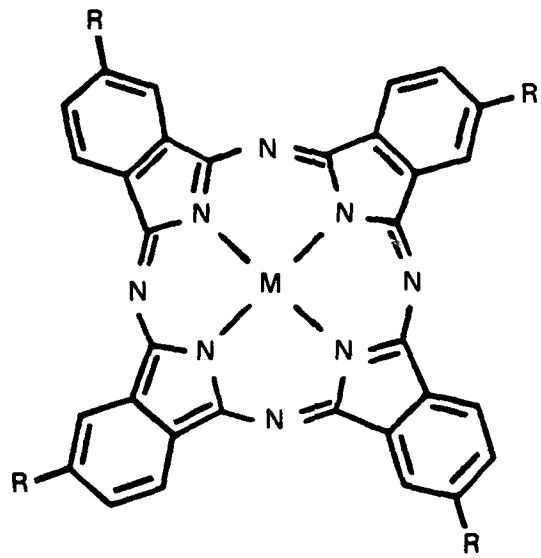
III OBJECTIVES AND APPROACH

The overall objective of our research is to develop new antidotes for cyanide intoxication based on metal complexes that have high binding constants for cyanide. The selection criteria for candidate antidotes is based on optimization of the activity of complexes such as Co(II)EDTA, which have been shown to be effective. Thus, the three specific objectives of this program are as follows: (1) optimize the metal ion to provide maximum in vivo efficacy coupled with low toxicity, (2) optimize antidotal activity by altering the chelate to increase competitive binding of cyanide ion, and (3) optimize stability and solubility in water of the potential antidotes.

Our approach is iterative. Test compounds are prepared on a small scale and their chemical and physical characteristics are evaluated. We measure the detailed kinetics and equilibria of cyanide binding to the test compounds. On the basis of these evaluations, new target compounds are identified (or a family of compounds) and promising candidates are resynthesized in 3- to 10-gram quantities to support in vivo tests and evaluation by MRDC staff.

The project was conducted in two parts: First, the metal complexes were synthesized and characterized, and second, the kinetics and equilibria of cyanide binding to the metal complex test compounds were measured. The metal complexes investigated are shown in Table II.

Since the beginning of this project, we have concentrated on the synthesis of macrocyclic compounds that fall into Class 3 (Table II) phthalocyanine complexes. Their structure is shown in Figure 4. Phthalocyanine compounds were chosen because they have the following desirable properties: (1) high stability and solubility, (2) wide choice of complexing metal, (3) a reactivity that can be varied by varying the peripheral substitution group (R), (4) a structure resembling the porphyrins that are present at the active site of cytochrome oxidase.



TSPC R = SO_3^- Na^+

TAPC R = NH_3^+ Cl^-

TCPC R = CO_2^- Na^+

M = Co(II), Co(III), Fe(II), Fe(III), Ni(II), Cu(II), Ru(III), Pd(II), O = Mn(IV), O = V(IV).

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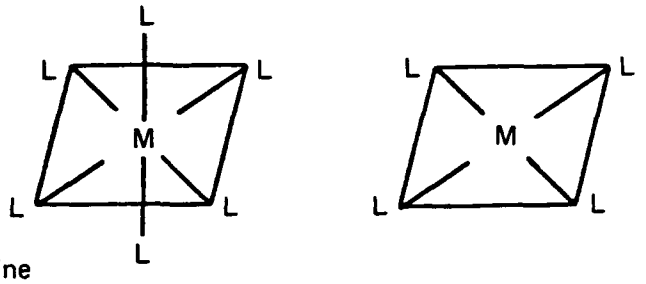
FIGURE 4 STRUCTURE OF PHTHALOCYANINE COMPLEXES

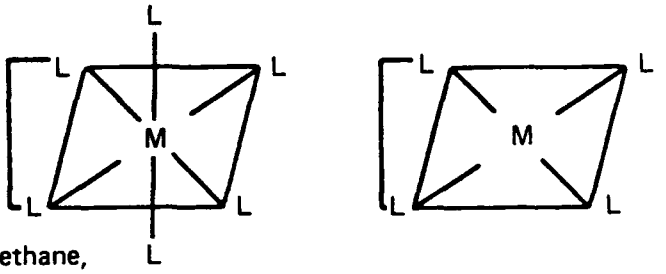
Table II
PROPOSED METAL COMPLEXES

Class 1 - Non-chelated Complexes



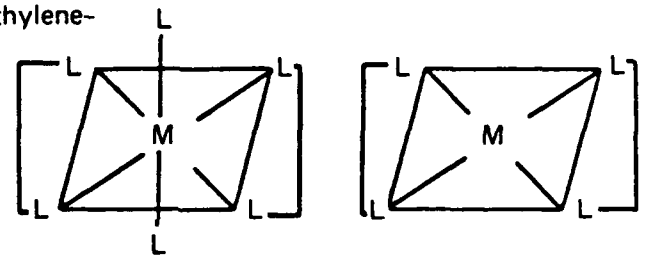
L = H_2O, NH_3, pyridine, or morpholine

Class 2 - Mono-bidentate Complexes

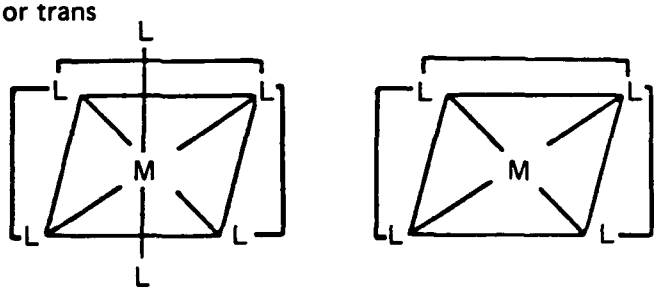


L L = 2,2'-bipyridyl, 1,2-diaminoethane, 1,3-diaminopropane, 2,3-diamino-2,3-dimethylethane, 1,2-diamino-cyclohexane, tetramethylethylene-diamine, or symdiethyl-propanediamine.

Class 3 - Bis-bidentate Complexes

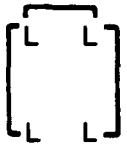


cis or trans

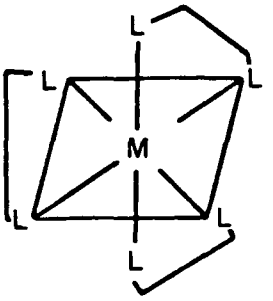


cis or trans

or Tetradentate Complexes

where  = 1,4,8,11-tetra-azocyclotetradecane, 1,4,7,10-tetraazaclecane, 1,4,8,11-Tetrazaundecane, 1,5,8,12-Tetraaza-dodecane, tris(2-aminoethyl) Amine, or triethylenetetramine.

Class 4 - Tris-bidentate Complexes



where M = Co(II), Co(III), Fe(III), Mn(II), and Pd(II)

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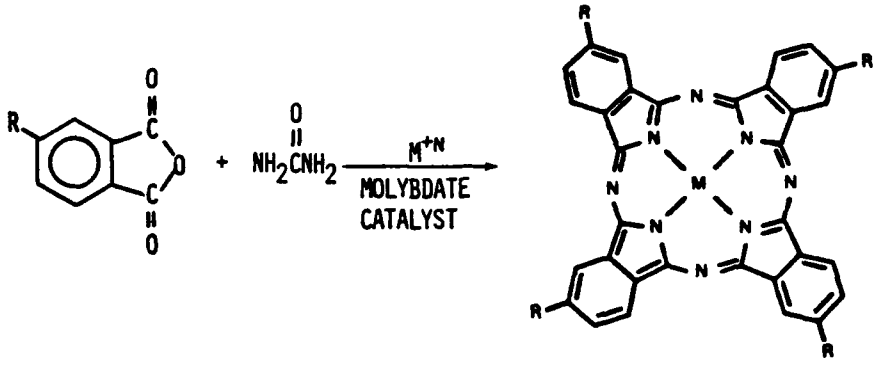
IV RESULTS AND DISCUSSION

Syntheses

The phthalocyanine complexes are synthesized by the phthalic anhydride-urea process.[38] Inorganic metal salts are used both as template and as the metal source. Ammonium molybdate is an effective catalyst for these reactions:



Depending on the substituent group on the phthalic anhydride (R), a wide variety of substituted phthalocyanine compounds can be made. For our purpose, we chose the sulfonic and carboxylic acid derivatives. We have also prepared the tetramino derivative of phthalocyanine by using the 4-nitrophthalic anhydride and subsequently reducing the four nitro groups with sodium sulfide. Acidification of these tetramino phthalocyanine complexes gives the quaternary salts.

