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Presented at the Dahlem Workshop on the Importance of Chemical Speciation in Environmental Processes, Berlin, September 2-7, 1984

THE INTERACTION OF METALS AND METALLOIDS WITH BIOMEMBRANES

R.J. Mehlhorn

September 1984

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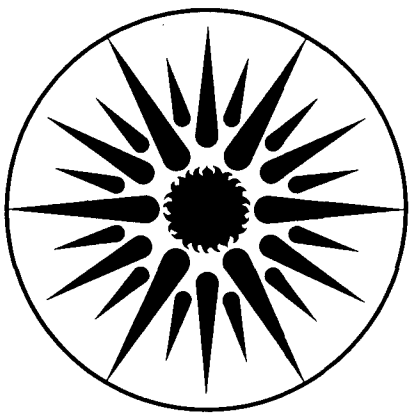

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Prepared for the U.S. Department of Energy under Contract DE-AC03-76SF00098

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LBL-17925

For: Dahlem Workshop on
The Importance of Chemical Speciation
in Environmental Processes
Berlin; September 2-7, 1984

THE INTERACTION OF METALS AND METALLOIDS WITH BIOMEMBRANES

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Abstract. The interaction of metal(loid)s with membranes can have a significant role in their biological effects. To the extent that membranes prevent the entry of these species into cells, they will inhibit damaging effects. However, many interactions with membranes can result in direct damage, or, more seriously, can lead to an amplication of toxicity. Direct damage can include structural alteration such as a disruption of vital lipid-protein associations or introduction of new surface charges. More substantial damage can occur when metals or metalloids act catalytically to impair membrane function, e.g., by providing ion conductance pathways or by promoting free radical reactions which may cause massive oxidation of membrane lipids. Recognition of factors involved in the interaction of compounds with membranes may be useful in devising meaningful strategies for speciating metal(loid)s in biological environments.

Biological cells are surrounded by membranes which usually prevent the entry of toxic molecules into the cell and which control the flow of nutrients and ions between the external fluid and the cytoplasm. Other membrane functions include providing an essentially two-dimensional matrix where collisions among molecules will occur more frequently than in aqueous environments, providing a nonpolar environment where organic chemical reactions can occur, providing a low dielectric region which allows membranes to act as electrical capacitors, and providing a macromolecular assembly which

serves as an anchoring surface for cell structural elements. Mammalian cells contain numerous subcellular compartments whose membranes usually account for the bulk of the total membrane content of the cell. The membrane interior is hydrophobic and this hydrophobicity enables membranes to serve as a barrier to the passage of polar molecules. Among the toxic substances that cells seek to avoid are most heavy metal ions, e.g., mercuric ions, organic complexes of metal ions, e.g., amino acid complexes with certain transition metals, and organometallic substances, e.g., methyl mercury. One purpose of this review is to consider the known permeability properties of membranes so that the potential toxicity of a metal or metalloid can be assessed in terms of its ability to enter cells, and to provide a framework for identifying classes of compounds which are the most interesting candidates for speciation study.

Metal(loid)s can exert deleterious effects on cells through a variety of mechanisms. They can replace essential structural components because they mimic them, as in the case of arsenate which closely resembles phosphate and can therefore undergo similar metabolic transformations or selenium which can sometimes substitute for sulfur. Another toxicity mechanism is catalytic activity, particularly for transition metal ions and certain of their complexes. If these transition metals can participate in electron transfer reactions with cellular reductants or oxidants then destructive free radical chain reactions are possible, including the membrane destruction process of lipid peroxidation. Hydrophobic or amphiphilic organometals or metal ion complexes can bind to membranes and alter structure-function relationships. Organotin compounds are known to transport anions across membranes and can cause transmembrane gradients to collapse; other organometallics may exhibit similar activities. Many heavy metals and metalloids interact strongly with thiols and therefore have the capacity to block enzyme activities and deplete cells of glutathione and other vital sulfhydryl compounds. Metabolic

processes involving organometallic substances can liberate free radical species into the cell and these free radicals can initiate chain reactions with considerable destructive potential. Although this list of toxicity mechanisms is not exhaustive it points out the complexity of effects that must be considered in dealing with effects of metal(loid)s on biological membranes.

