Hydrogen Selenide Ion Adsorption to Colloidal Elemental Selenium

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Hydrogen selenide ion (HSe^-) has an important role in the metabolism of the essential trace element selenium. Several redox reactions of selenide were found to be dominated by the amount of colloidal elemental selenium (Se°) generated during the reaction. The following reaction of selenide with the disulfide, oxidized glutathione (GSSG), was used as an example: $HSe^- + GSSG + H^+ \rightarrow Se^\circ + 2$ GSH. The resulting thiol is reduced glutathione (GSH; γ-glutamylcysteinylglycine). By following this reaction with polarography, it was seen that the ratio of colloidal selenium produced to selenide unreacted was a constant 2.1 ± 0.1, and was the only factor found to determine the extent of oxidation. This is best explained by the hypothesis that freshly generated colloidal selenium adsorbs selenide readily; no evidence for polyselenide formation was found. Adsorption of selenide should be considered in any reaction involving the oxidation of selenide to colloidal selenium.

Introduction

Studies on the biochemistry of the essential trace element selenium have revealed an important metabolic role for hydrogen selenide ion (HSe^-) [1, 2], and have provided motivation to study the chemistry of selenide more closely. While examining redox reactions of selenide with the view of better defining the electrode potential (E°) of the elemental selenium (Se°)/selenide couple [3], we found several reactions which were dominated by a non-equilibrium process related to the amount of colloidal elemental selenium generated. We have examined this phenomena in more detail concentrating on the following reaction of selenide with the disulfide oxidized glutathione (GSSG) due to its biological interest:

$$HSe^- + GSSG + H^+ \longrightarrow Se^\circ + 2\ GSH \qquad (1)$$

The resulting thiol, reduced glutathione (GSH; γ-glutamylcysteinylglycine), is the most abundant free thiol in the body tissues; reaction 1 is analogous to the biological action of glutathione reductase, an enzyme intimately associated with selenium metabolism. Disulfide reduction by selenide was first studied by Woods and Klayman [4] for its synthetic utility. GSSG, GSH [5] and selenide [6] are all polarographically active; by utilizing a polarographic cell as the reaction vessel, reaction 1 is easily monitored.

Experimental

Selenide solutions were prepared under nitrogen in anaerobic glassware by hydrolysis of aluminum selenide [7] (Al_2Se_3; purchased from Alfa Products); 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 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, as the reaction with oxygen is moderately fast [8]. Oxygen was removed from solutions in the cell by bubbling with nitrogen for 20 minutes prior to the introduction of selenide aliquots. Selenide solutions are crystal-clear until oxidized, at which time they form a colloidal suspension of red amorphous selenium.

Data were collected with a Sargent-Welch Model XVI polarograph equipped with a thermostated 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. Ten minutes was found to be sufficient time between selenide additions when the reaction temperature in the polarographic cell was 40 °C. Glutathione (purchased from Sigma) was standardized by potassium bromate ($KBrO_3$) titration [9]. Colloidal selenium in 0.1 *M* phosphate buffer (pH 7) was prepared by the air oxidation of selenide; these colloidal solutions were stable for days when protected from light. Colloidal selenium was also standardized by bromate titration [10].

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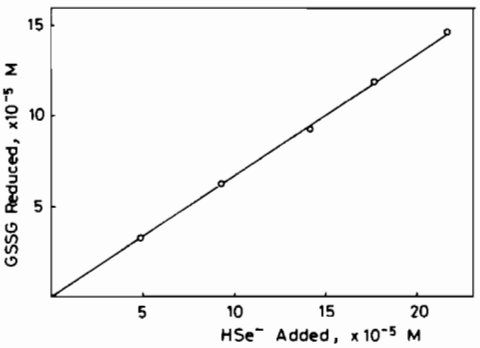

Fig. 1. The addition of selenide aliquots to 1.68×10^{-4} *M* GSSG in 0.1 *M* phosphate buffer (pH 7) at 40 °C; the slope of the resulting regression line and its 95% confidence interval is 0.68 ± 0.04.

Results

The result of adding selenide aliquots to a solution of GSSG in the polarographic cell is summarized in Fig. 1. This reaction was monitored by measuring both the polarographic waves of GSSG and GSH, but could not be monitored with the selenide wave. In spite of the fact that the reaction stoichiometry indicated unreacted selenide should be present, the selenide wave was found to be virtually absent; in other words a secondary process was removing selenide from the reaction pathway, an observation consistent with previous studies [4].