Both the sulfonic and the carboxy derivatives are prepared in the form of sodium salts to increase their water solubility. Purification of these sodium salts of phthalocyanine compounds is difficult. A Considerable amount of water is always found in the solid product. Because of the water content and the high formula weight, elemental analyses do not always provide adequate assurance of purity. Magnetic susceptibility measurements have been suggested as an alternate

analytical method. Table III lists the magnetic moments of four of the tetrasulfophthalocyanine complexes. They are in good agreement with those values reported by Busch and Weber.[39] The magnetic moment for Fe(II)TSPC has not been reported. A value of 3.3 BM indicates the Fe(II) is at an intermediate spin state between S = 1 and S = 2. This property is usually observed in the iron (II) porphyrin compounds, which are structurally very similar to the phthalocyanines.[40]

UV-visible spectra also provide valuable information on the purity. In the preparation of Co(III)TSPC, incomplete oxidation of Co(II)TSPC may result in a major impurity. The UV-visible spectrum of Co(II)TSPC has an intense absorption at 658 nm that shifts to 672 nm upon oxidization to Co(III)TSPC. Similarly, Fe(II)TSPC may be contaminated by Fe(III)TSPC because of insufficient reduction or subsequent air oxidation. Absorptions at 634 nm due to the Fe(III)TSPC and at 670 nm due to the Fe(II)TSPC can be used as an indication of purity. Some of the UV-visible spectral data are listed in Table IV.

Table III

MAGNETIC MOMENTS OF THE PHTHALOCYANINE COMPLEXES

Compound	Magnetic Moment, μ_{eff} (B.M.)	
	This work[a]	Reported[b]
Co(II)TSPC	1.95 ± 0.08	1.88 ± 0.05
Co(III)TSPC	0.73 ± 0.09	0.74 ± 0.05
Fe(II)TSPC	3.30 ± 0.05	
Fe(III)TSPC	2.08 ± 0.01	1.80 ± 0.05

[a]Measured by ^1H NMR operated at 89.55 MHz in ca. 10^{-2} M D_2O solution at 27°C.

[b]From References 38 and 39

Table IV

UV-VISIBLE ABSORPTION DATA OF METAL PHTHALOCYANINE COMPLEXES

Complex	λ_{max} (nm) (10^{-4} ε) Absence of CN^-	+ 100-fold CN^-
Co(II)TSPC	214 (7.13)	205 (7.04)
	320 (7.32)	245 (8.76)
	630 (6.19)	280 (5.87)
	658 (6.3)	347 (5.13)
		662 (6.40)
		676 (15.7)
Co(III)TSPC	214 (6.50)	244 (8.10)
	284 (5.04)	280 (5.53)
	326 (5.36)	348 (4.82)
	672 (8.25)	616 (3.29)
		676 (14.04)
Fe(III)TSPC	221 (8.44)	220 (11.48)
	322 (8.74)	314 (7.65)
	430 (1.95)	352 (5.58)
	610 (3.45)	372 (5.98)
	670 (11.50)	610 (3.95)
		672 (14.41)
Fe(II)TSPC	218 (7.24)	217 (7.48)
	285 (4.01)	287 (4.10)
	328 (5.87)	328 (6.02)
	634 (7.12)	634 (7.17)

[a]Solution concentrations were ca. 1 x 10^{-5} M.
[b]Cell length equals 1 cm.

Kinetics

The kinetics of cyanide binding to the test metal complexes were studied using three different techniques. First, UV-visible changes in the spectra of the phthalocyanines are observed with added cyanide ion. This technique is most effective for kinetic measurements under pseudo-first-order conditions where cyanide is in excess. However, this standard technique is limited to the excess cyanide regime and is an indirect technique. Hence, attempts were made to develop a technique that directly measures the cyanide concentration and would be applicable either to pseudo-first-order conditions where the metal complex is in excess or to second-order conditions. Our second attempt involved an IR technique, but this was unssuccessful. Hence, a third technique using a cyanide ion selective electrode (ISE) was developed.

In the first technique, changes in the UV-visible spectra were used to study cyanide binding to these phthalocyanine compounds. A gradual change in the UV-visible spectrum after addition of cyanide ion to the Co(II)TPSC is shown in Figure 5. Isobestic points are observed at λ = 286, 338, and 469 nm. Isobestic points indicate conversion from starting material to product without an intermediate or decomposition. The Co(III)TSPC and the Fe(II)TSPC behave similarly. These spectral changes indicate the formation of the cyanide bound complex, which can be expressed by the following equation:

$$\text{L-MTSPC} - \text{H}_2\text{O} + 2\text{CN}^- \xrightarrow{k_{obs}} \text{NC-MTSPC-CN} + \text{H}_2\text{O} + \text{L} \qquad (14)$$

where L = H_2O for the Co(II) and Fe(II) complexes, and L = OH^- for the Fe(III) and Co(III) complexes. The absorption data of the cyanide adducts, based on the addition of a large excess of cyanide ion to the metal phthalocyanine solution, are listed in Table IV. Unfortunately, the Fe(III)TSPC did not provide significant spectral change for kinetic study.

The rate of cyanide binding to the Co(II), Co(III), and Fe(II)TSPC was studied by using UV-visible spectral detection addition of a 10-fold

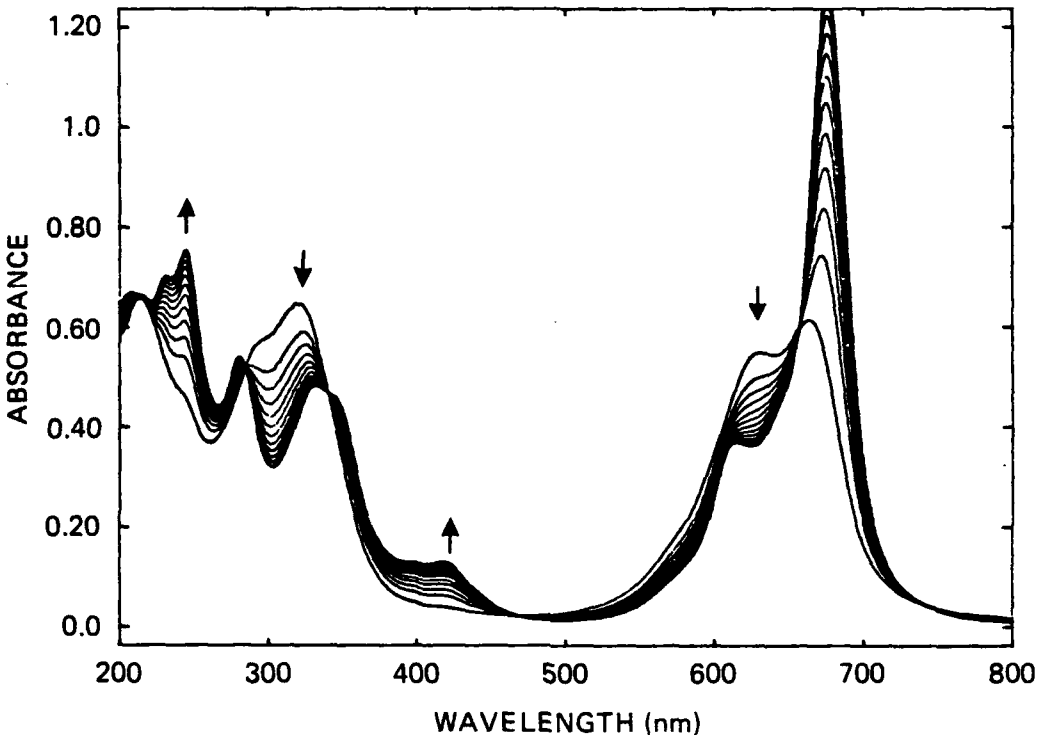


FIGURE 5 UV-VISIBLE SPECTRA OF Co(II)TSPC (1×10^{-5} M) IN PRESENCE OF KCN (1×10^{-4} M)AT 25°C IN pH 8 AQUEOUS SOLUTION

Time Intervals = 1 min.

excess of cyanide ion. Figure 6 shows a pseudo-first-order plot of the Co(II)TSPC reaction. The rate constants are listed in Table V along with the rate of mesohemin for comparison. The results show that Fe(II)TSPC reacts about three times faster than the cobalt analogs and that the two cobalt complexes react at the same rate. This indicates that the nature of the metal ion [e.g., Fe(II) versus Co(II)] has a significant effect on the rate of cyanide binding. Iron appears to have a higher affinity for cyanide ion than does cobalt. Apparently the oxidation state of the metal ion Co(II), compared with Co(III), has little effect on the rate of cyanide binding.