MEMBRANE PERMEABILITY PROPERTIES

Small cations and anions are usually not permeable to membranes and require transport systems to enter cells. Such transport systems are not always absolutely specific for a given ionic species and this lack of specificity enables toxic metal ions to enter cells. For example, in the bacterium *S. aureus*, CdII appears to use the same transport system as MnII does (21). Similarly, the toxic arsenate anion enters bacterial cells by the phosphate pathway (22). Ions may also cross membranes by forming uncharged complexes with organic ligands which surround the ion with a hydrophobic shell. Complexes which include large hydrophobic groups, like valinomycin, can cross the membranes as charged species.

For nonionic solutes the permeability is a function of their hydrophobicity. A reasonably quantitative relationship between permeabilities and the number of hydrogen bonds that a molecule can form as well as its content of hydrophobic residues, e.g., methylene groups, has been provided by Stein (23). Small molecules like glycerol cross membranes quite rapidly and such molecules cannot effectively be prevented from entering cells. Stein's formulation of permeabilities indicates that the permeability of small molecules decreases by a factor of about ten for every two hydrogen bonds that can be formed, e.g., for each OH residue. Thus, sugars, which can form a significantly larger number of hydrogen bonds than glycerol, do not enter cells at appreciable rates unless their uptake is facilitated by transport systems.

A useful technique for studying rates of solute permeability

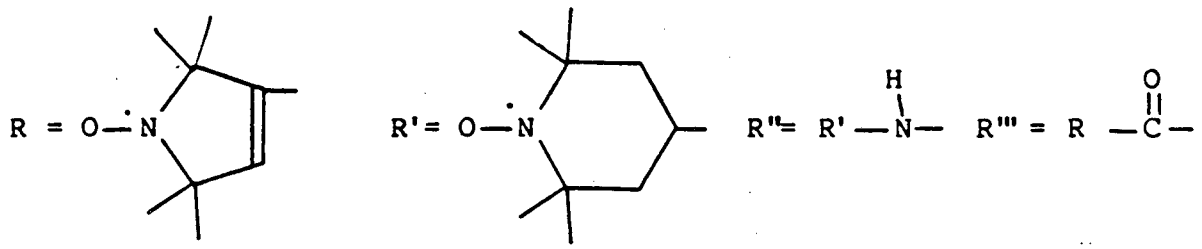
for molecules which can be spin-labeled, i.e,. labeled with a paramagnetic nitroxide reporter group, is electron spin resonance (ESR) spectroscopy (11). With this technique it is possible to measure the concentrations of spin labels on both sides of a membrane interface simultaneously by perturbing the external signal with impermeable paramagnetic transition metal complexes. These complexes broaden the spectra of the nitroxides in a collision-dependent process, thus allowing a simultaneous determination of spectral intensities associated with both aqueous compartments. When this technique is applied to the measurement of uptake kinetics of a spin label it is sufficient to add a large concentration of the paramagnetic transition metal complex to eliminate the external signal completely, and to monitor the time-dependent increase in the internal spin label signal. Manganese-EDTA has proven to be the most useful line broadening agent for these studies; it seems to be quite impermeable and broadens lines at low concentrations relative to other paramagnetic agents that have been tested.

The ESR method has been used to obtain the permeabilities of a large variety of available probes (13) and this information is useful for inferring some generalities about factors that control these permeabilities. The table below shows the uptake kinetics for a series of probes and a wide range of rates is evident. It is clear from this table and from Stein's analysis that uncharged molecules, e.g., uncharged organometallics and neutral metal ion complexes will enter cells at a rapid rate. It is noteworthy that charged species can also enter cells provided that the ionic sites are surrounded by lipophilic residues, as exemplified by the triphenylphosphonium nitroxide. The bulk of the large phosphonium ion does not prevent its entry into the vesicles. In some membranes smaller ions can also enter, perhaps by moving across membranes as ion pairs. This seems to be the case for the CAT8 nitroxide in the table which was observed to accumulate within cell envelope vesicles of halobacteria to the same extent in the presence and absence of a light-

induced electrical transmembrane potential.

