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Kinetics of the Reaction between Hydrogen Selenide Ion and Oxygen

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Hydrogen selenide ion (HSe^-) reacts with oxygen in the following manner: $HSe^- + 1/2O_2 \rightarrow Se^0 + OH^-$. Interest in the kinetics of this reaction comes from the fact that selenide is an important product in the metabolism of the essential trace element selenium. Using polarography to monitor both selenide and oxygen, we have found the reaction exhibits complex kinetics, including autoaccelerating behavior and the generation of reactive intermediates capable of inducing reactions in other substances present. Probable intermediate species include superoxide, peroxide and polyselenides. The reaction is slow with respect to diffusion controlled reactions, but fast with respect to the time required to prepare solutions for biological study. Selenide concentrations greater than 10^{-6} M *decay to give solutions of predominantly colloidal elemental selenium less than 3 minutes after exposure to atmospheric levels of oxygen.*

Introduction

Hydrogen selenide ion (HSe^-) reacts with oxygen in the following manner:

$$HSe^- + 1/2O_2 \longrightarrow Se^0 + OH^- \qquad (1)$$

In aqueous solution the resulting elemental selenium (Se^0) is an insoluble polymer which initially forms a colloidal suspension of red amorphous selenium; for this reason, it is useful to classify the reaction as a heterogeneous redox polymerization. Interest in the kinetics of this reaction comes from the fact that selenide is an important product in the metabolism of the essential trace elemental selenium [1]. Red blood cells, for example, have been shown to release a selenium metabolite [2] which behaves like exogenously added selenide [3]. It is an interesting question how long selenide persists in an oxygen-rich environment like the blood plasma, and one that has not been addressed. A similar question relates to the common practice of preparing 'selenide' solutions by dissolving sodium selenide (Na_2Se) in oxygen-containing aqueous solution [4–7]. How long does selenide persist under these conditions? By analogy to the closely related oxidation of aqueous sulfide [8], selenide might well be expected to decay slowly over several hours, but selenide was in fact found to disappear much more rapidly.

Using the electrochemical technique of polarography to monitor both selenide and oxygen concentrations, we have found the reaction between selenide and oxygen to be remarkably complex in its kinetics, showing an autoaccelerating pattern of behavior (see Fig. 1). The reaction is slow compared to diffusion controlled reactions, but fast with respect to the time required to prepare a solution for biological study. Selenide solutions above 10^{-6} *M* decay quickly, resulting in solutions which are predominantly colloidal selenium less than 3 minutes after exposure to atmospheric levels of oxygen; when elemental selenium is present initially, the rate of decay is even faster. It is worth nothing that commercially available sodium selenide contains appreciable levels of elemental selenium. The reaction can induce reactions in other substances present, a point of potential practical interest, and probably proceeds via a free radical chain mechanism.

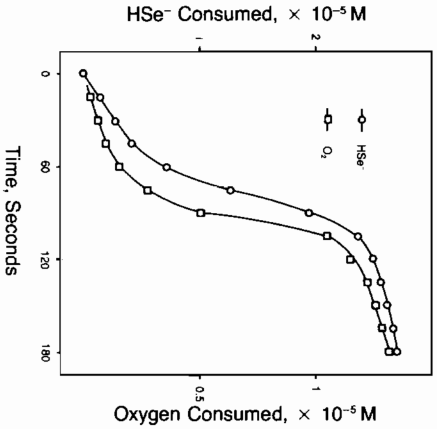

Fig. 1. Relative rates of disappearance of selenide and oxygen on addition of 3.00×10^{-5} *M* HSe^- to a 0.1 *M* phosphate buffer (pH 7) at 25 °C which contained 4.63×10^{-5} *M* dissolved oxygen; selenide was monitored polarographically at +0.05 V, and oxygen at −0.80 V.

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Experimental

Methods

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 [9]; 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 small volumes (*e.g.* 100 ml) required only tens of mg aluminum selenide, largely reducing the hazards associated with hydrogen selenide generation. 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 oxygen; on oxidation, selenide forms a colloidal suspension of red amorphous elemental selenium.

Selenide has a well-defined polarographic wave [10], as does oxygen; in the region of positive voltages (*e.g.* +0.05 V), the selenide wave can be monitored without interference from oxygen. Selenide was added from the buret to an oxygen-containing buffer solution in the polarographic cell, and the resulting disappearance of selenide monitored with time by maintaining the polarographic voltage at +0.05 V; the concentration of selenide subjected to rate analysis was from 1×10^{-6} *M* to 4×10^{-5} *M*. The rate of disappearance of oxygen was recorded by monitoring the solution at a voltage which reflected only oxygen concentration (*e.g.* –0.70 V); as the noise level was found to be much higher when monitoring oxygen disappearance, the oxygen wave was not routinely followed. Data was collected with 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.

