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Selenium Detoxification of Heavy Metals: a Possible Mechanism for the Blood Plasma

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Among the activities of the essential trace element selenium is the ability to reduce the toxicity of heavy metal ions like cadmium(II) and mercury(II). Detoxification often depends on the metabolic reduction of selenium to hydrogen selenide; the mechanism generally advanced to explain such selenium/metal interactions is that selenide combines with heavy metal ions to give a metal selenide which is metabolically inert. However, this hypothesis does not consider circumstances where selenide is quickly removed by other reactions. Given the ease with which selenide is oxidized, such conditions are likely to occur in the blood plasma, an environment rich in oxidizing agents and a site for many selenium/metal interactions. Using polarography to monitor both selenide and cadmium, we have found that selenide reacts rapidly in vitro with the disulfide bonds present in bovine serum albumin in preference to forming cadmium selenide. We hypothesize that a similar reaction occurs in the blood plasma with the disulfide bonds of plasma proteins to generate thiol groups on the protein involved, and that these newly formed thiols are responsible for the observed reduction of metal toxicity through the ability to chelate heavy metal ions.

Introduction

The ability of selenium to reduce the toxicity of heavy metals has been studied intensively in recent years [1]. Many selenium-mediated detoxifications depend on the metabolic reduction of selenium to hydrogen selenide; it has been suggested that selenide combines with heavy metal cations like mercury(II) and cadmium(II) to give a metabolically inert metal selenide. While this is an appealing hypothesis, it does not consider circumstances where selenide is rapidly removed by other reactions. Such conditions are very likely to occur in the blood plasma, an environment rich in compounds capable of rapidly oxidizing selenide and a site of many selenium/metal interactions. Red blood cells have been shown to release a selenium metabolite [2, 3] which behaves like exogenously added hydrogen selenide [4]; this metabolite is found bound primarily to the albumin fraction of the plasma proteins [2]. When cadmium(II) is present, chromatography of the plasma shows that selenium binding shifts, and is found associated with cadmium in a high molecular weight plasma protein complex [4]. Is this interaction due to cadmium selenide formation? We would like to suggest an alternate hypothesis based on the observation that selenide reacts rapidly with the disulfide bonds of BSA (bovine serum albumin), and in preference to forming cadmium selenide. We suggest that selenide is rapidly oxidized in the blood plasma by the disulfide bonds present in plasma proteins like albumin, and that the resulting thiol groups on these proteins are available to chelate metals like cadmium(II), thereby rendering such metals less toxic.

Experimental

Selenide solutions were prepared under nitrogen in anaerobic glassware by hydrolysis of aluminum selenide (Al_2Se_3; purchased from Alfa Products) as suggested by Waitkins and Shutte [5]; hydrolysis gives volatile hydrogen selenide, which is subsequently trapped in 0.1 *M* phosphate (pH 7) as hydrogen selenide ion. Preparation of m*M* concentrations in small volumes (*e.g.* 100 ml) requires only tens of mg of aluminum selenide, largely reducing the hazards associated with selenide generation. An amperometric-style buret was used to deliver selenide directly to the polarographic cell without exposure to oxygen.

Data was collected on a Sargent-Welch Model XVI polarograph equipped with a thermostatted H-type cell and saturated calomel reference electrode (SCE); the dropping mercury electrode (DME) had a drop time of 4.60 sec and a mercury flow of 1.60 mg/sec with no applied voltage. Crystallized and lyophilized BSA was purchased from Sigma (A4378),

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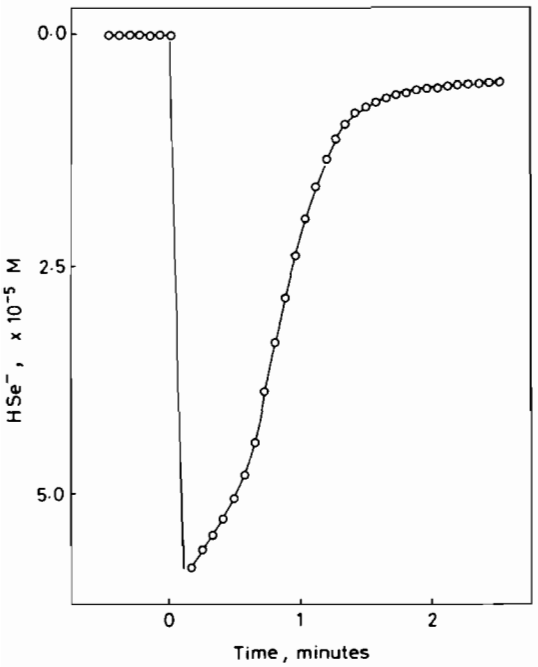

Fig. 1. Disappearance of selenide as monitored by polarography at a constant potential of −0.40 V (*versus* SCE); at time zero, 6.58×10^{-5} *M* hydrogen selenide ion was added to 3.7×10^{-4} *M* BSA (66,210 daltons) in oxygen-free 0.1 *M* phosphate buffer (pH 7) at 40 °C.

dissolved under nitrogen, and standardized by refractive index [6].