Table V

OBSERVED FIRST-ORDER RATE CONSTANTS OF THE CYANIDE BINDING

Compound	10^3 k_{obs} (sec^{-1})
Co(II)TSPC	1.6
Co(III)TSPC	1.5
Fe(II)TSPC	4.8
Mesohemin[a]	27.5

[a]Calculated from Figure 2 of Reference 40.

It is informative to compare the kinetic data of our system with results of a porphyrin system. Hambright and Chock have reported their kinetic study on cyanide binding to mesohemin, deuterohemin, and protohemin[41] (Figure 7). Wjem their results are converted to the conditions used in our system (i.e., $[CN^-] = 10^{-4}$ M, pH = 8), the phthalocyanine complexes react roughly 5 to 20 times slower than the porphyrin complex, $k_{obs} = 4.8 \times 10^{-3}$ and 2.75×10^{-2} s^{-1}, for Fe(II)TSPC and mesohemin, respectively. However, it is known that the cyanide binding rate is affected by the concentrations of hydroxide, cyanide, and metal complex.

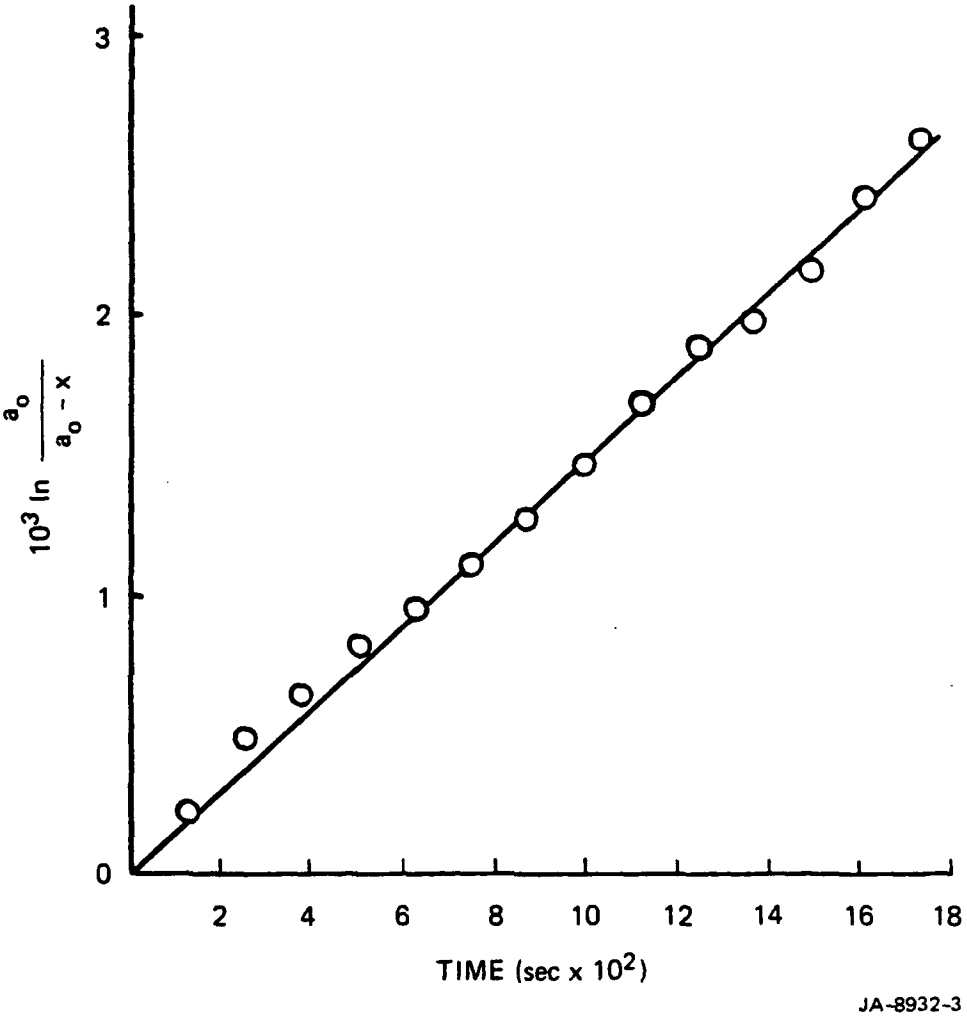


FIGURE 6 PSEUDO-FIRST-ORDER PLOT OF CYANIDE BINDING TO Co(II)TSPC AT AMBIENT TEMPERATURE, pH = 8, [Co(II)TSPC] = 1×10^{-5} M, [CN^-] = 1×10^{-4} M

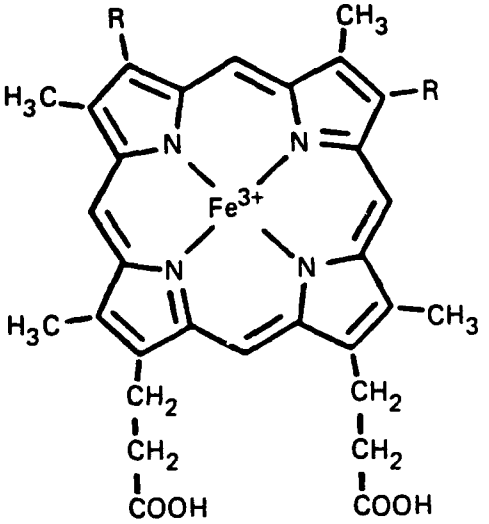


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FIGURE 7 STRUCTURAL DRAWING OF NATURAL PORPHYRINS

(a) Protohemin, R = $-CH=CH_2$;
(b) *Deuteroheimin*, R = H;
(c) Mesohemin, R = $-CH_2CH_3$

Attempts to use an FT-IR method to study the kinetics of cyanide binding to metal complexes failed. Aqueous potassium cyanide solution was placed in a cylindrical internal reflection cell (circle cell) with a ZnSe crystal. Unfortunately, the spectrum of pure water shows a broad weak peak centered at 2100 cm^{-1}. (Assignment of this band is uncertain.) The broad band overlaps with the cyanide vibrational peak ($\nu_{C\equiv N}$), which occurs at 2080 cm^{-1}. The cyanide peak cannot be observed at a concentration lower than ~1 M. No significant change in the absorptivity is observed when the concentration of potassium cyanide is increased to 2 M (due to water overlap). Because the maximum solubility of the metal tetrasulfophthalocyanine is about 0.2 M, it is not practical to study kinetics of cyanide binding to metal tetrasulfonated phthalocyanines by this technique. The solvent system could be changed to use a nonaqueous solvent such as dimethyl sulfoxide or dimethylformamide, which may allow us to study the reaction, but this type of solvent is not suitable for our research purpose.

The use of the cyanide ion-selective electrode was more successful. Reactions of cyanide with Co(II), Co(III), Fe(II), and Fe(III) tetra-

sulfophthalocyanine were followed by measuring the decrease in cyanide concentration. The results are plotted in Figure 8. The overall reaction is expressed by

$$L_2MPC + 2\ CN^- \underset{k_r}{\overset{k_f}{\rightleftharpoons}} [(NC)_2MPC]^{2-} + 2L \qquad (15)$$

where PC represents the phthalocyanine dianion (tetrasulfonated derivative), M is the metal cation, and L is the labile axial ligand water or hydroxide ion. Assuming the reaction is irreversible, that is, $k_f >> k_r$, then the rate law is

$$\text{Rate} = k_f[L_2MPC][CN^-]^2 \qquad (16)$$

With the metal complex acting as the limiting reagent, as the UV-visible method was applied, the reaction became pseudo-first order:

$$\text{Rate} = k^1_{obs}[L_2MPC] \qquad (17)$$

Because the ion-selective electrode measures the changes of cyanide concentration, we have to choose the cyanide ion as the limiting reagent. When an excess of metal complexes is used, the observed rate constant is pseudo-second-order.

$$\text{Rate} = k^2_{obs}\ [CN^-]^2 \qquad (18)$$

The integrated form of eq. (18) is

$$\frac{1}{[CN^-]} = k^2_{obs} \cdot t + \frac{1}{[CN^-]_o} \qquad (19)$$

According to equation (19), a plot of $1/[CN^-]$ versus t should be linear, with a positive slope equal to k^2_{obs} and an intercept of $\frac{1}{[CN^-]_o}$. All reactions we have studied can be fit to second-order lines with good correlations. Two examples are shown in Figure 9. The rate constants obtained are listed in Table VI.

