The Effect of Some Cystine Derivatives on Catalase Activity*

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The decreased liver catalase activity of animals bearing tumors is well known (17, 18). However, various explanations of this effect have been offered. One of these, as exemplified by the studies of Nakahara et al. (11, 12, 23–26), postulates that tumors release a factor designated as toxohormone, which exerts its effect on liver catalase. A similar view is also taken by other investigators (15, 16, 18).

An alternate suggestion is that the discussed inhibition is due to some aberrancy in cystine metabolism by tumor tissues. These show a marked increase in alkali-labile sulfur (18), and the level of cystine has been found by Schweigert et al. (29) to be present in markedly enhanced concentration in liver hepatomas as compared with adjacent normal tissue. The intraperitoneal administration of cystine also lowers liver catalase activity in vivo (30), but no increased blood level of this amino acid was noted in tumor-bearing rats.

Hargreaves and Deutsch (11) have reported that tumor "Kochsafts" are very active in inhibiting catalase in vitro as compared with those of other tissues. This finding has been criticized by Endo et al. (9), who reported that other tissue "Kochsafts" likewise inhibited catalase strongly. They suggest that this activity is due to sulfhydryl compounds. However, it was found that, whereas iodoacetate would reverse the in vitro inhibitory effect of cysteine on catalase, it was ineffective in reducing the activity of tumor "Kochsafts" (30).

The indicated aberrancy in cystine metabolism by tumors and the known inhibitory action of cysteine on catalase activity prompted an investigation on the effect of various known cystine metabolites on this enzyme. Such compounds might undergo marked elevation in liver tissue as the result of an increased rate of metabolism of this amino acid.

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MATERIALS AND METHODS

Beef liver catalase, crystallized six times and prepared by acetone fractionation (8), was utilized as the source of enzyme in in vitro experiments. It was found to be essentially pure, as evaluated by electrophoretic and ultracentrifugal assay. Its hydrogen peroxide-destroying activity, as evaluated by the method of Bonnichsen, Chance, and Theorell (4), varied between 2.2 and 2.7 × 10^7 liters/mole/sec. As in a previous investigation (11), the amount of catalase giving a first-order reaction constant of 1 × 10^-4 was designated as 1 unit. The dissociation constants of various catalase-inhibitor complexes were calculated from general equilibrium equations.

The cystine derivatives used in the various in vitro and in vivo experiments were cysteine, cystine disulfoxide (18), cysteine sulfinic acid (18), cysteine sulfonic acid (21), 2-amino-ethane-sulfinic acid (2, 14, 27, 33), cysteic acid, taurine, and the decomposition products resulting from the action of sodium sulfite on cystine disulfoxide.

The rapid autoxidation of cysteine and sulfonic acids makes difficult the preparation of standard solutions for experimental work. Such type compounds were always prepared in 0.01 M sodium sulfite, which prevents oxidation of the above. This level of sodium sulfite showed no appreciable in vitro or in vivo effects on catalase in control experiments.

Cysteine concentrations were determined by the method of Folin and Marenzi (10). Ascending paper chromatography experiments on Whatman No. 1 paper with the use of a saturated phenol-water (100:40 volumes) solvent was employed to determine the relative purity of compounds such as cysteine, cystine, cystine disulfoxide, and the sulfonic acids. The chromatograms were developed with ninhydrin.

RESULTS

In vitro studies.—Various levels of inhibitors and catalase in 0.1 M, pH 6.8, potassium phosphate buffer were incubated at 0–2° C. for 60–90 minutes. Sodium sulfite was present at a level of 0.01 M, as previously mentioned. The period of incubation employed was found sufficient to give maximum inhibition in all cases. These mixtures were then assayed for catalase activity, which was interpreted as free enzyme by reference to a control catalase activity-concentration curve of the type presented in Chart 1.

The results of the experiments with cysteine are shown in Chart 2. The value of the dissociation constant of cysteine-catalase was nearly 4 × 10^-5.

† The authors wish to acknowledge with thanks the helpful advice of Dr. Kiyoo Satoh during the preparation of these compounds.
This may be compared with the values for the analogous complexes of catalase with cyanide, 2- amino-ethane and cysteine sulfinic acids, and the products of the decomposition of cystine disulf oxide by sodium sulfite (see Table 1). It is apparent that cysteine was the strongest inhibitor of the cystine derivatives studied, although it was weaker than cyanide. Our value for the dissociation constant of the cyanide-catalase complex was somewhat lower than that of other investigators (6, 7, 20, 22, 32, 34). The value of $4 \times 10^{-8}$ for the dissociation of the cysteine-catalase complex is much lower than the $1 \times 10^{-3}$ for sulfhydryl groups reported by Stern (32). However, as previously mentioned, the failure to take into consideration the rapid loss of cysteine by oxidation may account for the higher values of the older literature.

