Hydrogen Selenide Ion and Colloidal Selenium in the Catalytic Oxidation of Thiols

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Abstract

The ability of selenium to act as a catalytic agent is an important factor in understanding the biological role of selenium as an essential trace element. Using polarography, we have found that hydrogen selenide ion (HSe^-) and its oxidation product, colloidal elemental selenium, can catalyze the aerobic oxidation of thiols like glutathione (GSH) and dithiothreitol (DTT). Under certain conditions, thiols can drive the reduction of colloidal selenium to give selenide, which subsequently reacts rapidly with oxygen to regenerate colloidal selenium; this catalytic cycle results in an increased rate of thiol oxidation. The state of aggregation of the lyophobic colloidal selenium is a major factor in catalysis; colloidal selenium is an effective catalyst when disperse, but loses activity as the colloid aggregates and the attendant surface area of the colloid decreases. Factors which increase the rate of colloidal aggregation likewise decrease the catalytic activity of selenide and colloidal selenium.

Introduction

The ability of selenium to act as a catalytic agent is an important factor in understanding the biological role of selenium as an essential trace element [1–4]. Among inorganic selenium compounds, selenite (SeO_3^{-2}) is recognized to be an effective catalyst in the oxidation of thiols by oxygen [1]. The mechanism of catalytic action has not been firmly established, although there is general agreement that selenite itself is not the agent directly responsible for catalytic activity. A role in the catalytic mechanism has been suggested for hydrogen selenide (H_2Se) [2, 4], or for the hydrogen selenide ion (HSe^-), which is the predominant species in solution at physiological pH. Selenide is a biological metabolite of selenite, and selenide plays a central role in the metabolic pathway of selenium [4]. As the catalytic activity of selenide has never been directly verified experimentally, we felt an examination of selenide in the aerobic oxidation of thiols would contribute to an understanding of the role of selenide in selenium-mediated catalysis, and in its role as an essential trace element.

Using the electrochemical technique of polarography, we have found that selenide and colloidal elemental selenium increase the rate of aerobic oxidation of thiols like glutathione (GSH) and dithiothreitol (DTT) through the following catalytic cycle:

$$Se^0 + 2RSH \rightleftharpoons HSe^- + RSSR + H^+ \quad (1)$$

$$HSe^- + \tfrac{1}{2}O_2 \longrightarrow Se^0 + OH^- \quad (2)$$

Reaction 1 has been studied in the absence of oxygen [5, 6]. The direction of equilibrium in this reaction depends on the electrode potential of the thiol/disulfide couple involved; equilibrium lies to the left with GSH, and to the right with DTT. Kinetic studies have shown reaction 2 to be relatively rapid: solutions above a concentration of 10^{-6} M selenide were found to be at least 95% oxidized within 3 minutes of exposure to atmospheric levels of dissolved oxygen [7]. The product of reaction 2, elemental selenium (Se^0), is a long-chain polymer which forms a colloidal suspension of the red amorphous allotrope in aqueous solution [8]. The more disperse the colloidal selenium, the greater the surface area, and the more actively the colloid participates in reaction 1; likewise, factors which encourage colloidal aggregation decrease the rate of thiol oxidation.

Experimental

Selenide solutions were prepared under nitrogen in anaerobic glassware by hydrolysis of aluminum selenide [9] (Al_2Se_3; purchased from Alfa Products); hydrolysis gives volatile hydrogen selenide which is subsequently trapped in 0.5 M phosphate buffer (pH 7) as hydrogen selenide ion. Preparation of mM