Treatment of Data

The initial rate law for the reaction can be written phenomenologically as:

$$\text{rate} = k[HSe^-]^x[O_2]^y \qquad (2)$$

where k is the rate coefficient, x is the reaction order in selenide, and y is the reaction order in oxygen. As described later, y was found to be zero order when oxygen was present at atmospheric concentrations. For this condition, equation 2 may be formulated as:

$$\ln \text{rate} = x \ln [HSe^-] + \ln k \qquad (3)$$

By collecting initial rates at several different selenide concentrations, the data can be fitted to a straight line: the slope from this plot will give the apparent reaction order in selenide, and the intercept will give the natural log of the rate coefficient. The most likely straight line and the 95% confidence interval associated with a given collection of data points was determined with a simple linear least squares computer program.

Results

Figure 1 shows the relative rates of selenide and oxygen disappearance when oxygen is present in comparable concentrations to selenide. Both selenide and oxygen show an initial rate of disappearance followed by a period of increasing rate; this pattern of 'autoacceleration' is a familiar one in polymerization reactions which involve free radical chain mechanisms [11]. Autoacceleration is also seen in the oxidation of selenide by disulfides (data not shown). LaMer and Kenyon [12] observed autoacceleration in the formation of the closely related polymer, elemental sulfur, from the acid decomposition of thiosulfate:

$$S_2O_3^{-2} + H^+ \longrightarrow HSO_3^- + S^0 \qquad (4)$$

These facts indicate autoacceleration is associated with the formation of the polymer structure. Factors influencing the rate of the reaction are discussed below.

Elemental Selenium

In aqueous solution at moderate temperature, elemental selenium initially forms a colloidal suspension of the red amorphous allotrope [13]; this is in distinction to the more thermodynamically stable allotrope of grey hexagonal selenium. Amorphous selenium is a polymer composed primarily of long linear chains of selenium atoms [15]. When colloidal selenium is present at the start of the reaction between selenide and oxygen, the initial rate of selenide disappearance is increased dramatically. This effect is influenced by the state of aggregation of the elemental selenium colloid and is difficult to quantitate. Reproducible data on the initial rate of reaction clearly depends on the ability to consistently prepare selenide solutions with very low levels of elemental selenium present.

Light

Exposing the reaction mixture to the light provided by a 100 watt bulb held next to the glass polarographic cell was found to increase the reaction rate compared to normal laboratory light. This effect was not due to an increase in the temperature of the reaction mixture which was adequately thermostatted. Running the reaction in a darkened cell gave essentially the same results as normal lab illumination.

Oxygen

As shown in Table I, the reaction with selenide was found to be zero order with respect to oxygen

TABLE I Rate Coefficients and Reaction Orders for the Reaction between Selenide and Oxygen.

Variable	ln k[a]	x[b]	n[c]
Standard Conditions[d]	1.49 ± 0.05	0.9	22
1.1×10^{-4} M O_2	1.5 ± 0.1	0.9[e]	10
5.6×10^{-5} M O_2	1.1 ± 0.1	0.9[e]	10
10 °C	0.41 ± 0.06	0.7	14
30 °C	2.22 ± 0.05	1.1	12
36 °C	2.78 ± 0.07	1.2	12
0.05 *M* Phosphate	0.9 ± 0.1	0.9	14
0.30 *M* Phosphate	2.84 ± 0.07	1.1	15
with 0.46 *M* NaCl	2.08 ± 0.05	0.9	15
3.4×10^{-7} *M* SOD[f]	1.4 ± 0.1	1.3[e]	12
1.2×10^{-6} *M* BSA[g]	0.8 ± 0.1	0.9	10
2.3×10^{-6} *M* BSA[g]	1.15 ± 0.08	0.9	12
0.1 *M* Mannitol	1.25 ± 0.06	0.9	11

[a] k is the rate coefficient in units of 10^{-5} M^{-1} $\sec^{-1}$; the 95% confidence interval follows the value of ln k. [b] x is the reaction order in HSe^-; the 95% confidence interval for x is ±0.1 unless otherwise noted. [c] n is the number of determinations in the regression line. [d] Standard Conditions were air-saturated 0.1 *M* phosphate buffer (pH 7) at 20 °C. [e] 95% confidence interval of ±0.2. [f] Superoxide dismutase (32,500 daltons). [g] Bovine serum albumin (66,200 daltons).

above a concentration of 1.1×10^{-4} *M* oxygen. As the concentration of oxygen is decreased, however, the reaction rate shows a dependence on oxygen. The bulk of the experimental data was taken under conditions where oxygen was present in excess: air-saturated buffers were used and the polarographic cell left open to the atmosphere. This simplified the acquisition of data as the oxygen concentration did not have to be adjusted or monitored; it also reflects the circumstances associated with many biological studies.

Temperature and Salt

The effect of both temperature and salt concentration is shown in Table I. The fact that both the rate coefficient and the apparent reaction order increase with temperature is an indication of the complexity of the processes involved in the reaction mechanism (data has been adjusted for the temperature dependence of the polarographic wave [15]). Like temperature, the effect of buffer concentration is complex. Increasing the concentration of the 0.1 *M* phosphate buffer to 0.3 *M* increases both the rate coefficient and the reaction order, whereas adding 0.46 *M* sodium chloride to the 0.1 *M* phosphate buffer causes an increase only in the rate coefficient. The basis of these effects is not known.

