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MECHANISM OF INHIBITION OF IODOTHYRONINE-5′-DEIODINASE BY THIOUREYLENES AND SULFITE

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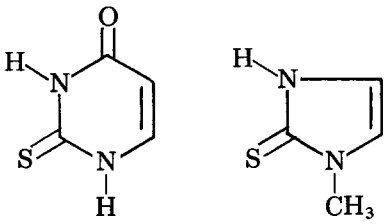
Summary

Previous studies have demonstrated that thiouracil inhibits the 5′-deiodination of 3,3′,5′-triiodothyronine uncompetitively with respect to substrate and competitively with respect to cofactor (thiol compounds). This paper shows that sulfite is also a strong inhibitor of this reaction showing a dose-dependent effect between 1 μM and 1 mM. The mode of inhibition is similar to that described for thiouracil. Dose-dependent inhibition was also observed with thiosulfate (0.01—1 mM), iodide and thiocyanate (both greater than 1 mM). No effect was exerted by up to 10 mM cyanide and up to 100 mM azide. Methimazole and thiourea were weak inhibitors above 0.1 mM but inhibition did not reach completion. These experiments were carried out in the presence of 1 mM dithiothreitol. The effect of thiouracil was found to be competitively obviated by methimazole and thiourea. However, the effeft of sulfite and that of methimazole or thiourea were additive. It is proposed that an enzyme-sulfenyl iodide is formed during deiodination (ping-pong mechanism). This sulfenyl iodide may be reduced by cofactor to yield native enzyme. It may also react with thioureylenes, yielding mixed disulfides, or with sulfite, yielding a thiosulfate. The enzyme-methimazole disulfide is apparently less stable than the enzyme-thiouracil complex. It is suggested that sulfite also reacts with the enzyme-thioureylene disulfide.

Introduction

2-Thiouracil (1) derivatives and 2-mercapto-1-methylimidazole (methimazole; 2) are used in the treatment of hyperthyroidism.



Thiouracil (1) Methimazole (2)

Scheme I. Structures of thiouracil and methimazole.

These drugs interfere with the biosynthesis of thyroxine by inhibiting thyroid peroxidase activity [1]. Of these compounds only the thiouracil derivatives have an additional effect on the deiodination of thyroid hormone in peripheral tissues [1]. It has been shown that thiouracil derivatives uncompetitively inhibit the 5′-deiodination of thyroxine, yielding 3,3′5-triiodothyronine (triiodothyronine) [2], and of 3,3′,5′-triiodothyronine (reverse triiodothyronine), yielding 3,3′-diiodothyronine (diiodothyronine) [3,4]. Thyroid hormone-deiodinating enzymes contain essential sulfhydryl groups and thiol compounds are required for activity [5]. Thiouracil has been shown to react selectively with sulfenyl iodides forming mixed disulfides [6]. It was, therefore, proposed that deiodination follows a ping-pong mechanism implying the intermediate formation of an iodo-enzyme complex where the essential -SH group is converted into a -SI group [4]. In the uninhibited reaction this E-SI complex is subsequently reduced by cofactor [4]. The present study was undertaken to test this hypothesis by investigating the effects on the 5′-deiodination of reverse triiodothyronine by agents such as sulfite known to react with a sulfenyl sulfur. In addition, the interaction of methimazole and thiourea with the enzyme inhibited by thiouracil or sulfite was studied. The results demonstrate that the mode of inhibition by sulfite is similar to that previously shown for thiouracil [4]. However, inhibition by thiouracil is competitively obviated by methimazole and thiourea, whereas the effects of the latter compounds and that of sulfite are additive.

Methods

The conversion of reverse triiodothyronine into diiodothyronine by rat liver microsomal fraction in the presence of dithiothreitol was measured essentially as described previously [7]. In short, usually 0.1 μM reverse triiodothyronine was incubated in 0.25 ml 0.05 M phosphate containing 3 mM EDTA, 1 mM dithiothreitol (unless indicated otherwise) and other substances to be tested (pH 6.5) with 7 μg of microsomal protein/ml for 20 min at 37°C. The reaction was stopped by the addition of 1 ml 0.06 M barbitone buffer containing 0.1% bovine serum albumin and 0.1% SDS (pH 8.6). The amount of diiodothyronine produced was measured with a specific radioimmunoassay in 50 μl of the extract [8]. The reaction was started by the addition of microsomes.