Table: Half times for nitroxide uptake into envelope vesicles of Halobacterium halobium

Structure	Trivial name	# H-bonds	Half time
R"'-3-amino-1,2-propanediol	Pyrglyc	6	40 sec
R"-tashimik acid	Tashik	8	30 min
R"'-glucosamine	Pyrglucosam	10	15 hours
R'-sulfate	Tempsulfate		70 min
R'-N-trimethylammonium	CAT1		280 days
R'-N-dimethyl,octylammonium	CAT8		10 min
R -triphenylphosphonium	KPHI		2.5 min



When metals or metalloids can cross membranes as charged complexes, then the possibility exists for substantial accumulation into compartments whose membranes maintain electrical potentials. Mitochondria generate large electrical potentials for the purpose of ATP synthesis and these compartments could accumulate cationic metal(loid) species to perhaps a hundredfold higher concentration than the surrounding aqueous phase. In view of the vital role of this organelle in cell metabolism, such accumulation of a toxic substance could result in a substantial amplification of

damage. In principle, metal complexes that are weak acids and amines can also be accumulated within alkaline and acidic subcellular compartments, respectively, provided that these residues are not strongly tied up as metal ligands and provided that the metal complex is sufficiently membrane permeable in the uncharged state.

The ESR technique has also shown promise for the measurement of permeabilities of transition metal complexes which broaden nitroxides. In this approach, the leakage of the paramagnetic transition metal into a cell or vesicle is observed as a decrease of the internal nitroxide spectral line heights as the lines become broadened. In preliminary studies we have observed that amino acid complexes like copper-valine cross the membranes of halobacterial membranes at a slow but easily measurable rate.

SOME EFFECTS OF METAL(LOID) INTERACTIONS WITH MEMBRANES

The interaction of a metal ion complex or an organometallic species with a biomembrane will depend on its hydrophobicity as outlined in the previous section. If the metal(loid) is quite nonpolar it will become solubilized in the hydrophobic center of the membrane and usually disrupt the ordered structure of the lipids, possibly altering vital lipid-protein interactions. The effect of small hydrophobic molecules on membrane structure has been generally characterized as a fluidity increase. Many studies have shown that such fluidity increases alter membrane function, e.g., activities of membrane bound enzymes are increased or decreased (18). However, gross membrane integrity, as reflected in a capacity to serve as a permeability barrier, seems to be retained in the presence of significant concentrations of such structural perturbers.

The notion that perturbations of membranes by exogenous lipid soluble solutes cause impaired excitability of membrane proteins forms the basis of the unitary hypothesis of general anaesthesia. This hypothesis unifies a considerable body of

evidence about the efficacy of many small molecules of diverse structures in causing anaesthesia. The list of these molecules includes the gas xenon, halogenated hydrocarbons, simple alcohols, ethers, indeed, the only requirement for activity appears to be hydrophobicity. Thus, the potency of these anaesthetics correlates quite well with their oil-water partitioning, with the exception of some very hydrophobic compounds, e.g., tetradecanol, which is inactive. A hallmark of these general anaesthetics is the relatively high concentrations that are required to elicit a physiological effect, e.g., millimolar aqueous concentrations for compounds having appreciable solubilities in both lipids and water. Binding studies in lipid bilayer model membranes have shown that these aqeuous concentrations correspond to a mole fraction of about Ø.Ø4 of the anaesthetic relative to the lipid, a rather high membrane content of the perturbing molecule (1Ø). This can be taken to imply that comparably high concentrations of a perturbing metal(loid) in a membrane would be required to cause significant effects on membrane function.