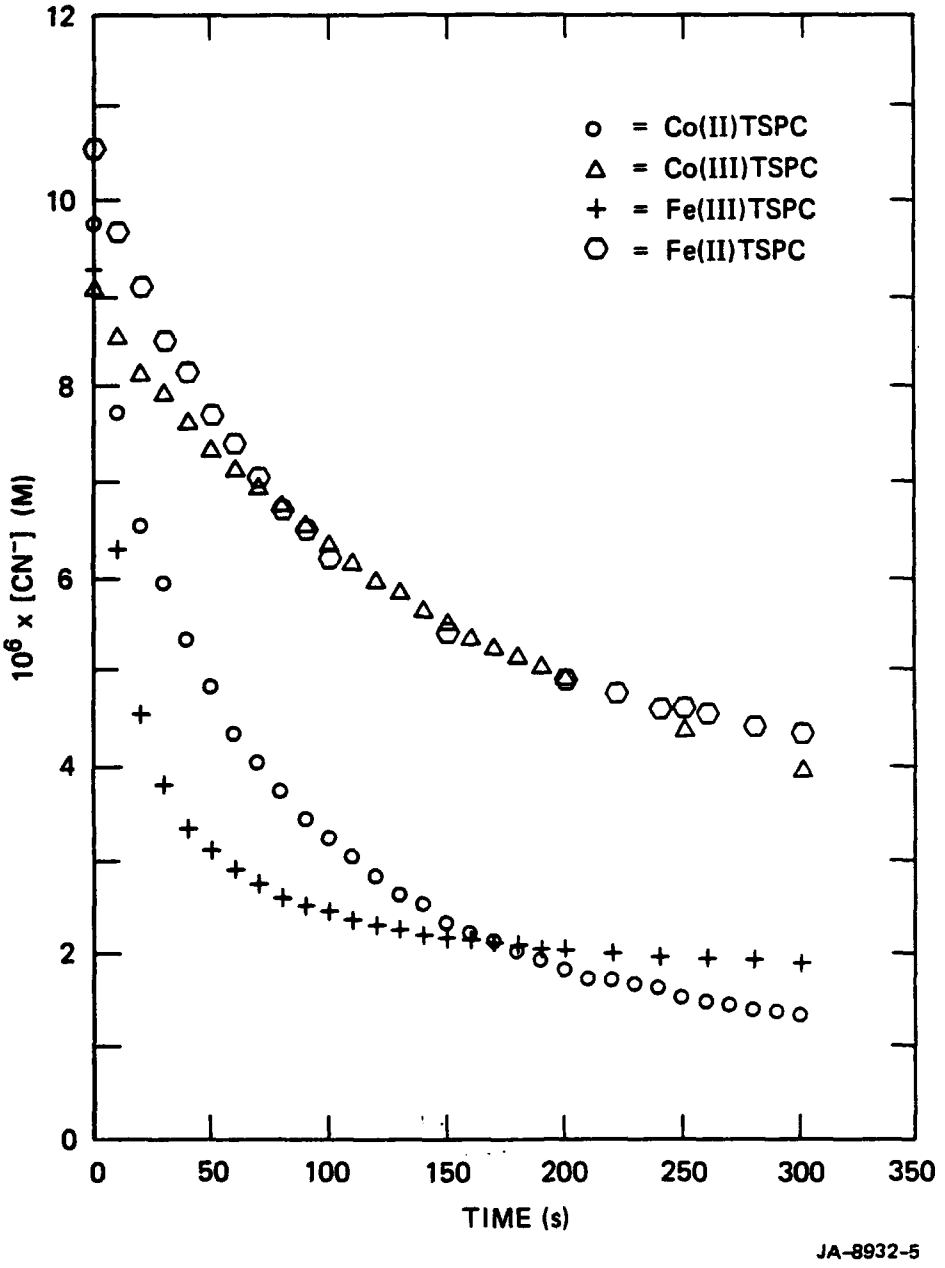


FIGURE 8 PLOT OF CYANIDE CONCENTRATION VERSUS TIME DURING THE REACTION OF CYANIDE BINDING TO THE METAL COMPLEXES

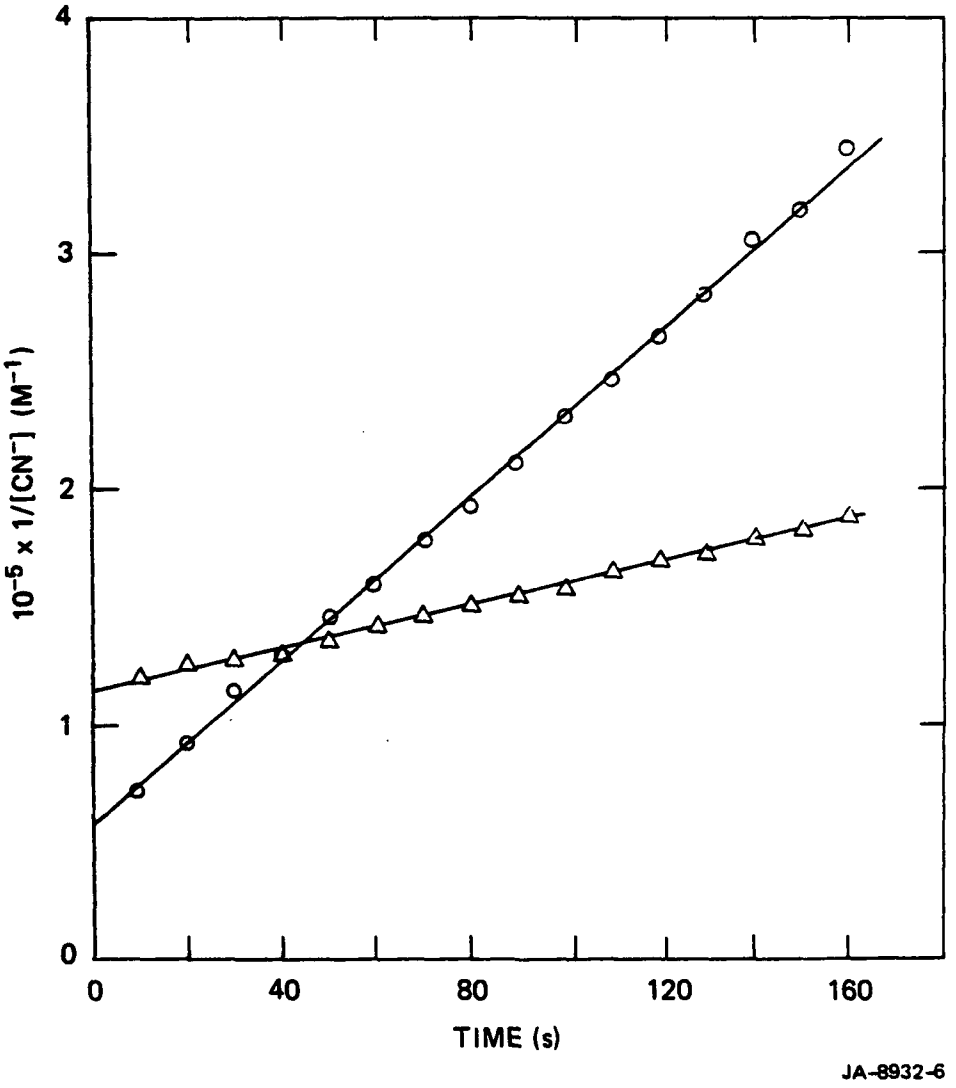


FIGURE 9 PSEUDO-SECOND-ORDER PLOT OF CYANIDE BINDING TO Co(II)TSPC, -o- AND Co(III)TSPC -△- AT AMBIENT TEMPERATURE, pH = 13, [Co(II)TSPC] = 1.3×10^{-4} M, [CN$^-$] = 0.95×10^{-5} M, [Co(II)TSPC] = 1.22×10^{-4} M, [CN$^-$] = 0.95×10^{-5} M

Table VI

THE RATE CONSTANTS OF CYANIDE REACTION WITH METAL COMPLEXES

Compound	$10^{-2} \times k^2_{obs}$ $(M^{-1} s^{-1})^a$	$10^3 \times k^1_{obs}$ $(s^{-1})^b$
Co(II)TSPC[c]	22.5 ± 0.8	1.6
Co(III)TSPC	4.7 ± 0.17	1.5
Fe(II)TSPC	5.8 ± 0.7	4.8
Fe(III)TSPC	27.1 ± 0.8	
Co(III) trien[d]	0.17 ± 0.017	
Co(III) cyclen[e]	0.53 ± 0.02	
Fe^{3+} enzyme[f]	0.02[g]	
Fe^{2+} enzyme[f]	2.0[g]	

[a] k^2_{obs} is the observed second-order rate constant obtained by the cyanide electrode method. Initial concentration of metal is about 1 x 10^{-4} M and initial concentration of cyanide is about 1 x 10^{-5} M. Reactions were run at ambient temperature.