Deiodinase activity was corrected for non-enzymic production of diiodothyronine as measured in extracts of control incubations. In the controls, microsomes were added after the barbitone-SDS buffer. Usually, the amount of diiodothyronine produced in the absence of enzyme was negligible (less than 5%) compared with that generated enzymically. Incubation and radioimmunoassay were performed in duplicate.

Results

At the concentration of microsomes used there is no significant binding of reverse triiodothyronine to non-enzymic constituents of this fraction and degradation of diiodothyronine is negligible [7]. Alterations in diiodothyronine accumulation by test substances either via an effect on substrate availability or via an effect on the stability of the product are, therefore, excluded. A dose-dependent inhibition of diiodothyronine production was observed with 0.01—10 μM thiouracil, 1 μM—1 mM sulfite, 0.01—1 mM thiosulfate and 1—100 mM iodide or thiocyanate (Fig. 1). Deiodinase activity was also lowered by methimazole and thiourea at concentrations above 0.1 mM. Inhibition by these compounds reached a plateau of only approx. 50% despite increasing their concentration above 10 mM. No effect was observed with up to 10 mM cyanide and up to 100 mM azide. A 50% reduction in deiodinase activity was obtained with 0.3 μM thiouracil, 0.02 mM sulfite, 0.2 mM thiosulfate and 20 mM thiourea, iodide or thiocyanate but not with even higher concentrations of methimazole.

The effect of addition of 0.04 or 0.1 mM sulfite on the kinetics of the reac-

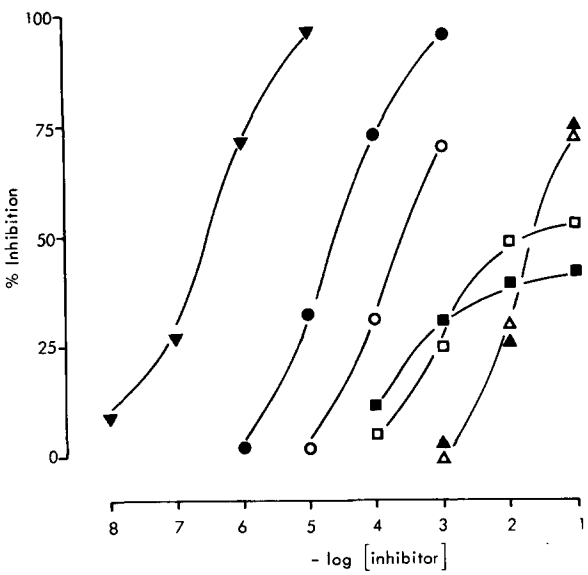


Fig. 1. Inhibition of the conversion of reverse triiodothyronine into 3,3′-diiodothyronine by various compounds. The following substances were tested: ▼, thiouracil (TU); ●, SO_3^{2-}; ○, $S_2O_3^{2-}$; ▲, I^-; △, SCN^-; □, thiourea, and ■, methimazole (MMI). For details see Methods. Results are means of 3—8 closely agreeing experiments.

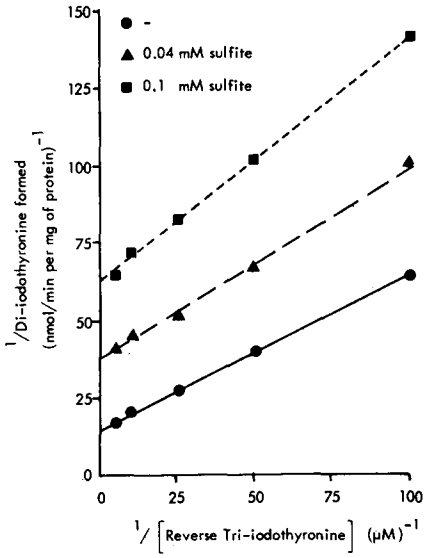


Fig. 2. Lineweaver-Burk plot of 3,3'-diiodothyronine production rate as a function of reverse triiodothyronine concentration and the effect of SO_3^{2-}. The following concentrations of SO_3^{2-} were tested: 0 (●), 0.04 (▲) and 0.1 (■) mM. For details see Methods. Results are means of three closely agreeing experiments.