Superoxide Dismutase

Oxygen is mechanistically constrained to 1-electron transfers [16]. This suggests the initial reaction between selenide and oxygen leads to the formation of the superoxide radical (O_2^-), as shown below:

$$HSe^- + O_2 \rightleftharpoons HSe^\bullet + O_2^- \tag{5}$$

Superoxide is a relatively stable intermediate, and is known to decay primarily by dismutation in aqueous solution [16]; the enzyme superoxide dismutase (SOD) influences reactions producing superoxide by catalyzing the dismutation of superoxide [17]. Reaction 5 suggests faster removal of this intermediate would be expected to increase the rate of selenide disappearance, and as seen in Table I, SOD was found to increase the reaction order in selenide dramatically. For the collection of this data, bovine SOD (purchased from Sigma; catalog number S8254) was present at a concentration of 30 units SOD/ml associated with 0.011 mg protein/ml. SOD has sulfide bonds which may be capable of directly oxidizing selenide, but the protein level was kept low to avoid this possibility; other proteins with disulfide bonds, like bovine serum albumin (BSA), do not show an effect on the reaction order even when present at much higher concentrations (see Table I). The dramatic increase in the reaction order seen with SOD is most likely due to its ability to catalyze the dismutation of superoxide, but it should be emphasized this is only indirect evidence for the involvement of superoxide.

Table I shows that as BSA concentration increases, so does the disappearance of selenide; this is due to the reaction of selenide with the disulfide bonds present in BSA, a reaction which also occurs in the absence of oxygen. The cause of the decrease rate at lower BSA concentrations is a matter of conjecture, but a similar slowing of the rate is also observed when mannitol is present. It is likely the slower rates are due to the trapping of reactive intermediates in alternate reactions available when these compounds are present.

Peroxide

Peroxide is normally formed as a result of the dismutation of superoxide [16]. Selenide added to peroxide containing solutions was found to be completely consumed in the time required for a single mercury drop (less than 4.60 sec). This suggests that if peroxide is formed as an intermediate during the course of the reaction between selenide and oxygen, the peroxide is consumed rapidly and does not contribute to the rate determining steps.

Induced Reactions

Induced reactions are associated with mechanisms which form reactive intermediates [18]. While not specific for only free radical intermediates, induced reactions often occur in free radical reactions. The reaction of selenide with oxygen was found to induce reactions in several diverse compounds, including ascorbate, EDTA (ethylenediaminetetraacetic acid), and the aromatic amino acid tryptophan. None of these compounds was found to react directly with selenide. In the case of tryptophan, the presence of 6.1×10^{-5} M tryptophan does not alter the reaction rate between selenide and oxygen in 0.1 M phosphate (pH 7) at 20 °C (data not shown). However, tryptophan fluorescence, excited at 220 nm and monitored at 350 nm, is quenched during the reaction, and does not return to prereaction levels. Tryptophan reaction products can be found using thin layer chromatography: Eastman cellulose plates were developed with n-butanol/acetic acid/water, in the ratio of 12/3/5 by volume, in a sandwich-type chamber. Tryptophan was identified by its fluorescence under UV light ($R_f = 0.56$), and the reaction products were detected with 1% ninhydrin spray ($R_f = 0.48$).

Discussion

Several lines of evidence indicate the reaction between selenide and oxygen proceeds via a free radical chain mechanism: the pattern of autoacceleration is often characteristic of free radical chain polymerizations [11]; the induced reactions seen with ascorbate, EDTA, and tryptophan are characteristic of a mechanism with reactive intermediates like free radicals; and the effect of the enzyme SOD suggests the superoxide radical is present during the reaction. In addition, the closely related reaction between aqueous sulfide and oxygen is thought to proceed via a free radical chain mechanism [8, 19]; Semenov [20] has also proposed a chain mechanism for the gas phase reaction between hydrogen sulfide and oxygen.

It is possible to construct a number of hypothetical free radical chain mechanisms for the reaction, but these mechanisms are based mostly on conjecture and are therefore not discussed here. Several points concerning possible intermediates are worth noting, however. It can be seen from Fig. 1 that selenide is initially consumed at a faster rate than oxygen. One explanation for this is that polyselenides are formed in the following manner:

$$\mathrm{HSe^- + Se_x{}^0 \longrightarrow HSe_{x+1}{}^-} \qquad (6)$$

Polyselenides are logical species to suspect in the transition from selenide monomers to selenium polymer. Relatively little is known about polyselenides, but they are known to be easily oxidized [21]. It is tempting to attribute autoacceleration to the formation of polyselenides which subsequently react at a faster rate with the oxidant present; this is consistent with the effect of colloidal selenium in dramatically increasing the reaction rate.

In summary, the reaction of selenide with oxygen is complex in its kinetics, demonstrating autoaccelerating behavior and generating reactive intermediates. The intermediate steps in the reaction subsequent to selenide disappearance remain an area of potential research, but probable intermediates include superoxide, peroxide and polyselenides. It is hoped that elucidation of the kinetics of this reaction will aid in the understanding of the biochemical behavior of selenide.

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