Results

Our original intent was to measure the binding constant of selenide to BSA, as we believed there was a possibility that selenide was binding to some plasma protein, possibly albumin [2], on release from the red blood cells; albumin is the most abundant protein in the plasma, and is known to bind a wide variety of substances [7]. The electrochemical technique of polarography was selected for this purpose on the basis of its use in similar binding studies [8]. When selenide was found to react with BSA rather than bind to it, polarography proved to be an effective method to monitor the reaction as well as to measure levels of cadmium(II), disulfides, thiols, and oxygen. The rate of selenide disappearance on addition to a solution of BSA is shown in Fig. 1; the disappearance of selenide is also associated with the appearance of a polarographic signal characteristic of cysteine residues (data not shown).

Although there is no polarographic evidence for selenide binding, the resulting elemental selenium binds readily to BSA. In aqueous solution, elemental selenium initially forms a colloidal suspension of the red amorphous allotrope [9]; BSA prevents precipitation of the selenium colloid through a non-specific hydrophobic-type binding. The ability of albumins to stabilize selenium colloids in this manner has been recognized for some time [10]. It seems likely the binding observed after release of selenide by the red blood cells [2, 3] is an indication of the rapid oxidation of selenide in the blood stream to colloidal selenium.

The tendency of selenide to undergo oxidation in preference to forming metal selenides can be illustrated in a series of simple experiments. Addition of selenide to an oxygen-free solution of cadmium(II) gives a brownish-black precipitate of cadmium selenide. If carried out in a polarographic cell, the decrease in selenide as well as cadmium(II) can be followed as a function of time; we found this reaction rapid enough to use as a convenient method to standardize selenide solutions. However, the presence of a chelating agent like EDTA (ethylene diamine tetraacetic acid) blocks the formation of cadmium selenide; even after 30 minutes, such a solution remains free of precipitate. The lack of reaction when EDTA is present is easily confirmed by polarography from the fact that the concentrations of both selenide and cadmium(II) remain constant. Selenide is known to react with disulfides in the following manner [11]:

$$HSe^- + RSSR + H^+ \longrightarrow Se^\circ + 2RSH \qquad (1)$$

The products are colloidal elemental selenium and the thiol. Addition of selenide to a solution containing cadmium(II), EDTA, and a disulfide like oxidized glutathione results in a rapid reaction between the disulfide and selenide, in which cadmium does not participate; this is apparent from the fact that the polarographic signal of cadmium(II) does not change on addition of selenide. BSA is both a disulfide source as well as a weak chelator of cadmium(II) [7]; when selenide is added to a solution of BSA and cadmium(II), the selenide disappears rapidly in the manner seen in Fig. 1. The polarographic signal of cadmium again remains unaltered, indicating cadmium selenide does not form.

Discussion

The behavior of selenide *in vitro* shows that heavy metal selenides, like cadmium selenide, do not form when selenide is rapidly removed by other reactions. Selenide released by the erythrocytes is likely to be quickly oxidized by oxidants like oxygen and disulfides present in the blood plasma; this does not of course preclude the formation of metal selenides in other locations in the body. It seems likely, therefore, that some other reaction of selenide, rather than the formation of metal selenides, is responsible for selenium interactions with heavy metal ions in the blood

plasma. The resulting thiol groups produced on disulfide oxidation of selenide are likely candidates for heavy metal detoxification, as chelation by thiol-rich proteins is known to be an effective method of reducing metal toxicity [12]. Further, the high molecular weight protein complexes which result may well be due to cross-linking of proteins by the metal ion through these newly formed thiol groups. The elemental selenium formed on disulfide oxidation would be expected to bind hydrophobically to the nearest available protein, namely the one containing the reacting disulfide group; the resulting protein complex would thus contain both selenium and cadmium. Although this mechanism may prove difficult to verify directly in the biological matrix, it should be given serious consideration on the basis of being consistent with the behavior of selenide *in vitro*; the idea that metal selenides form in the blood plasma should be reexamined.

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