[b] k^1_{obs} is the observed first-order rate constant.

[c] TSPC = 4,4',4",4" -tetrasulfophthalocyanine dianion.

[d] Co(III)trien = cis-aquohydroxo-triethylenetetramine cobalt(III) diperchlorate.

[e] Co(III)cyclen = 1,4,7,19-tetraazacyclododecane aquohydroxo cobalt(III) diperchlorate.

[f] Enzyme = cytochrome oxidase.

[g] Obtained by UV-visible method from Reference 43.

It is interesting to compare the kinetic data obtained by the UV-visible method[5] with the data obtained from the ion-selective electrode. The reaction rate based on the oxidation state and the nature of the metal decreases in the order $Fe^{2+} > Co^{2+} \approx Co^{3+}$, as measured by UV-VIS. However, the cyanide electrode method gives a different result in which the decrease is in the order $Co^{2+} > Fe^{2+} \approx Co^{3+}$.

The faster rate of loss of cyanide ion (measured by ion-selective electrodes) compared with the formation rate of the dicyanide complex of Co(II)TSPC (measured by UV-visible method) may be explained by referring to an observation of Busch and Weber.[38] The Co(II)TSPC is oxidized to Co(III)TSPC by molecular oxygen in the presence of two equivalents of cyanide ion. After the oxidation is completed, no trace of cyanide or cyanide derivatives can be detected. Apparently, the reaction of Co(II)TSPC with excess cyanide in the presence of oxygen is a combination of the following reactions:

$$Co^{2+}TSPC + 2CN^- \xrightarrow{k_{10}} [(NC)_2Co^{2+}TSPC]^{2-} \qquad (20)$$

$$[(NC)_2Co^{2+}TSPC]^{2-} + O_2 + OH^- \xrightarrow{k_{11}} (HO)Co^{2+}TSPC + ? \qquad (21)$$

$$(HO)Co^{3+}TSPC + 2CN^- \xrightarrow{k_{12}} [(NC)_2Co^{3+}TSPC]^- + OH^- \qquad (22)$$

The final product is a cobalt(III) dicyanide complex rather than a cobalt(II) dicyanide complex. This is supported by the UV-visible spectra of the cyanide addition adducts of Co(II)TSPC and Co(III)TSPC. Figure 10 shows that the two final products have practically the same spectra. Thus the rate constant obtained from the UV-visible method is based on following the formation of the cobalt(III) dicyanide complex, and the rate of cyanide decrease followed by the electrode method results from the combination of reactions (20) and (22). This observation also explains why the cyanide concentration in Figure 8 for Co(II)TSPC does not decline to an equilibrium value as is the case for Fe(III)TSPC.

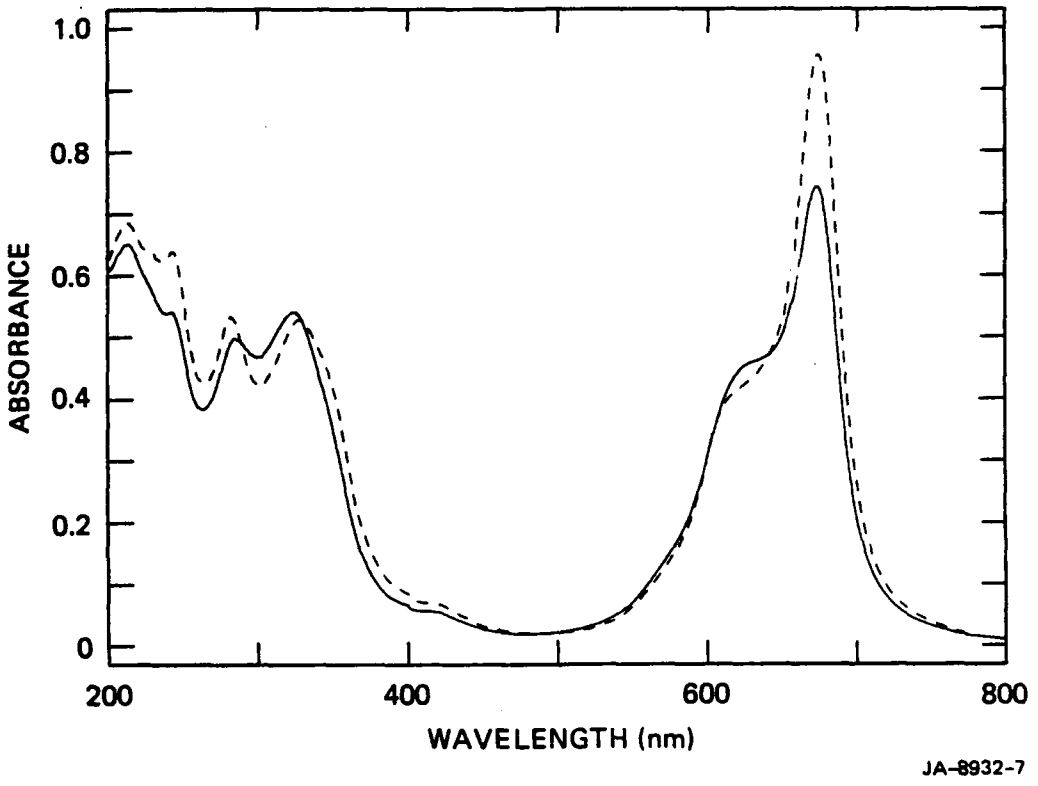


FIGURE 10 UV-VISIBLE SPECTRA OF Co(II)TSPC + 100 FOLD CN^- (SOLID LINE) AND Co(III)TSPC + 100 FOLD CYANIDE (DASHED LINE)

The rate constant k_f of reaction (15) can be calculated by equation (23) [from eq. (17)] or by equation (24) [from eq. 18)]:

$$k_f^1 = \frac{k_{obs}^1}{[CN^-]^2} \tag{23}$$

$$k_f^2 = \frac{k_{obs}^2}{[MPC]^2} \tag{24}$$

In theory, k_f^1 and k_f^2 should have the same value if we assume that the reaction is a simple one-step reaction as expressed by equation (15) and $k_f >> k_r$. However, the results listed in Table VII show that this is not the case. The rate constants obtained from the cyanide electrode method are approximately 10 to 100 times larger than those obtained from the UV-visible method. The concentration of the hydroxide ion, which affects both the ligand dissociation, eq. (25), and the cyanide ion concentration, eq. (26), may be a major factor causing the disagreement.[43]

$$(OH)(H_2O)MPC \rightleftharpoons (H_2O)MPC + OH^- \tag{25}$$

$$HCN + OH^- \rightleftharpoons CN^- + H_2O \tag{26}$$

The reaction rate is also influenced by the dimerization of phthalocyanine complexes. Further kinetic study of these reactions will result in a better understanding of the detailed reaction mechanism.

Nicholls and Chance used the UV-visible method to obtain the second-order rate constants of cyanide reaction with isolated cytochrome oxidase.[25] Their results, which are also listed in Table VII, show that the phthalocyanine complexes react much faster than the isolated enzyme (approximately 2 to 10 times faster than the reduced enzyme and 200 to 10,000 times faster than the oxidized enzyme).

Table VII

RATE CONSTANTS OF CYANIDE REACTION WITH METAL TETRASULFOPHTHALOCYANINE COMPLEX

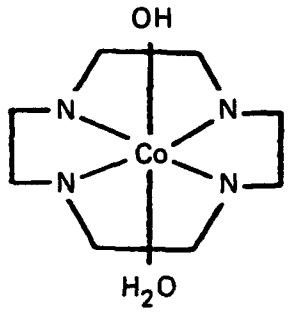
Compound	UV-Visible Method[a]			Cyanide Electrode Method[a]		
	$10^5 \times [CN^-]$	$10^5 \times [M^+]$	$10^{-5} \times k_f^1$	$10^5 \times [CN^-]$	$10^5 \times [M^+]$	$10^{-5} \times k_f^2$
Co(II) TSPC	0.99	10	1.58	0.95	11.32	198.8
Co(III) TSPC	0.99	20	1.49	0.91	11.22	41.9
Fe(II) TSPC	0.99	15	4.75	1.26	13.70	42.3
Fe(III) TSPC				0.94	12.2	222.1

*Rate constants k_f^1 and k_f^2 are defined by equations (23) and (24), respectively.

