Catalytic Oxidation of Glutathione and Other Sulfhydryl Compounds by Selenite

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(Received for publication, April 9, 1958)

In studies of the oxidation of sulfhydryl compounds (1), selenite has been found to be an active catalyst. Considering the biological importance of sulfhydryl oxidations and the implication of sulfhydryl compounds in selenium poisoning (2-5), more information on selenite-catalyzed oxidations is desirable. This paper reports studies of the catalytic oxidation of GSH by selenite and its reaction mechanism.

EXPERIMENTAL

Oxygen absorption was measured manometrically. After equilibration to 37° the manometer was closed to the atmosphere and the reaction was initiated by adding the solutions of catalyst and buffer from the side arms to the solution of sulfhydryl compound in the flask. All chemicals except CoA were analytical reagent or c.p. grade. The cysteine hydrochloride was purchased from the Fisher Scientific Company, GSH from Schwarz Laboratories, and CoA (approximately 75 per cent) from the Nutritional Biochemicals Corporation. Cysteine and GSH were chromatographically pure. Dihydrolipoic acid was a gift from Dr. J. A. Brockman of the American Cyanamid Company. Water was twice distilled in glass. Selenite and GSH were allowed to react by the method of Klug et al. (6) and the products were chromatographed on paper with the use of n-butanol-acetic acid-water, 50:25:25 by volume.

RESULTS

Catalysis of Oxidation of Sulfhydryl Compounds by Selenite and Other Metallic Salts—After preliminary comparisons of the catalytic activity of various metallic compounds, Na₂SeO₃, CuCl₂, FeCl₃, MnSO₄, and CoCl₂ were found to be the most active catalysts for the oxidation of sulfhydryl compounds. Results for the catalytic oxidation of cysteine, GSH, CoA, and dihydrolipoic acid are presented in Table I. The initial rate of cysteine oxidation was greater for Fe³⁺⁺ catalysis than for Cr³⁺⁺ catalysis (Table I); however, after 10 minutes, the rates for Fe³⁺⁺ and Cu⁺⁺ were 8.0 μl. of O₂ per minute and 12.6 μl. of O₂ per minute, respectively. Selenate and selenite were equally active catalysts for GSH oxidation. For GSH oxidation as given in Table I, selenate catalysis gave an initial rate of 19.1 μl. of O₂ per minute compared to 18.2 μl. of O₂ per minute for selenite.

Effect of Selenite Concentration, pH, and Temperature—Study of the catalytic oxidation of GSH over a wide range of selenite concentration showed (Table II) that the rate of oxidation was a function of selenite concentration up to 0.01 mole of selenite per mole of GSH. Loss of catalytic activity at the higher concentrations of selenite was caused by its reduction to metallic selenium which is not a catalyst. This formation of metallic selenium is readily seen as a brown color and suspended red particles. The data in Table III show that the oxidation of GSH by selenite increases with increasing pH, and it is inhibited at pH 4.2. At higher pH values, above 8.4, the rate of the oxidation decreases because of the formation of metallic selenium which is inactive as a catalyst.

The rate of oxidation increased as a function of temperature. GSH of 4.9 × 10⁻⁷ m in 0.1 m phosphate buffer, pH 7.1, was catalytically oxidized by 0.01 mole of selenite per mole of GSH at rates of 9.0, 13.0, and 18.0 μl. of O₂ per minute at temperatures of 17°, 27°, and 37°, respectively. From the Arrhenius relationship, the activation energy was found to be 5.9 kilocalories per mole. In a similar reaction system, 0.01 mole of CuCl₂ per mole of GSH gave oxidation rates of 0.4, 0.9, and 1.7 μl. of O₂ per minute at temperatures of 17°, 27°, and 37°. The higher activation energy of 12.2 kilocalories per mole for Cu⁺⁺ indicates that it is not as good a catalyst as selenite.

Inhibition of Selenite Catalysis—Tellurite and sulfite, salts of members of Group VI in the periodic table with selenium, were evaluated as inhibitors of selenite catalysis. Tellurite at equimolar concentrations was a good inhibitor (Table IV). Considering the chemical similarities, tellurite might compete directly with selenite. Tellurite also catalytically oxidized GSH, but its activity was much less than that of selenite. Sulfite did not inhibit selenite catalysis or catalyze GSH oxidation. When arsenite was allowed to react with GSH for 30 minutes before the addition of selenite, GSH oxidation was inhibited (Table V). However, when arsenite was first added to selenite before the addition of GSH, there was little inhibition of the oxidation. A compound of arsenite and GSH might be the active inhibitor of selenite catalysis. The mechanism of this inhibition was not studied in detail.