Large rigid hydrophobic metal(loid)s have the potential to decrease the fluidity of membranes as exemplified by cholesterol. Cholesterol addition to phospholipids in model membranes renders the membranes more rigid and also has the effect of eliminating phase transitions at high concentrations. Lateral diffusion of molecules within the plane of the membrane is also decreased upon cholesterol addition. Such fluidity effects are also unlikely to impair membrane integrity at low concentrations of a metal(loid).

Polyvalent cations and amphiphilic ionic compounds will alter the surface charge and the fluidity characteristics of membranes when they become bound. The extent of binding of amphiphilic species will depend on the size of the hydrophobic groups. It has been shown in mitochondria that strong ionic detergents have a potent inhibitory effect on respiratory function due to alteration of membrane surface

charge rather than the fluidizing effects of the detergents (12). Most membrane surfaces bear a net negative charge which usually causes membranes to repel each other, to attract cations and to bind peripheral proteins electrostatically. Thus, it is not surprising that compromised charge properties have serious effects on membrane integrity. This suggests that amphiphilic ionic metalloids or organometallic substances may exert a more destructive direct effect on membranes than neutral species. Polycations bind to membrane surfaces, including lipid headgroups and also alter surface charge. In thus binding they may also disturb regulatory functions of calcium or block critical membrane cation transport systems.

Another serious threat to cell survival is posed by metal(loid)s which profoundly alter membrane function, e.g., by stimulating ion exchange. An important case in point is the action of of triaryl- and trialkyltins. These organometallics have been shown to be potent anion exchangers across membranes. As expected, the efficacy of these compounds increases with their lipid solubility, e.g., tributyltin is much more potent than triethyltin in red cells, mitochondria and artificial membrane vesicles (19). The predominant anion in many biological environments is chloride and this anion is readily tranported across membranes as a neutral complex with the organotin cation. Hydroxide is also carried across membranes by this mechanism and the presence of the organotin compound therefore causes a rapid dissipation of transmembrane pH gradients as hydroxide anions are exchanged for the much more abundant chloride anions. The results of this process can be disastrous for subcellular compartments which require the maintenance of acidic or alkaline conditions, e.g., the lysosomes, where acidic pH is required for activation of digestive enzymes. One assumes that this well-characterized mode of action of trialkyl and triaryltins will also occur with other organometallic compounds which will tend to form partially covalent bonds with halides, resulting in lipid-soluble

neutral species. Data are needed on the effects of representative organometallic compounds and speciation efforts should concentrate on these as having particularly potent biological effects.

OXIDATION OF MEMBRANES BY METAL(LOID)S

Perhaps the greatest membrane hazard posed by metal(loid)s is the initiation of free radical oxidative damage processes. Recent research on biological damage mechanisms has suggested that free radical processes, particularly those involving so-called active oxygen molecules play a major role in toxicology, disease and aging (3,16). There are many species that can wreak havok with living cells, but free radicals are considered to be especially damaging because of their unique chemistry, i.e., their ability to participate in chain reactions which, once initiated, have the potential to spread rapidly among membranes and macromolecules.

It has become clear that many cellular redox processes liberate superoxide radicals into the cellular environment and that these are rapidly dismutated to hydrogen peroxide. In principle, the existence of superoxide radicals and hydrogen peroxide is sufficient to lead to the production of the extremely destructive hydroxyl radical, provided that traces of iron or copper ions are available for the Fenton reaction (9). The Fenton reaction has received considerable attention among biologists recently. In this process either ferrous or cuprous ions react with hydrogen peroxide or organic peroxides to generate the extremely reactive hydroxyl or alkoxyl radical.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH\cdot$$

$$O_2^- + Fe^{3+} \longrightarrow O_2 + Fe^{2+}$$

The radical will react with virtually all biological targets, including DNA, proteins and membrane lipids. It is well established that hydroperoxides are formed in aerobic cells