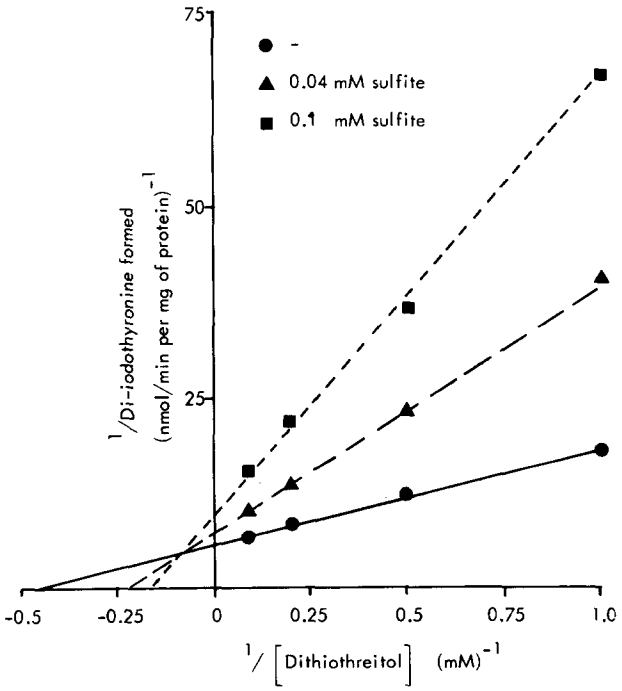
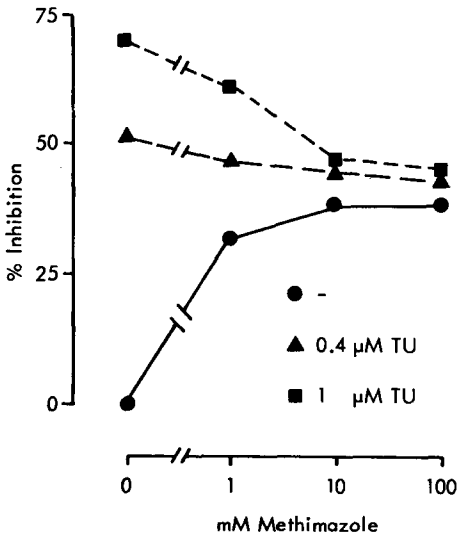


Fig. 3. Lineweaver-Burk plot of 3,3'-diiodothyronine production rate as a function of dithiothreitol concentration and the effect of SO_3^{2-}. The following concentrations of SO_3^{2-} were tested: 0 (●), 0.04 (▲) and 0.1 (■) mM. For details see Methods. Results are means of two closely agreeing experiments.

tion was investigated. This resulted in virtually parallel displacements of the Lineweaver-Burk plot of the deiodination rate versus reverse triiodothyronine concentration (Fig. 2). This indicates that inhibition by sulfite is largely uncompetitive with respect to substrate. Increasing the concentration of dithiothreitol alleviated the effect of sulfite although not completely. Analysis by means of a double-reciprocal plot of diiodothyronine production rate versus dithiothreitol concentration revealed that the reaction of sulfite with the enzyme was largely competitive with cofactor (Fig. 3). These conclusions were supported by Dixon plots [9] of Figs. 2 and 3. In addition, it was found that the replots [9] of the $1/v$ axis intercepts in Fig. 2 and of the slopes in Fig. 3 as a function of SO_3^{2-} concentration were linear.

In the experiments shown in Fig. 4 addition of 0.4 or 1 μM thiouracil alone resulted in a suppression of deiodinase activity by 52 and 70%, respectively. However, inhibition declined to 44 and 44%, respectively, in the presence of increasing concentrations of methimazole. This is virtually the maximum level of inhibition obtained with methimazole alone, i.e. 38%. The effects of thiouracil are, therefore, competitively obviated by methimazole.