Studies of Active Compound and Product Formed from Glutathione and Selenite—Chromatography of the products of the reaction of 1 mole of selenite and 4 moles of GSH in dilute HCl solution showed two components (Fig. 1). After ninhydrin treatment, the GSSG spot was purple and the spot for GS-Se-SG with the use of starch chromatography. It is also in accord with the views of Klug and Peterson (7) who separated the reaction products of GSH and selenious acid into components corresponding to GSSG and GS-Se-SG with the use of starch chromatography. It is also in accord with the views of Klug and Peterson (7) who separated the reaction products of GSH and selenious acid into components corresponding to GSSG and GS-Se-SG with the use of starch chromatography. It is also in accord with the views of Klug and Peterson (7) who separated the reaction products of GSH and selenious acid into components corresponding to GSSG and GS-Se-SG with the use of starch chromatography.
TABLE I

Effect of metal catalysts on rate of oxidation of sulfhydryl compounds

<table>
<thead>
<tr>
<th>Sulfhydryl compound</th>
<th>Phosphate buffer</th>
<th>pH</th>
<th>Initial rate for catalysts at 0.01 mole per mole of sulfhydryl compound</th>
<th>μl. O₂/min.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CuCl₂</td>
<td>FeCl₃</td>
</tr>
<tr>
<td>4.89 × 10⁻⁴ M cysteine</td>
<td>0.1</td>
<td>7.23</td>
<td>16.3</td>
<td>21.1</td>
</tr>
<tr>
<td>4.89 × 10⁻⁴ M GSH</td>
<td>0.1</td>
<td>7.26</td>
<td>2.4</td>
<td>0.9</td>
</tr>
<tr>
<td>9.61 × 10⁻⁴ M dihydrolipoic acid</td>
<td>0.21</td>
<td>7.00</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>7.85 × 10⁻⁴ M CoA</td>
<td>0.14</td>
<td>7.38</td>
<td>0.9</td>
<td>0.3</td>
</tr>
</tbody>
</table>

TABLE II

Effect of concentration of selenite on oxidation of GSH

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Initial</th>
<th>Color of reaction mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>mole Na₂SeO₃/mole GSH</td>
<td>μl. O₂/min.</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.3</td>
<td>Very pale yellow</td>
</tr>
<tr>
<td>0.0025</td>
<td>4.0</td>
<td>Yellow, red particles</td>
</tr>
<tr>
<td>0.0050</td>
<td>11.0</td>
<td>Brown, red particles</td>
</tr>
<tr>
<td>0.010</td>
<td>18.0</td>
<td>Yellow, red particles</td>
</tr>
<tr>
<td>0.10</td>
<td>10.0</td>
<td>Yellow, red particles</td>
</tr>
<tr>
<td>0.20</td>
<td>0.8</td>
<td>Brown, red particles</td>
</tr>
<tr>
<td>0.40</td>
<td>0.0</td>
<td>Brown, red particles</td>
</tr>
</tbody>
</table>

TABLE III

Effect of pH on selenite catalysis of GSH oxidation

<table>
<thead>
<tr>
<th>pH (0.1 M buffer)</th>
<th>Rate of oxygen absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>μl. O₂/min.</td>
<td></td>
</tr>
<tr>
<td>4.19 (Phosphate)</td>
<td>0</td>
</tr>
<tr>
<td>6.60 (Phosphate)</td>
<td>13.0</td>
</tr>
<tr>
<td>7.18 (Phosphate)</td>
<td>18.0</td>
</tr>
<tr>
<td>8.38 (Tris)</td>
<td>18.0</td>
</tr>
<tr>
<td>8.89 (Borate)</td>
<td>13.0</td>
</tr>
<tr>
<td>9.56 (Borate)</td>
<td>8.8</td>
</tr>
</tbody>
</table>

*The catalyst was 0.01 mole of selenite per mole of GSH.
†Tris = tris(hydroxymethyl)aminomethane.