The products resulting from the decomposition of cystine disulf oxide by sodium sulfite were distinctly stronger inhibitors than the expected sulfinic acid derivative. In analogy with the postulated decomposition of cystine disulf oxide and cystine by cyanide (21) and of cystine by sodium sulfite (5), the following reactions might be proposed for cystine disulf oxide with sodium sulfite.

\[
\text{R-S-S-R + Na}_2\text{SO}_3 \rightarrow \text{R-S-O-Na + R-S-S-O-Na}
\]

\[
\text{R-S-O-Na + R-S-S-O-Na}
\]

\[
\rightarrow \text{Sulfenate}
\]

\[
\rightarrow \text{Sulfinate}
\]

The paper chromatograms of the cystine disulf oxide-sodium sulfite reaction mixtures gave two ninhydrin-reactive spots. One of these had an $R_s$ close to that of the sodium salt of cysteine sulfinic acid ($R_s 0.2$), but the second was not identifiable in terms of the other cystine derivatives. The possibility of its being cysteine sulfenate is indicated by the above postulated equations. This very labile compound should be a strong inhibitor of catalase and, if formed, might be maintained in part by the presence of sodium sulfite. It will be pointed out later that other reactions of the cystine disulf oxide-sodium sulfite reaction products indicate that the catalase inhibition is not due to the sulfinate.

Mercaptoethanol and mercaptoaetic acid inhibit catalase to the same degree as cysteine. Neither cysteic acid nor taurine shows any activity, in agreement with previous findings (30).

Spectrophotometric studies.—A marked change in the absorption spectrum of catalase was seen...
following its reaction with cysteine. Of particular interest was the marked decrease in absorption in the region of the Soret band. The general characteristics of the curve are similar to those of catalase-tumor "Kochsaft" mixtures (19). If one assumes that the minimum value of the 405 m\(\mu\) optical density at high cysteine-catalase ratios is due entirely to this complex, the concentration of free enzyme at different cysteine:catalase ratios may be calculated from the following equations:

\[
\begin{align*}
(1) \quad FE_0 + BE_d &= E_{\text{read}}. \\
(2) \quad T &= F - B \\
(3) \quad F &= \frac{E_{\text{read}.} - TE_d}{E_a - E_d},
\end{align*}
\]

where \(T\) = moles of total enzyme, \(F\) = moles of free enzyme, \(B\) = moles of bound enzyme, \(E_a\) = molar extinction of the free enzyme at 405 m\(\mu\), and \(E_d\) = molar extinction of the bound enzyme at 405 m\(\mu\), and \(E_{\text{read}.}\) = optical density of mixture of enzyme and inhibitor.

Data from experiments on the determination of the dissociation constant of the catalase-cysteine complex by the spectrophotometric and activity methods are shown in Table 2. The results are in good agreement.

Cysteine sulfinic acid, 2-amino-ethane sulfinic acid, and the reaction products of cystine disulfoxide and sodium sulfite did not affect the absorption spectrum of catalase in the 350-520 m\(\mu\) range.

The effect of formol and iodoacetate on the inhibitory effect of various cystine derivatives.—Formol reacts with cysteine forming a thiazolidine derivative and thus effectively neutralizes the activity of the sulfhydryl group. Iodoacetic acid exerts the same effect through its well known alkylating reaction.

Solutions of formol and iodoacetate were reacted with the various cysteine derivatives in M/15 potassium phosphate buffer at pH 6.8. Sodium sulfite was included in the reaction mixture in all cases, since it was a component of the cystine disulfoxide decomposition reaction. Appropriate control experiments with the sodium sulfite were always performed. Under the conditions used, the reactions of formol and iodoacetate with cysteine had gone to completion at room temperature in 25 and 75 minutes, respectively. These reaction mixtures were then incubated with catalase for 1-2 hours at 0-2°C. following which interval the usual activity determination was performed. Data typical of experiments with formol and iodoacetate are presented in Tables 3 and 4, respectively. It can be seen that formol reversed the catalase-inhibiting action of all the compounds tested, as well as that of the tumor extracts. In control experiments it was seen that formol enhanced catalase activity in some cases.

