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Redox Reactions of Hydrogen Selenide Ion

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Hydrogen selenide ion (HSe^-) is an important product in the metabolism of the essential trace element selenium. Although its role in selenium metabolism is recognized, aspects of the basic chemistry of selenide have been ignored, particularly the tendency of selenide to undergo rapid redox reactions with biological oxidants. Using polarography, we have found that selenide reacts in vitro *with a variety of compounds including dehydroascorbic acid, quinones like vitamin K_1 and FAD (flavin adenine dinucleotide), and disulfides such as oxidized glutathione and lipoic acid. The fact selenide reacts readily* in vitro *suggests similar reactions may also occur* in vivo *with important biological consequences. Contrary to expectations, selenide was found not to reduce the disulfide bond of oxidized dithiothreitol (*trans*-4,5-dihydroxy-1,2-dithiane), indicating the commonly published value for the standard electrode potential of the selenium/hydrogen selenide ion couple is in error. The electrode potential is an important parameter to aid in anticipating possible redox reactions of selenide* in vivo.

Introduction

An important product in the metabolism of the essential trace element selenium is hydrogen selenide (H_2Se) [1]; in the physiological pH range, hydrogen selenide exists primarily as hydrogen selenide ion (HSe^-). Although the role of this ion in selenium metabolism is recognized, much of the basic chemistry remains uninvestigated, particularly the tendency of selenide to be rapidly oxidized by a variety of biologically relevant oxidants. Using the electrochemical technique of polarography, we have found selenide reacts rapidly with biological compounds like dehydroascorbic acid, quinones like FAD (flavin adenine dinucleotide), and other oxidants. The neglect of selenide redox reactions is emphasized by the fact that the commonly published value for the standard electrode potential of the elemental selenium/hydrogen selenide ion couple is in error. The standard potential is a useful value for predicting the direction of thermodynamically possible redox reactions, and, as such, is an important parameter in anticipating potentially interesting biological reactions of selenide. On the basis that selenide is rapidly oxidized *in vitro*, we suggest it is logical to suspect selenide also undergoes unidentified redox reactions *in vivo*. Detection of such reactions may prove difficult given the low levels present in the body, but *in vitro* reactions are easily monitored using polarography, giving clues as to what reactions might occur in the body.

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 & Shutte [2]; hydrolysis gives volatile hydrogen selenide, which is subsequently trapped in 0.1 *M* phosphate buffer (pH 7) as hydrogen selenide ion. Preparation of m*M* concentrations requires only tens of mg aluminum selenide, largely reducing the hazards associated with hydrogen selenide generation [3]. An amperometric-style buret was used to deliver selenide directly to the polarographic cell without exposure to air. Selenide solutions treated in this manner are crystal-clear until exposed to an oxidant; on oxidation, selenide forms a colloidal suspension of red amorphous elemental selenium obvious on visual inspection.

Selenide has a well-defined polarographic wave [4], as do many of the other compounds tested in the present study. Compounds were monitored for reaction with selenide in oxygen-free 0.1 *M* phos-

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TABLE I. Hydrogen Selenide Ion Reactions with Selected Oxidants Listed by Electrode Potentials at pH 7.

Oxidant/(Reductant)	$E^{o'}$, V[a]	Reaction with HSe^-
1,4-benzoquinone	0.293	yes
methylene blue	0.011	yes
riboflavin	−0.208	yes
FAD/($FADH_2$)	−0.219	yes
GSSG/(GSH)	−0.23	yes
lipoic acid	−0.29	yes
DTT (oxidized)[b]	−0.33[c]	no
H^+/H_2	−0.421	no
methyl viologen	−0.44	no

[a]From Loach [24] unless otherwise noted. [b]Dithiothreitol (oxidized) or *trans*-4,5-dihydroxy-1,2-dithiane. [c]Cleland [25].

phate buffer (pH 7) at 25 °C using a Sargent-Welch Model XVI polarograph equipped with a thermostatted H-type cell and saturated calomel electrode (SCE); the dropping mercury electrode (DME) had a drop time of 4.60 sec and a mercury flow of 1.60 mg/sec. Compounds were introduced into the polarographic cell at approximately 0.1 m*M* concentration, oxygen removed by bubbling with nitrogen for 20 minutes, and the selenide then added.

Results

Several of the compounds monitored for reaction with selenide are listed in Table I, along with their electrode potentials at pH 7. The electrode potential of selenide can be experimentally estimated by comparing its tendency to undergo redox reactions with compounds having established electrode potentials; Table I suggests the electrode potential for hydrogen selenide ion falls between that of lipoic acid and oxidized DTT (dithiothreitol). Individual reactions are discussed in more detail below.

Quinones

Quinones were found to oxidize selenide readily; the specific quinones tested were 1,4-benzoquinone, riboflavin, FAD (flavin adenine dinucleotide) and vitamin K_1 (in 50% ethanol). These reactions have not been described in the literature, and the widespread distribution of quinone compounds in the body makes the reaction with selenide of potential biological interest. Biological interactions with another quinone, vitamin E, have been noted by Diplock *et al.* [5].