Addition of 0.04 or 0.1 mM sulfite suppressed diiodothyronine production rate by 59 and 72%, respectively (Fig. 5). Now, in the presence of methimazole inhibition was further increased to a maximum of 79 and 87%, respectively. Inhibition by methimazole alone in this case was at the maximum 48%. The effects of sulfite appear, therefore, to be additive to that of methimazole. In the experiments described in Figs. 2—5 very similar findings were obtained by



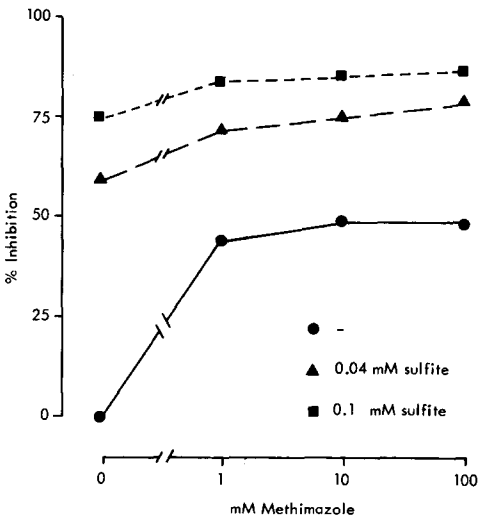


Fig. 4. Inhibition of the conversion of reverse triiodothyronine into 3,3′-diiodothyronine by the simultaneous addition of thiouracil (TU) and methimazole (MMI). TU was tested at concentrations of 0 (●), 0.4 (▲) and 1 (■) μM. For details see Methods. Results are means of four closely agreeing experiments.

Fig. 5. Inhibition of the conversion of reverse triiodothyronine into 3,3′-triiodothyronine by the simultaneous addition of SO_3^{2-} and methimazole (MMI). SO_3^{2-} was tested at concentrations of 0 (●), 0.04 (▲) and 0.1 (■) mM. For details see Methods. Results are means of two closely agreeing experiments.

using 0.4 and 1 mM thiosulfate instead of sulfite and 1—100 mM thiourea instead of methimazole.

It was found that the effects of the combined additions of thiouracul and sulfite, of thiouracil and iodide or of thiouracil and thiocyanate, tested in various proportions, were larger than the effects excerted by these compounds alone.

Discussion

When this study was in progress Leonard and Rosenberg [10] also reported on the effect of 6-propylthiouracil on the 5′-deiodination of thyroxine by rat kidney microsomal preparations. These investigators found that the inhibitory effect of thiouracil and propylthiouracil was attenuated by dithiothreitol, methimazole and thiourea but not by thiocyanate. They, however, noted a full restoration of deiodinase activity by 1 mM thiourea or methimazole, which in themselves did not inhibit triiodothyronine production. This discrepancy with our observation may be related to the differences in experimental conditions such as the choice of substrate and enzyme preparations, and the concentrations thereof, difference in dithiothreitol concentrations (0.1 mM [10] or 1 mM) and the absence [10] or presence (this paper) of air oxygen. In essence, nevertheless, their findings are in agreement with those presented by us previously [4] and in the present paper.

It has been demonstrated by Leonard and Rosenberg [10] and by us [4] that enzymic 5′-deiodination follows a ping-pong mechanism. Reaction of the first substrate (iodothyronine) with the enzyme results in the formation of an intermediate enzyme-complex, which by reaction with the second substrate (thiol cofactor) is converted back into native enzyme. Since it has been shown [5] that iodothyronine-deiodinating enzymes contain essential cysteine residues it is quite conceivable that during deiodination a sulfhydryl group is being oxidized. In consideration of the high reactivity of thiouracil towards sulfenyl iodides compared with ordinary disulfides [6] the formation of an E-SI complex in the catalytic cycle was implied [4]. The findings that thiouracil inhibits this reaction uncompetitively with substrate [2—4,10] and competitively with cofactor [4,10] support this hypothesis. They demonstrate that thiouracil reacts only with an intermediate in the deiodination process, being also the site of reaction with cofactor (see Fig. 6). It is not excluded, however, that inhibition by thiouracil is due to a reaction with some other form of sulfenyl sulfur such as an activated (protonated) disulfide [11—15]. To test this possibility the effect of several agents known to react with protein disulfides was investigated.

An intriguing observation was the high reactivity of SO_3^{2-} in contrast to the inactivity of CN^-. Cyanide is at least as reactive as SO_3^{2-} towards both disulfides [11—13] and sulfenyl iodides [6]. It should, however, be kept in mind that the present experiments were carried out in the presence of 1 mM dithiothreitol. The products of the reaction of SO_3^{2-} and CN^- with both a sulfenyl iodide and a disulfide are a thiosulfate and a thiocyanate, respectively. The difference in effect of sulfite and cyanide on the production of diiodothyronine may well be due to differences in the rate of regeneration of the

sulfhydryl group from these products by dithiothreitol. Iodide and thiocyanate were found to be weak inhibitors of 5′-deiodinase activity (Fig. 1).