In keeping with previous studies, iodoacetate was found to inhibit catalase slightly, to partially reverse the catalase-inhibiting action of cysteine, and to show little effect on the activity of the tumor extract (30). A further finding was the ability of iodoacetate to reverse the inhibitory action of the sodium sulfite decomposition products of cystine disulfoxide. Interestingly, iodoacetate enhanced the inhibitory action of cysteine sulfinic acid.

In vivo effects of various cystine derivatives on liver catalase activity.—Various compounds in M/15, pH 7.2, potassium phosphate buffer were administered by intraperitoneal injection to 150-190-gm. male rats. The low solubility of cystine and cystine disulfoxide necessitated their administration in the form of fine suspensions. The rats were killed by decapitation after stated intervals, the livers perfused with isotonic NaCl and homogenized in 8 volumes of distilled water. Catalase
activity and dry weight determinations were then carried out on the homogenates. The activity measurements were made within several hours, although no changes were noted in homogenates that were allowed to stand for 24 hours at room temperature. Freezing and thawing of the homogenates result in enhanced catalase activity, but these procedures were not employed, since variable results were obtained. A summary of the average period. Cysteine sulfonic acid would appear to be a stronger in vitro inhibitor than the 2-amino-ethane derivative, because it is converted to the latter inhibitory compound on its metabolic pathway to taurine.

**DISCUSSION**

The work reported is directed toward the general problem of determining whether the known effects of cystine and some of its derivatives is shown in Table 5. The detailed data are plotted in Chart 3 to show the individual variations encountered.

A considerable variation in the liver catalase activity within a given group is indicated. With the exception of 2-amino-ethane sulfonic acid, all the compounds tested except the sulfonic acid derivatives showed greater liver catalase-depressing activity than cystine. The former compound is converted to inactive taurine by rat liver (3) and shows a definite inhibitory effect only at the 2-hour effects of cystine in depressing liver catalase activity are related to the analogous in vivo effects of tumors or to the in vitro effects of tumor "Kochsafts."

Sulfhydryl compounds of the type exemplified by cysteine are strong inhibitors of catalase. Our value of $4 \times 10^{-4}$ for the dissociation constant of the catalase-cysteine complex may be compared with a value of $8 \times 10^{-7}$ for catalase cyanide. Relatively strong catalase inhibition was also shown by the sulfonic acid and the sodium sulfite decomposition products of cystine disulfoxide. The in-

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**TABLE 3**

<table>
<thead>
<tr>
<th>INHIBITOR</th>
<th>MOLAR CONC.</th>
<th>CATALASE ACTIVITY ($K \times 10^{10}$) IN PRESENCE OF</th>
<th>FORMOL*+ INHIBITOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Formol*</td>
</tr>
<tr>
<td>Cysteine</td>
<td>1.25 x 10^{-4}</td>
<td>0.80</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>5.0 x 10^{-4}</td>
<td>1.07</td>
<td>1.05</td>
</tr>
<tr>
<td>Cysteine sulfonic acid</td>
<td>1.0 x 10^{-4}</td>
<td>0.96</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>1.0 x 10^{-4}</td>
<td>0.96</td>
<td>0.97</td>
</tr>
<tr>
<td>Cystine disulfoxide- Na2SO3 reaction products</td>
<td>5.0 x 10^{-3}</td>
<td>0.96</td>
<td>0.97</td>
</tr>
<tr>
<td>Tumor &quot;Kochsaft&quot;</td>
<td>1.18</td>
<td>1.30</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* Final concentration of formol was $1 \times 10^{-2}$ M.