The slope of the line in Fig. 1 is 0.68 ± 0.04, which indicates that for every mole of selenide added, 0.68 mol of GSSG was reduced. The slope was not affected by the starting concentrations of GSSG or GSH; the concentration of GSH did not alter the reaction course even when present initially at 5.16×10^{-4} *M* GSH (along with 1.56×10^{-4} *M* GSSG; data not shown). Examining the stoichiometry closer, it can be seen that for every mole of selenide added, 0.68 mol was reduced, leaving 0.32 mol of unreacted selenide. Thus the ratio of elemental selenium produced to selenide unreacted is a constant 2.1 ± 0.1; it is clear that the only factor which determines the extent of oxidation is the selenium/selenide ratio.

Addition of selenide aliquots to a solution of 2.83×10^{-4} *M* colloidal elemental selenium, prepared at an earlier time, showed no effect due to the presence of elemental selenium (data not shown); that is for every mole of selenide added, one mole could be detected polarographically. Under these conditions no interaction between selenide and elemental selenium was noted.

Discussion

Woods and Klayman [4] suggested the reduced selenide activity in reactions like 1 was due to the formation of polyselenides in the following manner:

$$HSe^- + Se^\circ \rightleftharpoons HSe_2^- \quad (2)$$

This seemed logical when using only end-product analysis as did Woods and Klayman, but it is inconsistent with the data in Fig. 1. An equilibrium process like reaction 2 would be expected to trap progressively more selenide as the concentration of elemental selenium rose, in distinction to maintaining a constant selenium/selenide ratio. Another fact militating against polyselenide formation is the lack of a detectable reaction when selenide aliquots were added directly to colloidal selenium. If polyselenide formation was in fact responsible for the reduced selenide activity in reaction 1, it should also have lowered the selenide concentration in a like manner when selenide and colloidal selenium were mixed. It is worth noting Wood [11] cites evidence suggesting that polyselenide formation is a pH dependent process occurring only above pH 8.5. The pH curve of selenide titrated with potassium hydroxide was found by Wood to have a distinct break about pH 8.5, which he attributed to the onset of polyselenide formation. We conclude that polyselenides do not form under the reaction conditions here (pH 7), and that polyselenides are not likely to form as stable species under conditions of biological interest.

At moderate temperature, elemental selenium forms a colloidal suspension of the red amorphous allotrope [12]; such a colloid provides a large surface area for adsorption. Elemental selenium colloids prepared by hydrazine reduction of selenite (SeO_3^{-2}) adsorb species like hydrazine readily, endowing colloidal selenium with an unusual degree of stability [13]; selenium colloids apparently have an unusually strong tendency for adsorption. A trapping of selenide on the surface of freshly generated colloidal selenium can account for the data in Fig. 1. Adsorption would be expected to remove selenide in proportion to the amount of colloidal selenium generated, resulting in a constant ratio of elemental selenium to selenide adsorbed. Why this ratio is approximately 2 is a matter for speculation, but the same ratio can be seen in data taken under considerably different conditions [4]. As a colloid ages, two factors which reduce its capacity for adsorption are the decrease in surface area due to continued particle aggregation, and a filling of the available adsorption sites by whatever species are available. As might be anticipated, mature selenium colloids do not adsorb significant amounts of selenide.

Selenide adsorption is potentially important in several respects. Many reactions involving the selenium/selenide couple may be dominated by selenide

adsorption rather than thermodynamic factors; we found this to be the case while searching for reactions from which to recalculate the electrode potential of this couple [3]. Reactions involving relatively strong oxidants like oxygen still react with adsorbed selenide readily [8]. Adsorption apparently lowers the free energy of selenide to the point that reactions with weak oxidants like GSSG are no longer energetically possible, whereas reactions with strong oxidants remain thermodynamically favorable. Adsorption also has the potential for seriously interfering with the determination of selenide concentration. If a sample undergoes partial oxidation during preparation, much of the remaining selenide could be masked by adsorption; given the speed with which selenide reacts with oxygen [8], this is a very real possibility. Whether selenide adsorption has any biological significance remains to be seen, but in any condition involving selenide oxidation, selenide adsorption by the resulting colloidal selenium should be considered.

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