Oxidation–Reduction Reactions of Metal Ions

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Several metal or metalloid ions exist in multiple oxidation states and can undergo electron transfer reactions that are important in biological and environmental systems. There are endogenous metal ions such as iron, copper, and cobalt that participate in oxidation–reduction reactions with species of oxygen like molecular dioxygen, superoxide, and hydrogen peroxide. These reactions may be modulated by endogenous reducing agents such as glutathione, ascorbate, and tocopherol. The reactions can be described in terms of thermodynamics through the use of standard electrode potentials. A favorable reaction will depend on the concentrations of the reactants and may depend on the pH and/or on the presence of organic ligands that form complexes with the metal or metalloid. Arsenate (As(V)) can react with glutathione in buffered aqueous solutions to produce arsenite (As(III)) and oxidized glutathione. This reaction may be important in the methylation reactions of arsenic. Arsenic species can decrease the red blood cell levels of reduced glutathione, but the products of oxidation and the mechanism of oxidation are more complex than those found in water alone. Chromium (VI) is thought to interact with DNA after first reacting with a reducing agent such as glutathione to form lower oxidation states of chromium. These examples illustrate the importance of oxidation–reduction reactions for toxic metals and metalloids. — Environ Health Perspect 103(Suppl 1):17–19 (1995)

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Introduction

Chemical toxicity depends upon several chemical properties including reactions that have reached equilibrium (binding constants), acid base behavior, electron transfer reactions (oxidation-reduction) and reaction rates. Often these chemical terms are described as a component of metabolism or the mechanism of toxicity and are called "bioactivation," a process in which "reactive intermediates" are formed and subsequently react with critical endogenous compounds to form "adducts." These reactions are generally oxidations in which oxygen atoms are added to carbon or nitrogen atoms of a molecule.

Metals and metalloids can bind directly with endogenous compounds to form complexes. These complexes may or may not be the toxic compounds, but rather they may be a transport form of the metal ion or even a species that protects against the toxicity of the free metal ion. In addition, electron transfer reactions occur; some metal(loid)s transfer electrons between stable oxidation states and this process may lead to changes in the site and/or route of toxicity. There is a significant possibility for redox cycling between the oxidation states of metal ions such that electrons are transferred between endogenous compounds; this may create much greater effects than would be predicted on the basis of stoichiometric quantities (doses) of the metal ion alone.

The term "metal speciation" has been used to describe some of these chemical forms and the oxidation state and/or the composition of the metal complex in the biological system. When this concept of metal species is combined with the transport and toxicity of metals, its impact on the complexity of the system can be appreciated.

Oxidation–Reduction: Electron Transfer Reactions

Many important biological reactions occur with the transfer of one or more electrons either directly or with the transfer of oxygen atoms. There are several substances in the functioning organism that are involved with these reactions. These include metal ions at the active site of enzymes: Fe(II)-Fe(III), Cu(I)-Cu(II), Co(II)-Co(III); endogenous oxidizing agents: O_2^{-} (superoxide), H_2O_2 (hydrogen peroxide), O_2 (oxygen); and endogenous reducing agents: O_2^{--} , H_2O_2 , glutathione, ascorbate, tocopherol, NADPH.

The oxygen-related species arise from the conversion of molecular dioxygen to water by an organism to produce energy.

$$O_2 + e \rightarrow O_2^{\bullet-} + e \rightarrow H_2O_2 + 2e \rightarrow H_2O_2$$

The key step is the initial formation of the superoxide radical formed as a by-product of the use of dioxygen as an oxidant in respiration and oxidative metabolic processes. The amount of dioxygen converted to superoxide during aerobic respiration varies from 1 to 4% (1). Whenever an electron tranfer system operates in microsomes or mitochrondria, superoxide is produced. The radical can also be produced directly when xanthine is oxidized by xanthine oxidase and during phagocytosis when a burst of superoxide is produced by a membrane-bound NADPH oxidase in all phagocytic cells. In the red blood cell, superoxide can be formed from the autooxidation of oxyhemoglobin.