**TABLE 4**

<table>
<thead>
<tr>
<th>INHIBITOR</th>
<th>MOLAR CONC.</th>
<th>CATALASE ACTIVITY ($K \times 10^{10}$) IN PRESENCE OF</th>
<th>IODOACETATE+ INHIBITOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Iodoacetate*</td>
</tr>
<tr>
<td>Cysteine</td>
<td>5 x 10^{-4}</td>
<td>0.82</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>1 x 10^{-4}</td>
<td>0.60</td>
<td>0.62</td>
</tr>
<tr>
<td>Cysteine sulfonic acid†</td>
<td>1 x 10^{-4}</td>
<td>0.82</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>5 x 10^{-4}</td>
<td>0.82</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>5 x 10^{-4}</td>
<td>0.76</td>
<td>0.68</td>
</tr>
<tr>
<td>Cystine disulfoxide- Na2SO3 reaction products</td>
<td>2.5 x 10^{-4}</td>
<td>0.79</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>2.0 x 10^{-4}</td>
<td>0.79</td>
<td>0.58</td>
</tr>
<tr>
<td>Tumor &quot;Kochsaft&quot;</td>
<td>1.18</td>
<td>1.08</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* The concentration of iodoacetate was $1 \times 10^{-2}$ M.
† The concentrations of this inhibitor are approximate, since the preparations contained considerable cysteic acid at the time of these experiments.
hibitory action of the latter does not appear to be due to the sulfinic acid, since iodoacetate was effective in reversing its effect while augmenting the effect of the cysteine sulfinic acid. The possibility of cysteine sulfenic acid's being responsible was previously mentioned. In any event, a series of cystine degradation products has been shown to inhibit catalase both in vitro and in vivo. Other cystine metabolites, such as mercapto-pyruvic acid, 2-amino-2-carboxy-ethanethiosulfinic acid, and alaninethiosulfinic acid (31), have not been tested but are also probable inhibitors of this enzyme. An aberrancy in sulfur amino acid metabolism by tumors resulting in excess production of cystine, as suggested by the work of Schweigert et al. (29), might be expected to raise the liver level of the various metabolites which inhibit catalase, with a resultant decrease in the activity of this enzyme. The effects of testosterone and cortisone in elevating catalase activity in vivo (1) could be the result of a lowering of tissue amino acid levels with a fall in the amount of the discussed cystine metabolites. Test of this hypothesis must await suitable assay methods for these type compounds.

No definite conclusions can be reached as regards the compounds in tumor "Kochsafs" which inhibit catalase. The suggestion by Endo et al. (9), that this is owing to the action of sulphydryl groups, is not borne out by our present and previous studies (30) which show that iodoacetate partially reverses the inhibitory effects of cysteine but has little or no effect on tumor "Kochsaft" activity. However, "Kochsaft" material, in admixture with catalase, causes spectral changes similar to those of cysteine (19). The sulfinic acids and cystine disulfide-sodium sulfite reaction products produced no such changes. Spectrophotometric studies on various liver catalase preparations from rats bearing hepatomas reveal that marked changes in absorption spectrum are induced by the presence of the tumor (28).

The ability of "Kochsafs" of other tissues to inhibit catalase (9, 30) indicates that tumor tissue is not unique in this respect, although in our experi-

**TABLE 5**

THE EFFECT OF CYSTINE AND SOME OF ITS DERIVATIVES ON THE ACTIVITY OF RAT LIVER CATALASE

<table>
<thead>
<tr>
<th>Amount injected† (mg/100 gm)</th>
<th>Units catalase gm of liver</th>
<th>Per cent of normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitor*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>31</td>
<td>342</td>
</tr>
<tr>
<td>Cystine</td>
<td>8.5</td>
<td>256</td>
</tr>
<tr>
<td>Cystine (2 hr.)</td>
<td>8.5</td>
<td>167</td>
</tr>
<tr>
<td>Cystine disulfide</td>
<td>9.5</td>
<td>249</td>
</tr>
<tr>
<td>Cystine disulfide-Na2SO₄ reaction products</td>
<td>9.5</td>
<td>220</td>
</tr>
<tr>
<td>Cysteine sulfinic acid</td>
<td>12</td>
<td>194</td>
</tr>
<tr>
<td>2-amino-ethane sulfinic acid</td>
<td>8.5</td>
<td>230</td>
</tr>
<tr>
<td>2-amino-ethane sulfinic acid (2 hr.)</td>
<td>8.5</td>
<td>292</td>
</tr>
<tr>
<td>Cysteic acid</td>
<td>12</td>
<td>250</td>
</tr>
<tr>
<td>Tumor (Flexner-Jobling)</td>
<td>5</td>
<td>156</td>
</tr>
</tbody>
</table>

*The rats were sacrificed 24 hr. after administration of the various compounds except where otherwise indicated.
†The amounts of inhibitor are equivalent to 0.7 millimoles/kg body wt.

**SUMMARY**

Cysteine, cysteine sulfinic acid, 2-amino-ethane sulfinic acid, and sodium sulfite-cystine disulfide reaction products are all good inhibitors of catalase in vitro. They and cystine disulfide also lower rat liver catalase activity in vivo, with 2-amino-ethane sulfinic acid showing only a weak
activity. Only cysteine, of the above compounds, affects the absorption spectrum of catalase. Iodoacetate reverses the catalase-inhibiting action of cysteine, and of cystine disulfide-sodium sulfite reaction products, but not of cysteine sulfonic acid and of tumor "Kochsaft." Formol reverses the catalase-inhibiting action of all the above. Changes in the absorption spectrum of catalase are effected only by cysteine and "Kochsaft" material.

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The Effect of Some Cystine Derivatives on Catalase Activity

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