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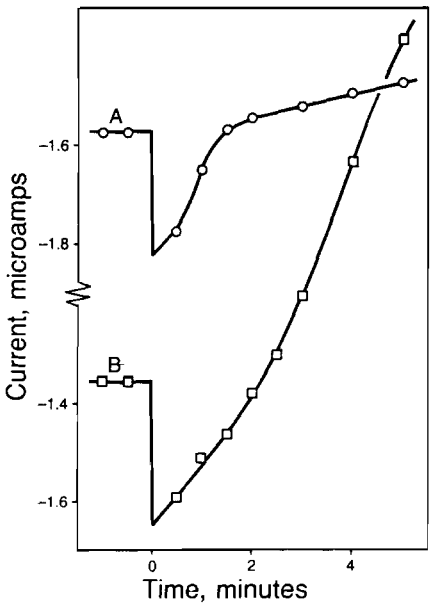

Fig. 1. The decay of the anodic waves of 1.61×10^{-5} M HSe^- added to 5.60×10^{-4} M GHS (Curve A), and of 1.90×10^{-5} M HSe^- added to 6.89×10^{-5} M DTT (Curve B); selenide was added to both thiols at time zero. The waves were monitored at +0.05 V *versus* the SCE in air-saturated (2.2×10^{-4} M oxygen) 0.05 M phosphate buffer (pH 7) at 25 °C.

concentrations in volumes of 100 ml requires approximately 50 mg 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 air. Solutions of red colloidal elemental selenium were prepared by air oxidation of selenide.

Reactions were monitored with a Sargent-Welch Model XVI polarograph equipped with a thermostatted H-type cell and a saturated calomel reference electrode (SCE). The dropping mercury electrode (DME) had a drop time of 4.98 s and a mercury flow of 1.60 mg/s with no applied voltage. Thiols like GSH [10] and DTT exhibit anodic polarographic waves, as does selenide [11]; such substances can be monitored in the presence of oxygen at positive voltages where the polarographic wave of oxygen does not interfere. Data was collected by setting the polarograph at +0.50 V *versus* the SCE, introducing an air-saturated thiol solution into the polarographic cell, and monitoring the decay of the resulting anodic waves on addition of the appropriate selenium-containing solution (see Fig. 1). Due to the characteristics of anodic waves, the signal is seen as the decay of a negative current (in microamps). Reactions were carried out in 0.05 M phosphate buffer (pH 7) at 25 °C unless otherwise noted. GSH and DTT were purchased from Sigma.

TABLE I. The Effect of Selenium on the Initial Rate of Aerobic Oxidation of Glutathione (GSH).

Selenium Added	Initial Rate[a], $\times 10^{-7}$ M min^{-1}	
	0.05 M buffer	0.1 M buffer
Without Selenium	0.8 ± 0.4	0.6 ± 0.3
1.79×10^{-5} M Se^0	2.0 ± 0.5	0.8 ± 0.4
1.88×10^{-5} M HSe^-	9.3 ± 0.6	4.9 ± 0.4

[a]As recorded by polarographic monitoring at +0.05 V *versus* the SCE; conditions were 1.8×10^{-4} M GSH and 2.2×10^{-4} M oxygen (air-saturated) in phosphate buffer (pH 7) at 25 °C.

Results

Table I shows the initial rate of aerobic GSH oxidation as a function of the selenium compound added. We see that selenide induces a significant increase in the rate of GSH oxidation, and that the increase is much less at the higher buffer concentration. Of particular interest is the fact that colloidal selenium also shows a slight but significant increase in the rate of GSH oxidation in 0.05 M phosphate buffer, but not in 0.1 M buffer. Tsen & Tappel [1] reported the appearance of elemental selenium was associated with the cessation of aerobic oxidation of thiols induced by selenite, and they concluded elemental selenium was not active in the catalysis of thiol oxidation. It is worth noting that Tsen & Tappel examined the activity of selenite in 0.1 M phosphate buffer at 37 °C, two conditions which encourage the aggregation of colloids relative to 0.05 M buffer at 25 °C [12, 13].