The concentration of these oxidizing species is kept under tight enzymatic control by superoxide dismutase (SOD) for superoxide and by catalase and glutathione peroxidase for hydrogen peroxide. The concentration of superoxide is maintained *in vivo* at 10^{-10} to 10^{-11} M, whereas hydrogen peroxide is maintained at 10^{-7} to 10^{-8} M (1).

In contrast to these low concentrations for the oxygen-based oxidizing species, some of the endogenous reducing agents are maintained at much higher concentrations. Ascorbate and glutathione concentrations in cell cytosol are found at millimolar levels and tocopherols are in high concentrations in the lipids in the cell (e.g., membranes).

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Metal(loid)s that can participate in oxidation-reduction reactions can react with these species either to protect against toxicity or to enhance toxicity. The well-known Fenton reaction between endogenous iron and hydrogen peroxide generates the extremely reactive hydroxyl radical.

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^{-} + OH^{-}$$

The hydroxyl radical can initiate lipid peroxidation and it reacts readily with proteins. The Fenton reaction, as written, oversimplifies the process, as it is doubtful that Fe(II) exists free in biological solutions and Fe(III) is very insoluble as its hydroxide at physiological pH. The actual species produced may be metal(loids) at higher oxidation states after subsequent reactions with the hydroxyl radical or other radicals formed, followed by reactions between the metal(loid) and cellular macromolecules (1). These reactions have been examined for the endogenous metals iron and copper, but it is likely that certain toxic metals also react with these endogenous oxidizing and reducing agents as well as the endogenous metal ions. These toxic metal(loid)s that change oxidation states are As(III)-As(V), Au(O)-Au(I)-Au(III), Cr(III)-Cr(VI),H g (O) - H g (I) - H g (II), Mn(II)-Mn(III)-Mn(IV), V(III)-V(V), [Ni(II)-Ni(III)]. Other toxic metals are unlikely to change oxidation states as a part of their toxic action. These are Al(III), Cd(II), Pb(II).

Oxidation–Reduction Reactions and Standard Reduction Potential

Chemical reactions can be described in terms of thermodynamics and the freeenergy change between products and reactants. An oxidation-reduction reaction which has reached equilibrium has a change in free-energy of zero. The freeenergy change of a chemical reaction can be described as a function of the cell potential of a hypothetical cell in which electron transfer occurs.

$$\Delta G^{0} = -nFE_{cell}^{0}$$
$$\Delta G = -nFE_{cell}$$

These cell potentials and the standard electrode potentials of the half cell reactions may be used to predict if specific reactions will occur spontaneously. A chemical reaction can be separated into its component half reactions that can be combined to determine if the reaction is thermodynamically favorable.

The example used is the reduction of Fe(III) by superoxide radical anion. This is an important reaction in the generation of the hydroxyl radical. If the potential for the reaction is a positive number, the reaction will be energetically favored.

$Fe(III) + e^- \rightarrow Fe(II)$	$E^0 = +0.77 V$
$O_2^{\bullet-} \rightarrow O_2 + e^-$	$E^0 = +0.33 V$
$\overline{\text{Fe(III)} + \text{O}_2^{\star-} \rightarrow \text{Fe(II)} + \text{O}_2 \text{E}_{\text{cell}} = 1.1 \text{ V}}$	

The reduction potential of the couple, O_2^{-}/O_2 , at 25°C at 1 atm of oxygen is -0.33 V at 1 atm of oxygen, but the sign is reversed when written as an oxidation. Other important reducing agents are: glutathione -0.23 V, NADPH -0.32V, and vitamin C -0.058 V (1).

Effect of pH on Reduction Potential

If the reaction requires hydrogen or hydroxyl ions to balance the equation, then the pH of the solution will affect the cell potential. Thus, it is possible that a reaction could change from being favorable to being unfavorable based on the pH of the solution.

Effect of Complex Formation on Reduction Potential

Metal ions are electropositive in nature and have a very high affinity for electronrich molecules-so much so that they avidly bind the oxygen atom in water molecules in aqueous solutions. Biological systems have a large variety of functional groups on molecules that are rich in electrons; carboxylic acids, heterocyclic nitrogens, amines and thiol groups on amino acids, vitamins, proteins, lipids, RNA and DNA provide strong binding sites for metal(loid)s. Binding to these electronrich molecules changes the electron density around the metal(loid) and, thus, changes the reduction potential of the metal(loid).

