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14. ABSTRACT
The objectives of the proposed research are to synthesize model compounds to mimic enzymes and to evaluate enzymatic activities and the stabilities of metal reactive centers and biocatalysts encapsulated in the nanochannels of MCM-41 and other mesoporous materials. The following spectroscopic techniques to characterize the materials and to elucidate the reaction mechanism: XRD, FT-IR, UV-visible, EPR and EXAFS have been applied. Efforts in the first six months of this study were concentrated to elucidate the mechanistic pathways in the degradation (oxidation) of polycyclic aromatic hydrocarbons of polycyclic aromatic hydrocarbons by cytochrome c enzymes immobilized in mesoporous materials. Studies of other parts of the proposed research were to follow.

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Progress Report for AFOSR on Taiwan – AFOSR Nanoscience Initiative
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Abstract

The objectives of the proposed research are to synthesize model compounds to mimic enzymes and to evaluate enzymatic activities and the stabilities of metal reactive centers and biocatalysts encapsulated in the nanochannels of MCM-41 and other mesoporous materials. We have applied the following spectroscopic techniques to characterize the materials and to elucidate the reaction mechanism: XRD, FT-IR, UV-visible, EPR and EXAFS. We have concentrated our efforts in the first six months of our study to elucidate the mechanistic pathways in the degradation (oxidation) of polycyclic aromatic hydrocarbons of polycyclic aromatic hydrocarbons by cytochrome c enzymes immobilized in mesoporous materials (Part c of the proposed research). Studies of other parts of the proposed research are in progress.

Progress Report

(1) Effect of spin configuration on the reactivity of cytochrome c immobilized in mesoporous silica.

Cytochrome c (cytc), a heme protein with positive electric charge, is immobilized in the nanochannels of mesoporous silica (MPS) by either electrostatic force or covalent bonding. The electrostatic interaction between cytc and MPS arises from the introduction of aluminum into the framework of MPS to produce the negative charge on the porous surface (Al-MPS). The covalent bonding arises from the binding between heme iron center and the –SH group of the mercapto- triethoxysilane in the thiol-functionalized MPS (MPS-SH). The nanochannels of MPS provide the confining spaces that could prevent cytc from protein unfolding and preserve its activity. Cytc immobilized in Al-MPS exist in high spin state as inferred from ESR and UV-Vis studies. This is different from the native cytc, which shows primarily the low spin state. The high spin state arises from the replacement of Met-80 ligands of heme Fe (III) by water or silanol group on the silica surface, which could open up the heme groove for easy access of oxidants to iron center and facilitate the catalytic activity. MPS-SH-supported cytc could exist in both high and low spin states. The low spin state arises from the replacement of axial ligands of heme Fe (III) by the –SH group which could cause the poisoning of active site and decrease the catalytic activity toward the decomposition of H_2O_2. In ESR spin trapping experiments, we show that cytc catalyzes a homolytic cleavage of the O-O bond of hydroperoxide and generates a protein cation radical (g = 2.00). Possible mechanisms for MPS-cytc catalytic oxidation of hydroperoxide is proposed based on the spectroscopic characterizations of the systems.

The results of these studies were presented at the 9th Spin Chemistry International Conference held at Oxford University, England in September 2005. A manuscript based on this study acknowledging the support of Taiwan – AFOSR Nanoscience Initiative was submitted and accepted to be published in *Molecular Physics* (a PDF file is attached herewith).[1]

(2) Model dinuclear copper center to mimic tyrosinases in mesoporous materials.

The tyrosinases (tyrs) catalyze the initial step in the formation of the pigment melanin from tyrosine in vertebrates and fungi, and tyrs also oxidize monophenols (like tyrosine) to *ortho*-

diphenols and *ortho*-quinones. Recently, Solomon *et al.* reported a model binuclear copper complex, μ-η^2:η^2-peroxo dicopper(II) (PDC) complex, to mimic tyrs, which was shown to oxidize phenols to catechols at low temperatures.[2] Upon phenolate addition at low temperatures in solution (–120°C), a reactive intermediate consistent with a bis-μ-oxodicopper(III)-phenolate complex, with the O–O bond fully cleaved, was observed experimentally. The subsequent hydroxylation step has the hallmarks of an electrophilic aromatic substitution mechanism, similar to tyrs. Overall, the evidence for sequential O–O bond cleavage and C–O bond formation in this model compound suggests an alternative intimate mechanism to the concerted or late stage O–O bond scission generally accepted for the phenol hydroxylation reaction performed by tyrs.

We have synthesized the model compound which is air sensitive. We have performed a few experiments to study the chemical reactivity and the stability of this model compound immobilized in the nanochannels of MPS materials. We observed that the confining spaces of the mesopores of MPS solids can provide some protection and stabilize some of the not yet identified reaction intermediates speculated in Solomon's study. Detailed studies are in progress.

(3) Model compounds to mimic superoxide dismutases (SOD).

The role of SOD in biological systems is to convert superoxide anion radicals, O_2^-, to hydrogen peroxides. Many model compounds have been synthesized and tested for their efficacies.[3] Most recently, another model compound, imidazolato-bridged complex (Cu(II)-diethylenetriamino -μ-imidazolato-Zn(II)-tris(aminoethyle)amine perchloarate) has been also reported to mimic CuZnSOD.[4] Immobilization of this model complex in the silica gel via hydrogen bonding has shown good SOD activity, but not in zeolite or MCM-41. It has been indicated that the configuration of paramagnetic center Cu(II) and a weak spin-spin interaction are important to achieve high SOD activity. We are trying to modify the mesoporous surface with either amine or other functional groups which could improve the binding with the model compounds and further improve the SOD activity. The modified surface of MCM-41 should also provide the needed separation of paramagnetic centers. We plan to apply the EPR techniques to study the magnetic properties of Cu(II). We also plan to study the details of the reaction pathways and the factors controlling the potency of free radical scavenging; especially the effects of immobilization on the SOD activity by different modified MPS materials, i.e., by Al substituted MPS to affect physical adsorption, or by modifying surfaces with functional groups (such as –NH_2 or –SH) to covalently binding with the model compounds. Detailed studies are in progress.

References:

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