We also studied two other Co(III) complexes: Triethylenetetramine cobalt(III) diperchlorate (Co(III)trien) and 1,4,7,10-tetraazacyclododecane aquohydroxo cobalt(III) diperchlorate (Co(III)cyclen) (Figure 11). The rate constants for their reaction with cyanide, which are listed in Table VI, are smaller than those obtained with the phthalocyanine compounds. The Co(III)trien has been tested as a cyanide antidote in vivo in a mouse model at WRAIR and proven to be effective in the pretreatment mode.

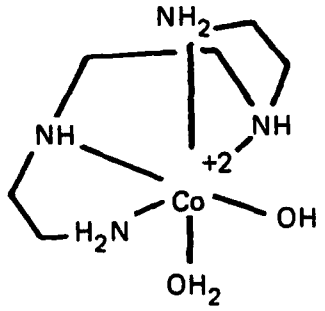
To study ligand effects on the rate of cyanide binding, we have synthesized other phthalocyanine analogs by varying the R group on the phthalocyanine ring (see Figure 4). Tetraaminophthalocyanine complexes of Co(II) and Fe(III) were synthesized and fully characterized. Unfortunately, the insolubility of these compounds in basic aqueous solution prevents the study of their reactivity toward cyanide binding.

The tetracarboxyphthalocyanine complexes are synthesized by a method similar to that for preparing tetrasulfophthalocyanine complexes. Higher yield can be achieved by carefully grinding the starting reagents to a fine powdery mixture. In some cases the reaction is conducted under a nitrogen atmosphere for a better yield. The cobalt(III) complex is prepared by oxidizing the cobalt(II) complex with bromine. The tetrasulfophthalocyanine complexes of palladium(II), copper(II), and nickel(II) are synthesized by the method described by Busch and Weber.[38] Moderate yields and good elemental analyses are obtained. The ruthenium(III), oxomanganese(IV), and the oxovanadium(IV) complexes are synthesized by the same method. However, these complexes are difficult to isolate. The pure manganese complexes are isolated in a very low yield (2%). Pure ruthenium and vanadium complexes have not yet been obtained, as indicated by the elemental analyses. The major impurity is sodium chloride, which coprecipitated with the desired product. Because sodium chloride will not affect the cyanide binding measurements, the kinetic measurements of these two complexes were studied by using excess amount of the crude products and quantifying the phthalocyanine concentration by UV-VIS.



$\cdot 2C\ell O_4^-$

1,4,7,10-Tetraazacyclododecane aquohydroxo cobalt (III) diperchlorate, Co(III) cyclen



$\cdot 2C\ell O_4^-$

cis-Aquohydroxo-tri-ethylenetetramine cobalt (III) diperchlorate, Co(III) trien

JA-8932-9

FIGURE 11 STRUCTURES OF THE Co(III) CYCLEN AND Co(III) TRIEN

Table VIII

THE SECOND-ORDER RATE CONSTANTS OF CYANIDE REACTION WITH METAL PHTHALOCYANINE COMPLEXES[a]

Compound	k_{obs} (M^{-1} s^{-1})
Co(II)TSPC[b]	2250
Co(III)TSPC[b]	470
Fe(II)TSPC[b]	580
Fe(III)TSPC[b]	2700
Co(II)TCPC	3900
Co(III)TCPC	180
Fe(III)TCPC	920
Pd(II)TSPC	28
Cu(II)TSPC	11
Ni(II)TSPC	28
Ru(III)TSPC	30
O=Mn(IV)TSPC	b
O=V(IV)TSPC	b
Co(III)cyclen[b]	53
Co(III)trien[b]	17
Oxid. cytochrome oxidase[b]	2
Red. cytochrome oxidase[b]	200

[a]Initial concentration of metal ion is approximately 1×10^{-4} M and that of cyanide ion is approximately 1×10^{-5} M. Reactions are run at ambient temperature.
[b]Too slow to be measured.

The observed second-order rate constants of Co(II)TSPC and Co(II)TCPC are 2250 M^{-1} s^{-1} and 3900 M^{-1} s^{-1}, respectively (Table VIII). The difference between these two complexes is the peripheral substitution groups: one contains sulfonates and the other contains carboxylates. The electronegativities of carbon and sulfur are about the same, but the sulfonate has more π-electrons than the carboxylate. Therefore, more π-electrons are being donated to the phthanocyanine ring system by the sulfonate and hence the electron density at the metal center is increased. As a result, the metal ion will have less affinity toward the negatively charged cyanide ion due to electron repulsion. This might explain the observation that the sulfonated cobalt(II) complex reacts slower with cyanide ion than the carboxylated complex. However, the opposite results were obtained from the cobalt(III) and iron(III) complexes, where the TSPC complexes bind cyanide more rapidly. Apparently the higher oxidation state of the metal compensates for electron repulsion effects.

Similar to CO or NO^+, the CN^- has empty π-orbitals that can accept electrons donated from the metal d orbitals. The formation of this π-back-bonding has been used to account for the stability of M-CO, M-NO, and M-CN complexes.[34] In comparison, increasing the electron-donating ability of the metal ion will further stabilize the metal-ligand bond. This explains why the sulfonated phthalocyanine complexes of Co(III) and Fe(III) have higher rate constants with cyanide than the carboxylated phthalocyanine complexes. The electron repulsion effect, which tends to weaken the metal-ligand σ bond, is not as important for a metal ion in a high oxidation state (+3) as it is for metal ion in a low oxidation state (+2). More experimental data will provide a better understanding of this electronic effect induced by the substitution group.

The rate constants of cyanide binding to various metal tetra-sulfonated phthalocyanines are listed in Table VIII. Although we tried to correlate the cyanide binding rate and the number of d electrons contained in the metal ions, such a correlation is not supported by our experimental results to date. For example, we expected Co(III) to have

a higher binding rate to cyanide compared with Co(II) due to the electron repulsion. However, our data show that the rate constant of cyanide binding to Co(II)TSPC is approximately five times larger than the rate of binding to Co(III)TSPC. The rate constants of cyanide binding to copper(II), nickel(II), palladium(II) and ruthenium(III) are roughly the same.

Basolo and Pearson[35] have suggested that the free ions of vanadium(II) and manganese(II) react much faster with cyanide ion than cobalt(III) or iron(III). We have prepared the manganese and vanadium complexes of tetrasulfophthalocyanine. The Mn(II)TSPC and the V(II)TSPC are very sensitive to oxygen and are isolated as the oxygenated complexes, O=Mn(IV)TSPC and O=V(IV)TSPC. The binding rates of cyanide to these two oxo-complexes are so slow that we are unable to accurately measure them by the electrode method.

V EXPERIMENTAL DETAILS

Materials

Monosodium salt of 4-sulfophthalic acid was prepared by adding one equivalent of sodium hydroxide to a 50% aqueous solution of 4-sulfophthalic acid (Kodak) and then dried under vacuum for 5 days. 1,4,7,10-tetraazacyclododecanaquohydroxo cobalt(III) diperchlorate (Co(III)cyclen) and triethylenetetramine carbonato cobalt(III) perchlorate were synthesized by Markus Hediger and recorded in the SRI final report for Project 4167. The aquohydroxo-triethylenetetramine Co(III) diperchlorate (Co(III)triene) was prepared by dissolving the carbonate analog in 0.1N perchloric acid and was subsequently neutralized by 1N NaOH. 1,2,4-Benzenetricarboxylic anhydride, urea, and sodium dithionite were obtained from Aldrich. Potassium cyanide, ammonium molybdate, ferrous sulfate, and cupric sulfate were supplied from Mallinckrodt. Cobalt sulfate, nickel acetate, manganese acetate, and vanadium acetate were purchased from alfa. Ruthenium trichloride was obtained from strem, sodium sulfide was obtained from Baker, and palladium acetate was obtained from Johnson Matthey on a loan program.