Inhibition by sulfite is largely uncompetitive with substrate and competitive with cofactor. Thus, while belonging to an entirely different class of compounds, the mode of inhibition of the 5′-deiodination of thyroid hormone by SO_3^{2-} is similar to that by thiouracil. These findings provide further evidence for the formation of an enzyme-sulfenyl group (E-S$^+$) in this reaction.

Alleviation of the inhibitory activity of thiouracil by methimazole may be due either to competition of these compounds for the sulfenyl group in the intermediate enzyme complex [6] (Fig. 6, Reactions 2 and 3) or to reaction of methimazole with the enzyme-thiouracil mixed disulfide. The reaction rate especially of methimazole but also that of thiourea with β-lactoglobulin sulfenyl iodide are much higher compared with thiouracil [6]. This is in contrast with the results presented in this paper. It is shown that inhibition at saturating concentrations of methimazole is far from complete. This suggests that the mixed disulfide of enzyme with methimazole is rapidly reduced by cofactor (Fig. 6, Reaction 5). Recent studies on the structure-activity relationship of thioureylenes have shown that the low activity of methimazole is primarily due to the methylation of N_1 [16].

The mode of inhibition by SO_3^{2-} is very similar to that of thiouracil. The different behaviour of methimazole with the SO_3^{2-}-inhibited enzyme compared with its action in the presence of thiouracil was, therefore, unexpected. It has been reported that besides methimazole other compounds such as SO_3^{2-} react with β-lactoglobulin-thiouracil disulfide [17]. This suggests that inhibition by sulfite is not prevented by methimazole but that reaction with SO_3^{2-} may even be accelerated by prior formation of the enzyme mixed disulfide with methimazole (Fig. 6, Reactions 3 and 6). It should be noted that in the presence of sulfite there is minor irreversible loss of enzyme activity, which is not overcome by increasing dithiothreitol concentrations (Fig. 3). It is not excluded, therefore, that the effects observed with the simultaneous addition of SO_3^{2-} and methimazole may be accounted for to some extent by this action of sulfite.

Fig. 6 is shown in an attempt to clarify the several observations described in

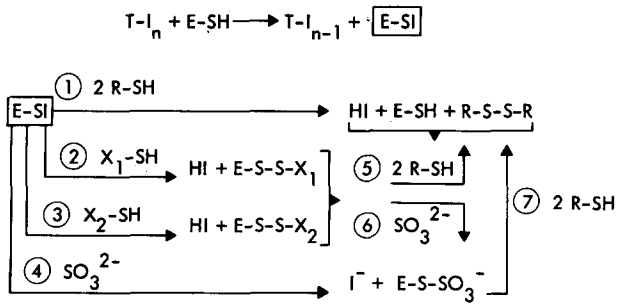


Fig. 6. Possible interactions of thiouracil (X_1-SH), methimazole (X_2-SH) and SO_3^{2-} with iodothyronine 5′-deiodinase. Unaltered thiouracil and methimazole are released in reactions 5 and 6. Similarly, unaltered SO_3^{2-} is a product of Reaction 7. T-I_n, iodothyronine.

this paper. Recent studies in our laboratory are compatible with this view, since it has been found [18] that binding of radioiodinated propylthiouracil to rat liver microsomal fraction is induced specifically by substrates of the iodothyronine-5′-deiodinase. This binding is prevented competitively by dithiothreitol, unlabelled propylthiouracil, methimazole and sulfite. Since, apparently, Reaction 5 for the enzyme-thiouracil mixed disulfide and Reaction 7 are slow compared with Reaction 1 (Fig. 6), thiouracil and sulfite may be regarded as dead-end inhibitors. This is substantiated by the finding of linear replots of Figs. 2 and 3 [9].

In conclusion, the present study provides further evidence that an enzyme-sulfenyl group, probably a sulfenyl iodide, is formed during 5′-deiodination of iodothyronines. In view of the lability of -SI groups in aqueous media [6] it will, however, be a difficult task to prove the actual formation of such a derivative of the 5′-deiodinase.

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