TABLE IV

Inhibition of selenite catalysis by tellurite

<table>
<thead>
<tr>
<th>Selenite concentration</th>
<th>Tellurite concentration</th>
<th>Initial rate</th>
<th>μl. O₂/min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>mole/mole GSH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>0</td>
<td>18.0</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>0.01</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>0.05</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.01</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.05</td>
<td>4.5</td>
<td></td>
</tr>
</tbody>
</table>

* Selenite and tellurite were first mixed together in the side arm of the flask, and then they were added to the flask containing the GSH solution to initiate the oxidation.

FIG. 1. Diagrammatic representation of the paper chromatograms showing GSSG and GSH-Se-SG. Column 1, the sample taken during selenite-catalyzed GSH oxidation; Column 2, products of the reaction of selenite and GSH; Column 3, pure GSSG.

Similarly, by paper chromatography the final product of the oxidation was identified as GSSG. The stoichiometry of oxygen absorption showing an uptake of 0.87 mole of O₂ per 4 moles of GSH is in agreement with this result. Mixing pure GSSG with selenite and oxygen gave no further reaction.

DISCUSSION

The catalytic oxidation of cysteine by Cu²⁺ and Fe³⁺ has been extensively studied, especially by Warburg (9). Comparison of selenite with Cu²⁺ and Fe³⁺ shows that it is also a good catalyst for cysteine oxidation. Before this study was undertaken, Cu²⁺ was known as the best catalyst for GSH oxidation (10, 11).
Thus, it was interesting to find that selenite was a far better catalyst.

Although it is well known that CoA and dihydrolipoic acid will undergo oxidation in a neutral or alkaline solution in the presence of heavy metals, this is the first intercomparison with other sulfhydryl compounds and a number of metal ions. In this comparison selenite was found to be a good catalyst for dihydrolipoic acid and CoA oxidation.

Because selenite is a most active catalyst for GSH oxidation, it is interesting to consider this reaction further. Painter (12) proposed that the general reaction between sulfhydryl compounds and selenious acid is as shown in Diagram 1.

\[
4RSH + H_2SeO_3 \rightarrow RSSR + RS-Se-SR + 3H_2O \\
\downarrow \\
RSSR + Se
\]

Diagram I

Klug and Petersen (8) and Petersen (7) found this reaction sequence to apply in reactions of cysteine and GSH with selenious acid. Although this reaction sequence accounts for the oxidation of 4 moles of GSH per mole of selenite, the mechanism for the oxidation of large amounts of GSH by molecular oxygen in the presence of catalytic amounts of selenite is unknown. From the results of this study showing that the catalytic oxidation of GSH by selenite proceeds through the formation of GS-Se-SG, an over-all reaction may be written (Equations 1 and 2).

\[
4GSH + SeO_3^{2-} \rightarrow GSSG + GS-Se-SG \\
+ 2OH^- + H_2O \\
2OH^- + GS-Se-SG + O_2 \rightarrow GSSG + SeO_3^{2-} + H_2O
\]

The ionic reaction in Equation 1 should proceed spontaneously. In Equation 2 the hydroxyl ion is not only a direct reactant but it can also increase the rate by ionization of the sulfhydryl group of GSH. Although Equation 2 represents an over-all reaction for GS-Se-SG oxidation the reaction mechanism must be more complex. The effect of pH on GSH oxidation is much too small for Equation 2 to be the rate-determining reaction.

Results of this study show some interesting correlations with the known biochemistry of selenium poisoning. After injection or oral ingestion of selenite, the experimental animal typically shows losses of GSH from the blood and organs (2–5). These decreases of GSH could be caused by its catalytic oxidation by selenite. Arsenite is effective in countering selenium poisoning (3), and this might be related to its inhibition of selenite-catalyzed GSH oxidation.

SUMMARY

1. Comparison with the action of Cu++, Fe++, Co++, and Mn++ showed that selenite was a good catalyst for the oxidation of cysteine, dihydrolipoic acid, and Coenzyme A, and was most active for glutathione oxidation.

2. Glutathione oxidation was a function of selenite concentration; the rate increased with increasing pH and the activation energy was 5.9 kilocalories per mole.

3. Tellurite and arsenite inhibited selenite catalysis.

4. Selenium diglutathione was an active intermediate in the oxidation.

Acknowledgment—The advice of Professor E. P. Painter is appreciated.

REFERENCES


Catalytic Oxidation of Glutathione and Other Sulfhydryl Compounds by Selenite
C. C. Tsen and A. L. Tappel


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