Figure 1 shows examples of the type of data obtained by polarographic monitoring when selenide is added to an air-saturated solution of GSH or DTT. With GSH (Curve A), the signal due to selenide addition disappears in approximately 2 minutes, and is easily separated from the subsequent slow oxidation of GSH. The rapid disappearance of selenide on exposure to oxygen-containing solutions is consistent with the kinetic studies of reaction 2 [7]. The effect of the added selenide on the rate of GSH oxidation is apparent long after the decay of the selenide wave, a fact that suggests the associated oxidation of GSH is actually due to a species other than selenide itself. The oxidation product of selenide, colloidal selenium, is a logical species to suspect. With the appearance of colloidal selenium, the solution is no longer homogeneous, and we must consider the effect of the physical state of the colloid on the rate of thiol oxidation; the more disperse a colloid, the more surface area it has, and the more it is capable of interacting with the surrounding solution [13].

As shown in curve B of Fig.1, the oxidation of DTT subsequent to selenide addition is much faster than the analogous oxidation of GSH. At the end of 4 minutes, about half of the initial DTT is oxidized, whereas less than 10% of the GSH is oxidized in a comparable period. The oxidation of DTT is so rapid that the disappearance of the anodic wave of DTT cannot be separated from the anodic wave of selenide as it can with GSH in Curve A. We know that selenide is rapidly generated from colloidal selenium by DTT in the absence of oxygen [6]; this fact coupled with the rapid oxidation of selenide on exposure to oxygen [7] leads to a catalytic cycle which quickly consumes DTT. In much the same manner, the addition of colloidal selenium to air-saturated DTT results in a rate of DTT oxidation similar to that seen in Curve B (data not shown). The rate of thiol oxidation in the catalytic cycle thus depends on the ability of a particular thiol to drive reaction 1 toward selenide, a factor which is related to the electrode potential of the thiol/disulfide couple involved. Given the reductive capacity of GSH, only a small amount of selenide is generated from elemental selenium, and the resulting rate of thiol oxidation is correspondingly much slower.

Discussion

We have demonstrated that selenide as well as colloidal elemental selenium are capable of inducing aerobic oxidation of thiols under certain conditions. The catalytic scheme represented by reactions 1 and 2 is similar to one proposed by Ganther [4] as an alternative to one proposed by Tsen & Tappel [1] for the catalytic action of selenite. It is worth noting that selenite is reduced by excess amounts of thiols like GSH to elemental selenium [14], but that selenide remains below detectable levels in this reaction. The ability of elemental selenium to participate in the catalytic cycle depends on the state of aggregation of the selenium colloid. Formation of colloidal selenium through the addition of selenide to an oxygen-containing reaction mixture has the advantage of producing a very disperse selenium colloid with a very high surface area available for reaction; a mature selenium colloid has much less surface area, and a flocculated colloid even less. Factors which increase the rate of colloidal aggregation likewise decrease the ability of selenide and colloidal selenium to act as effective catalystic agents; increasing such variables as ionic strength, temperature, and concentration of the colloid all contribute to an increase in the rate of colloidal aggregation [12, 13].

The danger of producing artifacts in biological studies of selenium metabolism through the addition of exogenous thiols has been noted before [3]; given the speed with which we see selenide and colloidal selenium interacting with DTT, this problem could be particularly acute with DTT addition. The use of DTT and its isomer, dithioerythritol, in studies of selenium metabolism is not uncommon [15–17], and seems unlikely to reflect physiological conditions.

It is interesting to consider the possible biological activity of colloidal elemental selenium. While elemental selenium is usually considered to be biologically inert [18], there have been suggestions that it may be active *in vivo* [19–22]. Colloidal selenium could remain very disperse in a biological system due in part to its ability to bind to proteins like albumin; albumin has been known to stabilize selenium colloids for some time [12], presumably through a nonspecific hydrophobic-type interaction. Colloidal selenium is also reduced under mild conditions *in vitro* to selenide [6], a compound to established biological activity. Whether elemental selenium is in fact biologically active remains to be determined, but the catalytic activity of colloidal selenium suggests it is a distinct possibility.

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