Dehydroascorbic Acid

The oxidized form of ascorbic acid, dehydroascorbic acid, is not stable at pH 7, but is stable in the pH range from 2 to 4 [6]; dehydroascrobic acid is easily prepared by air oxidation of ascorbic acid as described in Tolbert & Ward [6]. Selenide was found to react readily with dehydroascorbic acid in 0.1 *M* phosphate at pH 3, giving colloidal selenium; loss of volatile hydrogen selenide was minimal during the course of the reaction.

Biological interactions between selenium and ascorbate have been noted. Anderson & Moxon [7] correlated the survival rate of dogs injected with sodium selenite to the levels of ascorbate found in the blood. Hill [8] showed that ascorbic acid supplementation of chicks reversed the growth loss associated with chronic selenium toxicity. The biochemical basis of these effects is unknown.

Aromatic Nitro and Nitroso Groups

Hydrogen sulfide ion (HS^-) is well known for its ability to reduce aromatic nitro groups to amines [9]; it was anticipated that the closely related hydrogen selenide ion would react in an analogous manner. The two aromatic nitro compounds tested, martius yellow (2,4-dinitro-1-naphthol) and nitrofurantoin [N-(5-nitro-2-furfuryllidene)-1-aminohydantion], were both found to react with selenide. Nitrofurantoin is a common urinary tract antiseptic whose toxicity has been shown to be more severe in selenium deficient chicks [10].

Selenide also demonstrated the ability to reduce the nitroso groups of N,N-dimethyl-4-nitrosoanaline and nitroso R salt (3-hydroxy-4-nitroso-2,7-naphthalene-disulfonic acid disodium salt). Like many nitroso compounds, both these substances are presumed carcinogens.

Disulfides

Woods & Klayman [11] first reported the ability of selenide to reduce a variety of disulfides, including cystine, in the following manner:

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

We found selenide also reduces other disulfides of biological interest, including oxidized glutathione and the cyclic disulfide lipoic acid. However, another cyclic disulfide, *trans*-4,5-dihydroxy-1,2-dithiane (oxidized dithiothreitol), is not reduced by selenide. In fact, the reverse reaction occurs: a colloidal solution of amorphous elemental selenium is reduced by DTT to give selenide and the cyclic disulfide. This indicates the direction reaction 1 proceeds is determined by the redox potential of the disulfide/thiol couple; when the reduction potential of the thiol is strong enough, reaction 1 proceeds to the left. This fact suggests that the electrode

TABLE II. Comparison of Calculated Values for the Electrode Potential of the Selenium/Hydrogen Selenide Ion Couple.

Source	E^o, V	$E^{o'}$, V[a]
NBS Circular 500[b]	−0.51[c]	−0.72
Corrected[d]	−0.48	−0.69
NBS Note 270-1[e]	−0.23	−0.44
Corrected[d]	−0.20	−0.41
Present Estimate	−0.12 to −0.08	−0.33 to −0.29

[a]Electrode potential at pH 7. [b]Rossini *et al.*, [15]. [c]Commonly listed value. [d]Corrected for the standard free energy of formation for amorphous elemental selenium [23]. [e]Wagman *et al.*, [16].

potential of the selenium/hydrogen selenide ion couple falls between that of lipoic acid and oxidized DTT. It should be noted that this estimate of the electrode potential is only as good as the values for the disulfide/thiol couples involved, and that determinations of these couples are subject to error [12]. Aside from the electrode potential, it is apparent that the use of DTT in selenium metabolism studies, as in Banerjee and Sani [13], should be approached with caution.

Discussion

Most standard potentials for selenium compounds are obtained indirectly from thermodynamic calculations [14]. The standard source for thermodynamic data is the National Bureau of Standards (NBS) publication Circular 500 [15] and NBS Technical Note 270-1 [16]. The original source for the NBS data dates from 1887 [17]; problems with this data have been noted previously [18, 19]. The standard electrode potentials calculated using these two NBS sources are shown in Table II, along with the electrode potentials calculated for pH 7. The value of −0.51 V is that commonly found in tables of standard potentials [14, 20], and differs considerably from the value calculated from the present study. Calculations usually assume the formation of the most stable modification of elemental selenium, the gray hexagonal allotrope [21], but the modification which forms initially in aqueous solution at moderate temperatures is the less stable red amorphous allotrope [22]; therefore, corrections for the standard free energy of formation of amorphous selenium at 298 K of 5.06 kJ/mol [23] have been included in Table II.

The electrode potential of the selenium/hydrogen selenide ion couple is an important parameter in predicting the possible redox reactions in which this couple can participate. The fact that the redox potential is less negative than commonly thought suggests, for example, that some biological thiols may be able to generate selenide from colloidal selenium under favorable conditions. Given the ease with which selenide undergoes a variety of redox reactions with a variety of biologically relevant oxidants *in vitro*, similar reactions should be considered *in vivo*.

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