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Employing Permeability Coefficients To Understand the Biomobility and Bioaccumulation of Compounds Sensitive to the Environment

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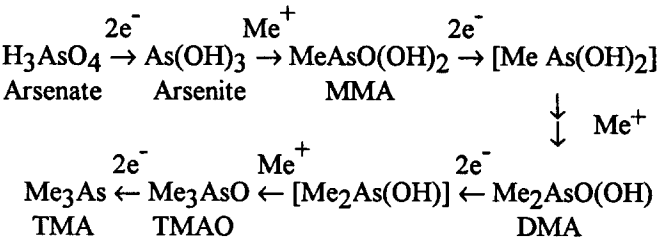
Arsenic is present in the environment in a wide variety of different chemical forms (Cullen and Reimer 1989) and although it occurs naturally, it is also introduced through use as herbicides and as a by-product of a number of industrial processes. Each of these different chemical forms possess different physical and chemical properties, toxicity's, mobility's, etc. and it is only when these are known, that the arsenic biogeochemical cycle can be fully understood. The biotransformation of arsenate to the volatile trimethylarsine by micro-organisms has been well documented. This bio-transformation seems to follow the stepwise mechanism of scheme 1 first proposed by Challenger (1945).



Scheme 1

The rate at which trimethylarsine (TMA) is produced from arsenate, arsenite, monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) by *Candida humicola* is pH dependent (Cox and Alexander 1973). For example, a culture of *C.humicola* inoculated with DMA (Cox and Alexander 1973) produced 87 nmols TMA at pH 5 while only 41 and 2 nmols TMA were produced at pHs 6 and 7 respectively. Under identical conditions, a culture inoculated with MMA produced 9, 6 and 0 nmols of TMA at pHs 5, 6 and 7 respectively.

There are considerable differences in the uptake of DMA and MMA by unicellular algae. When grown in a medium containing 2 ppm DMA *Isochrisis galbana* accumulated ~76% of the available arsenic (A. Tsang 1990). From medium

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containing 1 ppm and 5 ppm MMA, the same cellular concentration of *I.galbana* accumulated ~25 of the available arsenic. Likewise *Dunaliella tertiolecta* accumulated ~50% of DMA and ~19% MMA from media containing 0.5 ppm of the appropriate arsenical.

It is possible that the differences between DMA and MMA described above are simply due to differences in the initial rates of diffusion of these compounds into the cell. In fact, Cullen et al. (1990) have shown that arsenate is taken up by *C.humicola* via an active transport mechanism, whereas arsenite, MMA and DMA appear to enter the cell mainly by diffusion processes.

A conventional measurement of the ability of a compound to bioaccumulate is the n-octanol/water partition coefficient (Renberg and Sundstrom 1985). For example, Neely et al (1974) have demonstrated that the uptake of chlorobenzenes and chlorophenols into trout muscles correlates linearly with their n-octanol/water partition coefficients. This correlation is generally used because many of the organic compounds which are known or suspected to constitute a hazard to the environment are highly lipid soluble and, therefore, have relatively large partition coefficients (Tulip and Hutzinger 1978). These compounds also usually exist as a single molecular species in the environment and do not possess ionizable groups. By definition a partition coefficient is the distribution of a **single** molecular species between the two phases being considered (Hansch et al. 1971). Difficulties arise for solutes (such as DMA and MMA) which dissociate in solution because the exact identity of the species in each phase is rarely known. For example, in the partitioning of an aliphatic carboxylic acid with a pKa of 4.5 and the aqueous phase buffered at pH 8.5, only 1/1000th of the acid will be in the neutral form in the aqueous phase, and yet almost one-half of that present in the n-octanol phase will be the un-ionized species. Further difficulties arise because these hydrophilic compounds partition very poorly into the n-octanol. Thus, it is difficult to make reliable correlations between partition coefficients and bioaccumulation for these types of compounds. As an alternate to this type of model for bioaccumulation and biomobility, we suggest that the permeability coefficients for these types of molecules can be correlated to there biological behavior. This notion is tested using large unilamella vesicles (LUVs) and the diffusion coefficients for DMA and MMA.

MATERIALS AND METHODS

The NMR method for measuring diffusion coefficients which is summarized below has been previously described (Herring et al. 1992; Prestagard et al. 1979). LUVs in buffer (Hepes in D_2O) containing either DMA or MMA were formed from egg phosphatidyl-choline by using literature methods (Cullen and Nelson 1992; Hope time et al. 1985). They were passed through a Sephadex column and were eluted with buffer (Hepes in H_2O) to produce the necessary concentration gradient. The eluted LUVs were added to a NMR tube which already contained a solution of Mn^{2+} (shift agent), and TSP (spectral reference). ^{1}H-NMR spectra were obtained

at the appropriate time intervals by using a Bruker AM 400 spectrometer. The rate of change of the integral of the spectroscopic signals from the methyl groups on both sides of the membrane was measured to give the diffusion coefficients of the permeant (Herring et al. 1992). Typical time evolved spectra are shown in Figure 1.

