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RELATIVE BINDING CONSTANTS OF ARSENICAL-ANTIDOTE ADDUCTS DETERMINED BY NMR SPECTROSCOPY

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ABSTRACT

Proton nuclear magnetic resonance spectroscopy was used to determine relative binding constants for several arsenical-antidote adducts. It was found that BAL (2,3-dimercaptopropanol) and DMPS (2,3-dimercaptopropanesulfonic acid) had a higher affinity than DMSA (2,3-dimercaptosuccinic acid) for the two organic arsenicals studied.

INTRODUCTION

Antidotes to arsenic poisoning function by chelating and extracting tissue-bound arsenic. The most effective antidotes to date are dithiols, which form particularly stable cyclic adducts with arsenic. British anti-lewisite (BAL) was among the dithiol-containing compounds synthesized in the early 1940's in the search for a topical antidote to lewisite (1,2). Although BAL is not an ideal systemic antidote because of its inherent toxicity and unpleasant side effects, it has been the recommended arsenic antidote in the United States for the past 40 years (3). DMSA and DMPS are less toxic analogues of BAL (4) which have polar moieties (carboxylic and sulfonic acids) that make them less lipophilic, more water soluble, and considerably less toxic than BAL. Both have activity as arsenic antidotes *in vivo* and *in vitro*. However, we have observed that, on a mole to mole basis, neither competes as effectively for trivalent arsenic in cultured cells as BAL (5). We have recently investigated the structure of various arsenic-antidote adducts to gain information concerning electrostatic and steric factors which could lead to the development of better antidotes (6-8).

In this report, we used NMR spectroscopy to determine 'relative' binding constants for various arsenical-antidote complexes. The arsenicals used were phenyldichloroarsine (PDA) and lewisite oxide (LO: *trans*-2-chlorovinylarsine oxide) and the antidotes investigated were BAL, DMPS, and meso-DMSA. The results presented below indicate that the relative stability of the various complexes depends upon the nature, the charge, and stereochemistry of the antidote, as well as some other factors concerning the arsenical.

MATERIALS AND METHODS

The arsenicals, sulfhydryl compounds, and adducts were purchased or synthesized as previously described (6,8-10). Deuterated solvents were purchased from Merck, Sharpe, and Dohme (West Point, PA, USA) or Aldrich Chemical Co. (St. Louis, MO, USA).

All ^{1}H-NMR spectra were recorded on a Varian XL-300 FT NMR spectrometer operating at 300 MHz. The experiments were carried out at

ambient temperature and with a spectral window of 4,000 Hz. Normally, the experiments consisted of several data blocks (accumulated consecutively), each containing 640 transients. Each block was accessed separately so that the reaction could be monitored until equilibrium was confirmed.

Each experiment began with a fixed amount of arsenical-antidote adduct. Incremental amounts of a competing antidote were added and the concentrations of the components of the mixture were measured. Free arsenical concentration was too small to be measured by NMR, so it was not feasible to determine individual binding constants. However, the concentrations of the free antidote and the adducts could be ascertained, provided appropriate specific resonances were identified and integrated. The experiments were designed to span a wide enough concentration range of the competing antidote such that the above measurements allowed us to calculate relative binding constants. An example is given below for the system containing PDA, BAL and DMSA. The ratio of the two individual binding constants ($K_{relative}$) can be defined as:

$$K_{relative} = K_{BAL}/K_{DMSA} = [PDA{\cdot}DMSA][BAL]/[PDA{\cdot}BAL][DMSA]$$

The concentrations of the four components were measured or calculated from the spectral data. The product [PDA·DMSA][BAL] was plotted versus [PDA·BAL][DMSA] and $K_{relative}$ was determined from the slope. For each system studied, equal amounts of the arsenical-antidote adduct (typically 6 aliquots) were placed into 5 mm NMR tubes and were stored at -20 °C until needed. The concentrations ranged from 10 to 100 mM depending on solubility constraints. The competition binding experiment was initiated by adding competing antidote to one of the tubes followed by spectral evaluation until equilibrium was reached. Incremental amounts of competing antidote were then added in turn to the remaining tubes, and each reaction was monitored until equilibrium was reached. We added only one aliquot of competing antidote per tube to minimize potential sample degradation. Each tube thus yielded data for only one antidote concentration.

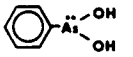
No single solvent system was found compatible with all combinations of arsenicals and antidotes under study. Several solvent mixtures were tried. The most practical solvent systems were a mixture of D_2O and methanol-d_4, D_2O and acetone-d_6, D_2O, and in one case methanol-d_4 was the only suitable solvent.