and attention has now shifted to the issue of whether transition metal ions are available in complexes which are compatible with the Fenton reaction. Considerable research is in progress to ascertain whether "loosely bound" iron or copper exists in cells under physiological conditions. A highly sensitive assay, based on the ability of bleomycin to form a complex with iron which can degrade DNA in the presence of a reductant, has been developed for the detection of iron in biological fluids (8). However, some iron complexes, like the EDTA complex, are potent catalysts for the Fenton reaction and yet would not be expected to be detectable in this assay. Additional analytical schemes will be required to identify other transition metal complexes whose toxicity may reside in a capacity to promote free radical reactions.

One consequence of oxidative processes in aerobic cells is lipid peroxidation. This process begins when some reactive free radical such as the hydroxyl radical enters a biological membrane and strips a hydrogen atom from a lipid alkyl chain. Under aerobic conditions an oxygen molecule adds to the alkyl radical. The resulting peroxyl radical abstracts a hydrogen atom from another lipid radical and a chain reaction ensues. Under normal conditions the resulting fatty acid hydroperoxides are probably reduced to alcohols by the protective enzyme glutathione peroxidase. However, if hydrophobic transition metal complexes are present in membranes these could react with such lipid hydroperoxides to promote an intramembrane Fenton reaction, leading to the production of alkoxy radicals which could initiate new chain reactions. Such chain branching reactions can, in principle, greatly accelerate the process of lipid peroxidation. It is conceivable that organic complexes of other transition metal ions, e.g., nickel, could also catalyze intramembrane chain reactions and therefore it is of interest to develop highly sensitive speciation of metals within membranes to identify other potentially damaging redox mediators.

Recently evidence has begun to accumulate showing that the microsomal P450 drug metabolizing system acts on organometallic compounds and that products of these enzyme activities initiate lipid peroxidation (15,25). The cytochrome P450 system oxidizes hydrophobic molecules to polar derivatives which are more readily excreted by the body, but occasionally this detoxification mechanism acts to produce a more toxic chemical species rather than a less toxic ones. Apparently this situation can occur when the enzyme reacts with organometallics like tetraethyllead or tetrabutyltin. These species are relatively innocuous until they are dealkylated by the cytochrome P450 enzyme. The products of this dealkylation include species which can disrupt membrane ion gradients via anion exchange and species which have been shown to initiate lipid peroxidation.

The sequence of events resulting from the metabolism of trialkyltins has been particularly well documented. Tetraalkyltins are converted to much more destructive trialkyltins and hydroxylated products. These species cause severe toxicity in animals, resulting primarily from damage to the central nervous system. The toxic effects have been ascribed largely to the inhibitory effects of trialkyltins on mitochondrial functions, including the anion exchange phenomena discussed earlier, as well as a direct inhibition of the mitochondrial ATP synthetase complex (1). In addition the metabolism of these compounds causes membrane lipid peroxidation in microsomes and in isolated hepatocytes (15,25) and conversion of cytochrome P450 to the inactive P420 species (15,17). It has been suggested that this lipid peroxidation is initiated by alkyl radicals which arise in the process of reductive metabolism of the alkyltins (15).

The occurrence of lipid peroxidation has been detected in an interesting non-invasive assay of hydrocarbons like ethane and pentane in the expired breath of animals. These hydrocarbons are thought to result from the decomposition of fatty acid peroxyl radicals arising from lipid peroxidation.

With this assay lipid peroxidation has been inferred to occur in rats that were injected with toxic doses of sodium selenite, but not with selenate (4). When the animals' diets were supplemented with the antioxidants vitamin E and selenium (at relatively low concentrations) the extent of lipid peroxidation was lower. A well-known effect of selenium is its reaction with thiols, resulting in their depletion from tissues. Such thiol depletion could weaken cellular antioxidant defenses. However, impaired protection cannot explain the lipid peroxidation, since it was found that lipid peroxidation could be demonstrated prior to the onset of significant losses of glutathione.