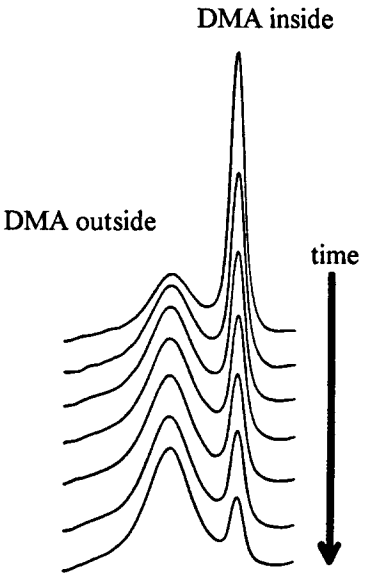

Fig.1

RESULTS AND DISCUSSION

The methyl resonance observed in this experiment is a composite signal made up of the resonance due to the unionized ($(CH_3)_2AsO(OH)$) and ionized ($(CH_3)_2AsO_2^-$) species. Consequently, if the diffusion of these two species across the membrane were comparable the rate of change of integral would be independent of pH. A study of the effect of pH on the rate of change of the integral of the composite methyl resonance for DMA shows that the apparent rate constant varies with $[H^+]$ in a linear fashion (Figure 2). The results indicate that the contribution of the ionized DMA to the rate of change of the integral is insignificant when compared to the that of unionized DMA contribution, probably less by a factor of two or three orders of magnitude. Thus, it is the neutral species that dominates the transport of DMA across the membrane.

The rate constant and permeability coefficient for the unionized DMA (corrected for pH effects) is found to be $1.08(\pm0.08)\times10^{-3}$ cm^3s^{-1} and $3.33(\pm0.44)\times10^{-8}$ cm/s respectively, from the experimental data. Similiar data for unionized MMA have also been measured and are $3.93(\pm0.05)\times10^{-4}$ cm^3s^{-1} and $5.44(\pm0.05)\times10^{-9}cms^{-1}$.

The diffusion coefficients for DMA are approximately three times larger than those for MMA. This result would lead to the prediction that a cell would be more likely to accumulate DMA than to MMA, if only passive diffusion of the neutral species were involved. This supposition is in agreement with the results described above for *I.galbana*, *D.tertiolecta*, and *C.humicola*. *D.tertiolecta* contains a cellular wall in addition to a membrane which may explain why the accumulation is greater in *I.galbana*. If the rate limiting step for the production of TMA by *C.humicola* from either DMA or MMA is the diffusion of the substrate into the cell then one would expect to see more TMA produced from DMA than from MMA and this is in agreement with the results described above. The observed pH dependence on the rates of TMA production by *C.humicola* from DMA and MMA may also be understood by this model as the diffusion rates for both arsenicals increase with decreases in pH.

DMA pH Study

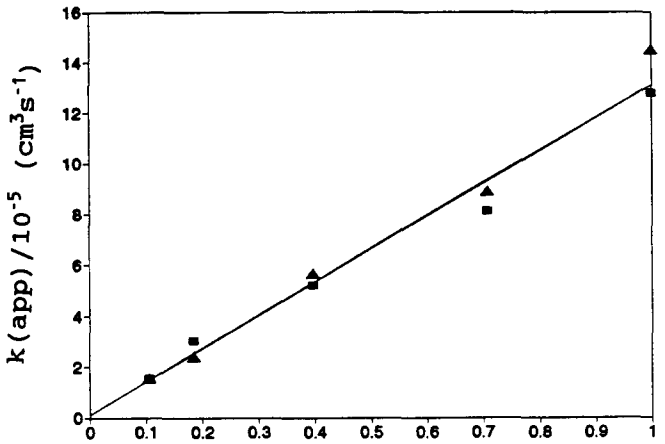

Fig. 2

In conclusion, NMR spectroscopy can be used to measure diffusion coefficients of hydrophilic compounds through LUVs. These coefficients can be used to make predictions concerning the biomobility and bioaccumulation of these compounds and other hydrophilic compounds. The technique has been applied to DMA and MMA where the effect of pH on the permeability is better understood . A simple consideration of partition coefficients, which have been shown to be similiar for DMA and MMA (Cullen and Nelson 1992), would lead to erroneous predictions about their biomobility and bioaccumulation. We intend to study the general applicability of these ideas with other environmentally important molecules.

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