For example, the standard reduction potential for the reaction, $Co(III) \rightarrow Co(II)$ is different when determined in water compared to the value obtained when complexed with ethylenediamine.

$$Co(III) + e^- \rightarrow Co(II) \qquad E^0 = +1.95 V$$
$$Co(en)3^{3+} + e^- \rightarrow Co(en)3^{2+} \qquad E^0 = -0.2 V$$

Examples of Oxidation Reduction Reactions with Metal(loid)s

Arsenic V Species with Glutathione

Inorganic arsenicals such as As(V) and As(III) are very toxic, but organisms have the ability to detoxify the arsenic through the formation of methylated metabolites: monomethylarsonic acid (As(V)) and dimethylarsinic acid (As(V)) in mammals and further to trimethylarsinic oxide (As(V))and trimethylarsine (As(III)) in certain bacteria and fungi (2). This metabolism appears to involve the formation of As(III)and As(V) species at various steps in the process. If the various steps in this process could be understood, the detoxification of arsenic may be able to be manipulated.

Reactions of arsenate and arsenite with glutathione were studied in aqueous buffer solutions to characterize the chemistry of that reaction (3). The reactions in solution were studied using high resolution nuclear magnetic resonance; the products of the reactions were isolated and characterized by mass spectrometry.

The results showed an electron transfer reaction between As(V) and glutathione to form As(III) and oxidized glutathione (GSSG).

$$\mathrm{AsO}_4^{3-} + 2 \ \mathrm{GSH} \rightarrow \mathrm{AsO}_3^{3-} + \mathrm{GSSH} + \mathrm{H_2O}$$

As additional GSH was added after the reduction to As(III) was complete, a complex between the As(III) and GSH formed.

$$AsO_3^{3-} + 3 \text{ GSH} \rightarrow As(SG)_3 + 3 \text{ OH}^-$$

This complex has a unique NMR spectrum, can be isolated as a solid, and gives a mass spectrum consistent with this structure. Analogous electron transfer reactions occurred between glutathione and the two methylated species of As(V) and the GSH complexes were formed.

$$CH_3AsO(OH)_2 + 4 GSH \rightarrow$$

 $CH_3As(SG)_2 + GSSG + 2 H_2O$

$$(CH_3)_2AsO(OH) + 3 GSH \rightarrow$$

 $(CH_3)_2As(SG) + GSSG + H_2O$

There is *in vivo* evidence for the reduction of arsenate to arsenite; both species are found in blood and urine after administration of As(V). However, there is no *in vivo* evidence for the corresponding methylated As(III) species. Their function is unclear, as they have never been identified in an organism, but their existence has been postulated in the methylation reaction; the methylation reaction has been thought to be an oxidative methylation that involves a change in oxidation state from As(III) to As(V) (4).

$$As(III)O_{3}^{3-} \xrightarrow{+CH_{3}} CH_{3}As(V)O(OH)_{2} \xrightarrow{+2e^{-}} CH_{3}As(III)(OH)_{2} \xrightarrow{+CH_{3}} (CH_{3})_{2}AsO(OH)$$

Thus, the role of glutathione could be to provide the electrons for the reduction of $A_s(V)$ to $A_s(III)$ and the GSH complex could be the substrate for the methylating enzymes.

The reduction of arsenic species by GSH has been studied in the red blood cell, where there is little likelihood of any methylating enzymes being present. When As(V) was incubated with red blood cells, there was a slow uptake of arsenic into the cells, followed by a reduction in GSH levels and the appearance of As(III). Instead of GSSG being formed, mixed disulfides of GSH and protein-SH appeared (GSSPro). This still shows that an oxidation of thiol groups accompanied the disappearance of GSH. However, the results appear more complicated than the solution chemistry reactions described above; when As(III) was incubated with the cells, GSH levels also declined. The reasons for this finding are unclear and are being investigated.