Some assumptions were necessary due to the complexity of some spectra, presence of isomers, overlap of resonances and/or solubilities. For example, DMSA and PDA·BAL are produced in the reaction between PDA·DMSA + BAL. The amount of free DMSA was assumed to equal the concentration of PDA·BAL observed. This was considered a valid assumption because no free PDA was detected. In the reaction between PDA·DMSA + DMPS, the amount of free DMPS was assumed to equal the initial concentration of DMPS minus the concentration of bound DMPS (overlap of resonances prevented the unequivocal integration of free DMPS).

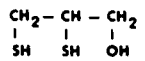
RESULTS AND DISCUSSION

Fig. 1 shows the ^{1}H-NMR spectrum for the competitive reaction of PDA·DMSA adduct with DMPS. We previously made the resonance assignments associated with each molecule (6,7). Two regions in the spectra are specifically indicated; these represent resonances for free DMSA and bound DMSA that are clearly discernible and do not overlap with other resonances.

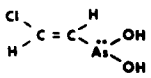
PDA (aq)



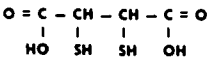
BAL



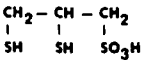
LO (aq)



DSMA



DMPS



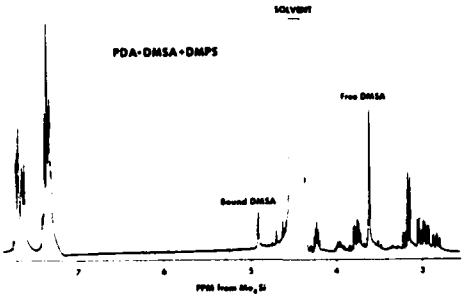


Fig. 1. ^{1}H-NMR spectrum of the equilibrium between the PDA·DMSA adduct and DMPS. Sample and spectral conditions are provided in the experimental section and Table 1. The initial ratio of PDA·DMSA to DMPS in this sample was 5:2.

Hence, their integral values were used to determine the concentrations of the equilibrium components for this system as described in the methods section. A representative plot of the data is shown in Fig. 2 in which the slope of the plot yielded $K_{relative}$.

The results obtained with PDA are summarized in row 1 of Table 1. When DMPS or BAL competed with DMSA for PDA, their binding constants both appeared to be ten times greater than that of DMSA. When BAL and DMPS competed, their binding constants were nearly identical (0.93). The lewisite oxide results are shown in row 2 of Table 1. The binding constant of DMPS with LO was 12.5 times greater than that of DMSA, a result similar to that observed with PDA. When BAL and DMSA were the competing antidotes, the binding constant for BAL was approximately twice that for DMSA. Thus the BAL/DMSA result obtained with LO was consistent with that obtained with PDA, though the difference was not as striking.

The above results indicate that the relative binding constants of the three disulfhydryl compounds toward organic arsenic are ranked as follows: BAL $\simeq$ DMPS > DMSA. The binding constants of BAL and DMPS were an order of magnitude higher than that of DMSA in all but one case. The exception was the LO/BAL/DMSA experiment, where the relative constants differed by only two-fold. The latter must be regarded cautiously, because it was the only experiment done in a nonaqueous solution.

The three antidotes studied above form identical 5-membered heterocyclic rings upon reaction with arsenic. Therefore, differences in equilibrium constants must be due to substituents on the carbon backbone. Thus, the weaker binding of DMSA may be explained by steric hindrance due to its bulky carboxyl groups. Charge could also be a factor in the case of ionizable molecules, although it is not a straightforward explanation. BAL, a neutral molecule, and DMPS (with its ionizable sulfonic acid moiety) have essentially identical binding constants, whereas DMSA, with two ionizable groups, has a considerably lower binding constant. Other factors, such as the environment around the arsenic atom (solvation, possible interactions of the arsenic atom with the adduct functional groups) and pH may also influence the relative binding constants.

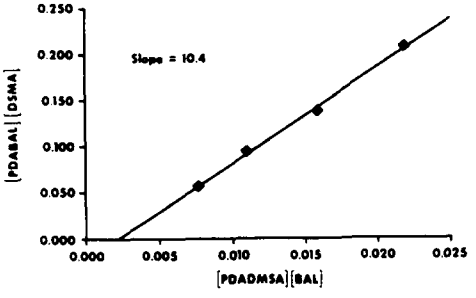


Fig. 2. Plot of the NMR equilibrium data for the PDA·DMSA/BAL system. $K_{relative}$ is derived from the slope.

Table 1
Relative Equilibrium Constants Determined for Various Arsenical-Antidote Adducts

Arsenical	Competing Antidotes		
	DMPS/DMSA	BAL/DMSA	DMPS/BAL
PDA	10.4[a]	10.1[b]	0.93[c]
LO	12.5[a]	1.76[d]	----[e]

[a] Solvent was D_2O. [b] Solvent was a 3:2 mixture of acetone-d_6/D_2O. [c] Solvent was a 1:1 mixture of methanol-d_4/D_2O. [d] Solvent was methanol-d_4. [e] No solvent mixture was compatible with these compounds.

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