Physical Measurements

UV-visible spectra were measured with a Hewlett-Packard 8450A spectrophotometer. NMR spectra were recorded on a JEOL FX90Q spectrometer. Microanalyses were performed by Galbraith Laboratory or measured on a Perkin Elmer 240-XA elemental analyzer. Magnetic susceptibilities were determined by the Evans Method. In a typical run, a solution of about 10^{-2} M D_2O of the metal complex containing 1% of tert-butyl alcohol as an internal reference is placed in a 5-mm NMR tube equipped with a 2-mm coaxial inner tube. A 5% tert-butyl alcohol in D_2O without the metal complex is placed in the inner tube, which serves as an external reference. The difference between the chemical shifts of the

two references is measured using ^1H NMR. The mass susceptibility, χ, of the metal complex is calculated according to the following equation:

$$\chi = \frac{3\Delta f}{2\pi f m} + \chi_o + \frac{\chi_o(d_o - d_s)}{m} \qquad (27)$$

where Δf is the frequency separation between the two lines, f is the frequency at which the proton resonances are being studied, m is the mass of the metal complex contained in 1 mL of solution. χ_o is the mass susceptibility of the solvent, d_o is the density of the solvent, and d_s is the sample solution. The last term is neglected in our calculation. Magnetic moment, μ_{eff}, can be calculated by the following equation.[17]

$$\mu_{eff} = 2.84 \sqrt{\chi_m \cdot T} \qquad (28)$$

Correction factors due to the solvent and the metal-free phthalocyanine, -0.72×10^{-6} and -550×10^{-6}, respectively, are applied.

Kinetic Measurements

UV-Visible Method

Solutions of the phthalocyanine complexes and of the KCN were prepared with double distilled water at pH 8 adjusted by NaOH solution. All measurements were done at ambient temperature. For Fe(II)TSPC, the solution was prepared and transferred to the typical cell in a glove box under nitrogen atmosphere. The reactions followed pseudo-first-order conditions in which a 10-fold excess of cyanide ion over the metal complexes was used. The calibration curve for the reaction product (i.e., the cyanide binded metal TSPC), was measured with the addition of about a 100-fold excess of cyanide ion to the solutions of MTSPC; absorbance was measured after at least one hour standing.

Cyanide Electrode Method

The concentration of free cyanide is measured by an Orion cyanide ion-selective electrode connected to a Orion EA940 pH/ISE meter. Standard potassium cyanide solution (1×10^{-1} M in 0.1N NaOH) is

prepared weekly and stored in a plastic bottle. All solutions are prepared with deionized water. Ionic strength is adjusted by adding 1 mL of 10N NaOH (ISA, Orion) to every 100-mL solution and the pH is measured to be 13.0 ± 0.2. In a typical run, 100 mL of H_2O containing 1 mL of ISA and a stir bar are added to a 150-mL beaker. The solution is stirred thoroughly at a constant speed. In 0.2-mL increments, 1 x 10^{-3} M KCN solution is added stepwise to the solution until the total volume of KCN added is equal to 1 mL. The potential reading after each increment is recorded and used for calibration. Then 5 mL of aqueous solution containing the metal (~1.2 x 10^{-3} mole) is added to the final cyanide solution and the cyanide concentration is measured at 10-s intervals.

Syntheses

Tetrasodium Salt of Cobalt(II)4,4',4'',4'''-tetrasulfophthalocyanine•3 Hydrate, Co(II)TSPC•3H_2O

The procedure is adapted from that reported by Busch and Weber.[38] A mixture of monosodium salt of 4-sulfophthalic acid (57.8 g, 0.2 mol), urea (360 g, 1.2 mol), ammonium chloride (10.3 g, 0.2 mol), ammonium molybdate (1.2 g, 0.001 mol), cobalt(II) sulfate 7-hydrate (28 g, 0.06 mol), and nitrobenzene (60 mL) are added to a 50-mL, three-necked flask fitted with a reflux condensor and a mechanical stirrer. The mixture is heated slowly to 150°C for 1/2 h with continuous stirring or until gas evaluation ceases. The temperature is raised to ~ 180°-190°C and maintained for 5 h. Nitrobenzene (200 mL) is slowly added while the mixture is still hot. The solid product is filtered, washed with 500 mL of methanol, and then ground to a powder. Then it is washed again with 500 mL of methanol. The remaining solid is added to 1 L of 1 N HCl saturated with NaCl. The solution and accompanying undissolved material are heated to a boil for 5 min, cooled, and filtered. The HCl treatment is repeated once. The solid collected from filtration is dissolved in 1 L of hot (~ 80^0) 0.1 N NaOH solution. Insoluble impurities are immediately separated by filtration. Sodium chloride (300 g) is added slowly to the solution, and the solution is then heated at 80°C for

3 h. The product is obtained by filtering the hot solution. This precipitation process is repeated two additional times. The solid collected is washed with 500 mL of 80% aqueous ethanol and then boiled for 4 h in 400 mL of absolute ethanol. The blue product is filtered and dried under vacuum at 150°C for 24 h (yield 36.3 g, 70%).

Anal. Calcd. for $C_{32}H_{18}N_8O_{15}S_4Na_4Co$: C, 37.18; H, 1.76; N, 10.84. Found: C, 37.07; H, 1.77; N, 10.77.

Tetrasodium Salt of Cobalt(III) 4,4',4'',4'''-tetrasulfophthalocyanine•3 Hydrate, Co(III)TSPC•3 H_2O

The Co(II)TSPC is oxidized by air in the presence of 2 equivalents of cyanide ion to Co(III)TSPC. Then, to 250 mL of 0.5 M Co(III)TSPC (6.12 g in 250 mL, 6.25 mmol) at pH 8 is added 10 mL of an 0.125 M sodium cyanide solution (0.163 g, 12.5 mmol). Air is bubbled through the solution for 4 days. Solvent is evaporated under reduced pressure. Ethanol (100 mL) is added and the mixture is heated to boiling. The solid is collected by filtration and is extracted for 3 h in a soxhlet extractor with 300 mL of absolute ethanol. The dark-green solid is collected and dried at 150°C under vacuum for 24 h (yield 6 g, 96%). Anal. Calcd. for $C_{32}H_{19}N_8O_{16}S_4Na_4Co$: C, 36.58; H, 1.82; N, 10.66. Found: C, 34.79; H, 1.81; N, 10.62.

Tetrasodium Salt of Hydrox Iron(III) 4,4',4'',4'''-tetrasulfophthalocyanine•3 Hydrate, Fe(III)TSPC•3H_2O

The preparation procedure is similar to that for Co(II)TSPC described above, with the substitution of ferrous sulfate for cobalt sulfate. The yield was 40%. Anal. Calcd. for $C_{32}H_{19}N_8O_{16}S_4Na_4Fe$: C, 36.69; H, 1.83; N, 10.69. Found: C, 36.02; H, 1.84; N, 10.58.

Tetrasodium Salt of Iron(II) 4,4',4'',4'''-Tetrasulfophthalocyanine•3 Hydrate• Fe(II)TSPC•3H_2O

This compound is obtained by the reduction of the Fe(II)TSPC with sodium dithionite. All manipulation is done in a nitrogen-filled glove box. Fe(III)TSPC (3.47 g, 3 mmol) is dissolved in 200 mL H_2O (degassed

by bubbled nitrogen for 1 h). An aqueous solution of sodium dithionite (0.3 M) is added dropwise to the Fe(III) TSPC solution until the UV-visible spectrum shows complete disappearance of the 632-nm peak, which is attributed to the Fe(III) species. Sodium chloride (100 g) is added and the solution is allowed to stir for 3 h. The powder product is filtered, washed with 30 mL of 95% ethanol, and recrystallized from water and ethanol. The pure product is collected and dried at 150°C under vacuum for 24 h (Yield 2.5 g, 73%). Anal. Calcd. for $C_{32}H_{18}N_8O_{15}S_4Na_4Fe$: C, 37.29; H, 1.76; N, 10.87. Found: C, 37.43; H, 1.81; N, 11.18.

Tetrasodium Salt of Metallotetrasulfophthalocyanine [metal Cu(II), Ni(II), Pd(II), Ru(II), O=Mn(IV), O = V (IV)]

The preparation procedures for these tetrasulfophthalocyanine complexes are similar to that of preparing the cobalt(II) analog, which is described above. The analytical data for the compounds prepared are listed below:

Cu(II)TSPC • $3H_2O$, Yield: (69%). Anal. Calcd. for $C_{32}H_{18}N_8O_{15}S_4Na_4Cu$: C, 37.02; H, 1.75; N, 10.79. Found: C, 37.22; H, 1.94; N, 10.45.

Ni(II)TSPC • $5H_2O$, Yield: (41%). Anal. Calcd. for $C_{32}H_{22}N_8O_{17}S_4Na_4Ni$: C, 35.94; H, 2.07; N, 10.48. Found: C, 35.97; H, 2.24; N, 10.21.

PD(II)TSPC • $3H_2O$, Yield: (62%). Anal. Calcd. for $C_{32}H_{18}N_8O_{15}S_4Na_4Pd$: C, 35.55; H, 1.68; N, 10.36. Found: C, 36.10; H, 2.12; N, 10.73.