Ganther has advanced the hypothesis that methylmercury toxicity is due primarily to free radical processes (7). He cites the protective effects of vitamin E and selenium in support of this hypothesis. Since methyl radicals are produced in a simple reaction mixture containing methyl mercury, ascorbate and copper, he suggests that oxygen radicals arising in the course of aerobic metabolism may suffice to cause scission of the carbon mercury bond in cells. This hypothesis explains the significant observation that much lower levels of selenium than those of the administered methylmercury can eliminate toxic symptoms and is also entirely consistent with a role of lipid peroxidation in methylmercury poisoning.

An example of lipid peroxidation involving an inorganic heavy metal is seen in mercuric chloride toxicity (26). In rats mercuric chloride induces acute renal failure, which results from extensive membrane destruction following lipid peroxidation (5). However, in this case lipid peroxidation appears to be related to some effect of mercury on thiols. A major involvement of thiol destruction in the damage process is suggested by substantial losses of glutathione following mercuric chloride administration to rats (5). Thiol reactions are a common theme of heavy metal and metalloid chemistry. Generally, thiol loss has been deemed to exert its

destructive effect directly through the loss of crucial enzymes; however, it is now clear that free radical oxidative processes must also be considered in seeking an understanding of heavy metal toxicity mechanisms. A further effect of mercuric chloride in promoting oxidative damage is its inhibitory action on cellular antioxidant systems. In studies of rat kidney mercuric chloride intoxication, the enzymes which guard against superoxide and hydrogen peroxide accumulation, i.e., superoxide dismutase, catalase and glutathione peroxidase suffered activity losses of 48, 32 and 25% respectively, 24 hours after subcutaneous mercuric chloride administration (5).

Lipid peroxidation has also been implicated in the toxicity of lead. Measurements of pentane in the breath of rats which were fed lead-containing diets indicated that this metal promotes lipid peroxidation (20). Furthermore, dietary supplements of vitamin E and selenium decreased the concentrations of expired ethane.

Cadmium damage to testes also appears to involve lipid peroxidation (14). However, in experiments where the effects of cadmium on rat lung were studied, a protective response to selenium was clearly evident, yet no indications of lipid peroxidation could be found, in fact the traditional assays of TBA-reactive materials revealed unexpected decreases in the cadmium treated animals.

Silver administration causes a decrease in glutathione peroxidase levels in animals (24), and thus silver would be expected to cause increased susceptibility to oxidative damage. Indeed, animals deficient in vitamin E and in selenium exhibit greater susceptibility to silver, which can be counteracted by nutritional supplements with these and other antioxidants (2).

A commonly observed consequence of lipid peroxidation is

protein polymerization (crosslinking). This can be a result of the combination of two free radical residues to produce a covalent bond, but a more likely process is disulfide formation as thiols undergo oxidation, e.g., by reacting with lipid hydroperoxides. Such crosslinks may be difficult to repair because the new bonds may be deeply imbedded within the hydrophobic membrane core where they may not be accessible to reduction by thiols. Thiols are also susceptible to crosslinking by more direct effects of metal(loid)s, as in the formation of selenotrisulfides (6).

In summary, a great variety of destructive effects of metal(loid)s with membranes can be envisioned. Of the many species that may interact with membranes and that are significant in the environment only a few have been adequately characterized. However, the available data on toxicity mechanisms provides important clues about general characteristics of classes of metal(loid)s which may promote membrane destruction and which therefore warrant special scrutiny.

Acknowledgement. Supported by NIH AG-04818 and the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

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Problems and open questions

1. What are some metal(oid) species that are likely to accumulate within subcellular compartments in response to electrical potentials?

2. What kinds of reactions are likely to occur within membranes

between metal(loid)s and organic free radicals? Can the products of these reactions propagate damage to distant targets?

3. Can we devise effective strategies for characterizing trace metal(loid) species solubilized in membranes?

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

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