Carcinogenicity of Chromate

Chromate (Cr(VI)), has been found to be carcinogenic to humans based on the results of several epidemiologic studies (5). The target organ after inhalation of aerosols is the respiratory tract. The exact mechanism by which Cr(VI) compounds cause cancer is not known, but the oxidation state is critical because the other most common oxidation state, Cr(III), is not carcinogenic. It is known that Cr(VI) is taken up by cells through an anion channel that is probably also used to transport sulfate and phosphate. Cr(VI) has insufficient interactions with DNA in vitro to be considered the carcinogen; significant chromium-DNA binding was observed in vitro only when Cr(VI) was incubated with DNA in the presence of agents capable of reducing Cr(VI) (5). These findings have led to the hypothesis that Cr(VI) is taken up by the cell in preference to other forms of Cr and once inside the cell, reacts with reducing agents to form the carcinogenic

species. Electron spin resonance spectrocopy evidence indicates that there are several possible carcinogenic species including Cr(V), Cr(IV), thiyl (GS·) and hydroxyl (HO·) radical intermediates (5). The reactions shown below are written as one-electron transfers until the stable Cr(III)oxidation state is reached, although there is no evidence to suggest this mechanism.

$$\begin{array}{ll} Cr(VI) + GSH & \longrightarrow Cr(V) + GS \\ Cr(V) + GSH & \longrightarrow Cr(IV) + GS \\ Cr(IV) + GSH & \longrightarrow Cr(III) + GS \end{array}$$

A number of nonenzymatic and enzymatic materials have shown the ability to reduce Cr(VI). The nonenzymatic reductants include glutathione, ascorbate (vitamin C), vitamin B2, and tocopherol (vitamin E). The enzymatic reductants include the mixed-function oxidase enzymes (cytochrome P450 and cytochrome b₅), mitochondrial enzymes (complexes I, II, and IV of the electron transport chain), and possibly DT-diaphorase (5). Of these, most of the work has focused on glutathione; it is the most likely candidate along with ascorbate, to reduce Cr(VI) because of its high concentrations in the cell cytosol.

Formation of Arsine from Arsenite in the Environment

Arsine is the most toxic form of arsenic, with a recommended maximal exposure to arsine of only 0.05 ppm. The toxicology of arsine is not clearly understood, but one major effect is the hemolysis of red blood cells followed by other effects including kidney failure and death.

Arsine is made in the laboratory by hydrolysis of arsenides with acid (e.g., Zn_3As_2) or by reduction of arsenic trioxide with reducing agents such as sodium borohydride or zinc in acid. It is commonly stated that arsine is accidentally generated when arsenic salts react with nascent hydrogen (abnormally reactive hydrogen), but this seems unlikely because the reduction of arsenous acid by hydrogen is not thermodynamically favored.

$$3H_2 + HAsO_2 \longrightarrow AsH_3 + 2H_2O$$

E = -0.36 V

The typical conditions that resulted in accidental arsine formation include water solutions of arsenic, acid or base, and a metal in its elemental form. Some examples include a) arsenic and an aluminum ladder in water, b) acid sludge in a galvanized bucket, c) flue dust mixed with sulfuric acid and metallic zinc, d) an aluminum tank containing sodium hydroxide solution, or e) metallic aluminum dross and hot water. It is more likely that the arsenic reacts with the metal after the metal oxide coating on the surface is removed by the acid or base. The role of the water is to dissolve the metal ion after it is formed.

$$2AI(0) + HAsO_2 + 6H^+ - ----$$
$$2AI(III) + AsH_3 + 2H_2O$$
$$E = +2.96 V$$

Reactions of this type with aluminum, zinc, iron, cadmium, nickel, tin, and lead in the metallic form are thermodynamically favored. Therefore, arsine may be generated in unexpected places if the solution conditions are appropriate.

Conclusions

Several metal species react in biological and environmental systems to participate in electron transfer reactions in which they change oxidation states. As different species are formed, there are significant changes in toxicity. Our understanding of these processes is incomplete and the determination of the actual metal species responsible for toxicity promises to be the next frontier in metal toxicity.

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