O=Mn(IV)TSPC • $3H_2O$, Yield: (2%) Anal. Calcd for $C_{32}H_{18}N_8O_{16}S_4Na_4Mn$: C, 36.76; H, 1.74; N, 10.72. Found: C, 38.07; H, 2.39; N, 10.48.

Co(II)4,4',4",4" -tetranitrophthalocyanine

Cobalt(II) chloride hexahydrate (9 g, 0.038 mol), 4-nitrophthalic acid (25.76 g, 0.122 mol), urea (31 g, 0.517 mol) and ammonium molybdate

(0.2 g, 0.0002 mol) were finely ground and placed in a 500-mL three-necked flask equipped with a mechanical stirrer, a reflux condensorand a glass stopper. Nitrobenzene (130 mL) was added and the mixture was heated in an oil bath at 130°C for 1/2 h with continuous stirring. After gas evolution ceased, the mixture was heated to 190°C for 5 h and filtered hot. The solid was washed with 500 mL of methanol and dried. The crude product was 800 mL 1N HCl, boiled for 1 h, cooled to room temperature, filtered, and washed with 200 mL H_2O. The purple powder was treated with 800 mL 1N NaOH and heated to 90°C for 2 h. The product was filtered and washed with 200 mL H_2O. The HCl and NaOH treatment was repeated twice. The product was finally washed with 200 mL methanol and dried under vacuum for 16 h. Yield, 18.3 g (80%).

Co(II)4,4',4",4" -tetraaminophthalocyanine Tetrahydrochloride

Co(II) 4,4',4",4" -tetranitrophthalocyanine (5 g) was finely ground and placed in a 250-mL round-bottomed flask. Excess of sodium sulfide nonahydrate was added and stirred at 50°C for 5 h. The mixture was allowed to cool and stand overnight. The clear solution was decanted and the slurry was filtered, and then washed with 50 mL H_2O. The solid was added to 500 ml of 1N HCl and heated to boiling for 1/2 h. The solution was cooled to room temperature and filtered. The solid was collected, added to 1L of 1N NaOH, boiled for 2 h, filtered, washed first with 50 mL H_2O and then with 200 mL methanol. The solid was added to 500 mL of 1N HCl, boiled for 1/2 h, and cooled to room temperature. The product was collected by filtration, washed with 50 mL H_2O and 200 mL methanol, and dried at 150°C under vacuum for 3 h. Yield, 4.5 g (85%). Anal. Calc'd: C, 49.44; H, 3.11; N, 21.62. Found: C, 49.75; H, 2.38; N, 22.29.

Chloro-Fe(III)4,4',4",4" -tetraaminophthalocyanine Tetrahydrochloride

This compound was prepared by the same method used above except that ferrous sulfate heptahydrate was used in place of cobalt chloride.

Yield ~30%). Anal. Calc'd: C, 47.47; H, 2.99; N, 20.76. Found: C, 49.57; H, 3.04; N, 20.31.

Tetrasodium Salt of Cobalt(II)-4,4',4",4" -tetracarboxy-phthalocyanine • 3 Hydrate, Co(II)TCPC

The following solid reagents were finely ground in a mortar: 1,2,4-benzenetricarboxylic anhydride (19.2 g, 0.1 mol), urea (36 g, 0.6 mol), ammonium chloride (1 g, 0.02 mol), ammonium molybdate (0.5 g, 0.0005 mol), and cobalt(II) chloride hecahydrate (7.14 g, 0.03 mol). The powder reagents and nitrobenzene (300 mL) were charged in a 500-mL three-necked round-bottom flask fitted with a mechanical stirrer and two reflux condensors. One condensor was connected to a nitrogen inlet and the other was connected to a bubbler. The mixture was heated in an oil bath to 160°C for 5 h. A slow steam of nitrogen was constantly sweeping through the system during the heating period. After the mixture cooled, the greenish black solid was crushed and washed with 500 mL of methanol. The solid was boiled in 500 mL of 1 N hydrochloric acid for 1 h and then filtered. The solid was washed with 200 mL of water and added to 2 L of 1 N sodium hydroxide solution; this solution was heated to 90°C and held at that temperature for 6 h. The blue solution was filtered hot. Sodium chloride (~300 g) was added to the filtrate, which was then heated to boiling for 1 h. The solution was allowed to cool and stand overnight. The blue product was collected by filtration and washed with 300 mL of 95% ethanol. After the product dried in a vacuum desicator overnight, it was further purified in a Soxhlet extractor with 600 mL of 80% ethanol-water for 6 h and then dried at 100°C under vacuum for 3 h. Yield: 19 g (85.5%). Anal. Calcd for $C_{36}H_{18}N_8O_{11}Na_4Co$: C, 48.61; H, 2.04; N, 12.60. Found: C, 48.33; H, 2.27; N, 12.88.

Tetrasodium Salt of Hydroxy-cobalt(III)-4,4',4",4" -tetracarboxy-phthalocyanine • 3 Hydrate, Co(III)TCPC

Co(II)TCPC (10 g, 0.011 mol) was dissolved in 2 L of H_2O. Bromine (1.2 g, 0.015 mol) in 100 mL of methanol was added dropwise to the above solution over a 3-h period with continuous stirring. This oxidation

reaction was monitored by following the absorption at 628 nm. Sodium hydroxide (40 g) was added to the green solution. The solution was concentrated to about 700 mL. Then sodium chloride (150 g) was added to the solution, and the solution was heated at 80°C for 1 h. The solution was allowed to cool, and the green product was collected by filtration. The sodium hydroxide treatment was repeated once and the product was washed with 100 mL of 95% ethanol. After drying under vacuum overnight, the solid product was ground to a fine powder and was extracted for 6 h in a Soxhlet extractor with 600 mL of 80% ethanol-water. The product was then dried at 100°C under vacuum for 3 h. Yield: 9.5 g (95%). Anal. Calcd for $C_{36}H_{19}N_8O_{12}Na_4Co$: C, 47.70; H, 2.11; N, 12.36. Found: C, 46.97; H, 2.19; N, 13.06.

Tetrasodium Salt of Hydroxy Iron(III)-4,4',4",4" -tetracarboxyphthalocyanine • 3 Hydrate, Fe(III)TCPC

The preparation procedure was similar to that for Co(II)TCPC described above, except that ferrous chloride was substituted for cobalt chloride. Yield: (26.5%). Anal. Calcd for $C_{36}H_{19}N_8O_{12}Na_4Fe$: C, 47.86; H, 2.12; N, 12.40. Found: C, 48.09; H, 2.08; N, 13.35.

VI CONCLUSIONS

The goal of this project was to develop metal complexes that rapidly and tightly bind cyanide to form stable cyanide complexes that are excreted and can be used as antidotal drugs for cyanide intoxication. Important parameters to be studied included the rate of binding (k), the equilibrium binding constant (K), the solubility, the stability, and the toxicity of the complexes. In this project we studied the effect of metal ion and peripheral functional group derivatization on the rate of binding and on the solubility of very stable phthalocyanine complexes. Specific objectives were to develop a better understanding of the role of the metal ion, its oxidation state, and the electronic effects of the ligand on the binding of cyanide ion and to correlate those effects with in vivo efficacy against cyanide intoxication.

The results show that cobalt does bind cyanide ion with a very fast rate and would be the most likely choice as an effective antidote. However, the rate of binding is dependent on the oxidation state of the metal and electronic effects due to substituents on the ligand. The target rate constants to be achieved or surpassed were the rates reported for the isolated enzyme, 2 M^{-1} s^{-1} for oxidized cytochrome oxidase and 200 M^{-1} s^{-1} for the reduced form. All the Co and Fe phthalocyanines achieve the target rate, with the best case being the Co(II)TSPC where k = 3900 M^{-1} s^{-1}. All the other metal complexes tested fall below the rate for the reduced form of cytochrome oxidase.

The effect of the oxidation state of the metal and the electronic effect of the ligand are as expected. The cyanide binding has a component due to σ-donation of electrons from the cyanide to the metal and a component due to π-back-donation from the metal to cyanide. The effect of increasing electron density at the metal is to weaken the

σ contribution and strengthen the π. The rate of binding is strongly correlated with the σ-bond.

Twelve of the complexes developed in this project have been submitted to WRAIR for toxicity testing and *in vivo* antidotal efficacy testing. The results of these studies will be correlated with our *in vitro* data to guide future research.

VII ACKNOWLEDGMENT

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