

Iodide Binding and Regulation of Lactoperoxidase Activity toward Thyroid Goitrogens*

(Received for publication, January 3, 1978, and in revised form, August 1, 1978)

Jean Luc Michot, Jacques Nunez, Michael L. Johnson,‡ Gaetano Irace,§ and Harold Edelhoç‡

From the Unite de Recherche sur la Glande Thyroide et la Regulation Hormonale, Institut National de la Santé et de la Recherche Médicale, Le Kremlin Bicetre, France

The effects of the antithyroid goitrogens, methylthiouracil and methylmercaptoimidazole, on the oxidation of *N*-acetyltyrosylamide at pH 8.8 by lactoperoxidase have been evaluated in the presence and the absence of iodide. At pH 8.8, iodide is not oxidized. In the absence of iodide, the two antithyroid drugs inactivate lactoperoxidase by a second order process. When iodide is added before methylthiouracil or methylmercaptoimidazole, enzyme inactivation does not occur as rapidly and both goitrogens are readily oxidized. The kinetics of the oxidation reactions have been analyzed in order to obtain the equilibrium constant of the iodide-lactoperoxidase complex. Essentially the same iodide dissociation constant, *i.e.* 2×10^{-5} M, was found by studying its effects on the kinetics of oxidation of the two antithyroid drugs. A large difference absorption spectrum is observed in the Soret region between native lactoperoxidase and lactoperoxidase inactivated by methylthiouracil.

Recently, it has been shown that the thyroid peroxidase (1, 2) is the enzyme responsible for iodination as well as coupling of tyrosyl residues in thyroglobulin to form the hormonal residues, *i.e.* thyroxine and 3,3',5-triiodothyronine (3, 4). It has been known for a long time that a group of related thiourea-like substances act *in vivo* to inhibit iodination of thyroglobulin (5, 6). Although these substances are metabolized, the mechanism of their action remains unclear.

Several mechanisms have been proposed to explain the behavior of these compounds. Maloof and Soodak (7), Morris and Hager (8), and Taurog (9) have reported that thiourea-like compounds, *i.e.* thionamides or thioureylenes (antithyroid drugs), are oxidized by different peroxidases only in the presence of iodide. Morris and Hager (8) have studied the effect of these drugs on tyrosine iodination catalyzed by the enzyme chloroperoxidase. They have proposed that inhibition of the iodination reaction depends on a "competition between the drug and tyrosine towards a common intermediary complex formed between the enzyme and an oxidized form of iodide, $E - I^+$, which is used to oxidize the thiourea-like compound." Working with thyroid peroxidase, Taurog (9) found that this

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address, Clinical Endocrinology Branch, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Md. 20014.

§ Recipient of United States Public Health Service Fellowship 1F05TW02573-01. Present address, Institute of Biological Chemistry, 1st Faculty of Medicine, University of Naples, Italy.

pathway is acceptable when the drug to iodide ratio is low. Taurog reported that under these conditions, the drug is rapidly oxidized and an "escape" of iodination from inhibition occurs after a lag period. For higher drug to iodide ratios, no oxidation of the drug was noticed and no escape observed. Taurog proposed, therefore, that in these conditions, the drug inhibits the formation of the enzyme-oxidized iodide complex (9).

In the above experiments, inhibition by the antithyroid drugs was measured by an iodination assay. Consequently, it is difficult to distinguish the role of iodide since it is involved in both reactions, *i.e.* iodination and antithyroid drug oxidation. We, therefore, decided to study the effect of iodide and the antithyroid drugs on the oxidation of *N*-acetyltyrosylamide. This reaction is catalyzed by lactoperoxidase at a high pH where iodination does not occur. Thus, the effect of the antithyroid drugs could be studied (with and without iodide) to elucidate the role of iodide.

EXPERIMENTAL PROCEDURES

Materials—Lactoperoxidase was purchased from Boehringer Mannheim GmbH or Sigma Chemical Co. *N*-Acetyltyrosylamide was purchased from Sigma Chemical Co. All other products and chemicals were of the highest purity available.

Methods—Lactoperoxidase was obtained as a suspension in 3.2 M ammonium sulfate solution or as a lyophilized powder. H_2O_2 solutions were standardized by permanganate titration. The buffer used throughout this work was 0.01 M glycine and 0.09 M NaCl, pH 8.80.

Oxidation of *N*-Acetyltyrosylamide—An Aminco-Bowman spectrofluorometer was used to monitor the oxidation of *N*-acetyltyrosylamide. Bayse *et al.* (10) have followed this reaction by the accompanying absorption changes. The excitation wavelength was 310 nm and the emission wavelength was 410 nm. The spectrofluorometric measurements are based on the fact that the product of *N*-acetyltyrosylamide oxidation, *i.e.* bis-(3,3'-*N*-acetyltyrosylamide), has a maximum absorption near 310 nm and emission peak at 410 nm. The absorption and emission properties of bityrosine and related biphenols have been described by Andersen (11).

Rates were measured for at least 3 min at room temperature in a final volume of 2.0 ml of buffer (pH 8.80) containing constant amounts of lactoperoxidase, H_2O_2 , and *N*-acetyltyrosylamide. The peroxide concentration was always present in excess with respect to enzyme and substrate, *i.e.* *N*-acetyltyrosylamide.

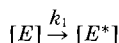
Absorption Measurements—A Cary spectrophotometer model 15 was employed for absorption measurements.

Differential Rate Equations—The kinetics of (a) methylthiouracil and MMI¹ oxidation, (b) the lactoperoxidase inactivation by methylthiouracil and MMI, and (c) the effect of iodide on these two processes have been analyzed with the aid of a computer program, MLAB (12), based on a least square curve fit to the simultaneous solution of three differential equations.

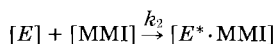
At pH 8.8 in the glycine buffer used for these experiments, lactoperoxidase undergoes a slow autoinactivation which is irreversible. Lactoperoxidase is also irreversibly inactivated by the antithyroid

¹ The abbreviations used are: MMI, methylmercaptoimidazole; ATA, *N*-acetyltyrosylamide; bi-ATA, bis-(3,3'-*N*-acetyltyrosylamide).

drugs such as MMI and methylthiouracil. The first differential equation includes the first order dependence of lactoperoxidase decay to an inactive form, E^* ,



and a second order dependence of inactivation by the antithyroid agents (the equations for methylthiouracil are the same as for MMI and will not be shown).

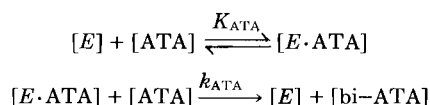


Therefore, the differential equation describing the inactivation of the enzyme includes both the decay rate of the enzyme in the absence of goitrogen (autoinactivation) and the rate of lactoperoxidase inactivation by MMI.

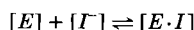
$$\frac{d[E(t)]}{dt} = -(k_1 + k_2[\text{MMI}(t)])[E(t)] \quad (1)$$

where the free MMI concentration, $\text{MMI}(t)$, the free enzyme, $E(t)$, and the total active enzyme, $E_t(t)$, are all functions of time.

The concentration of free lactoperoxidase, $E(t)$, is measured with *N*-acetyltyrosylamide as a substrate and involves the formation of bis-(3,3'-*N*-acetyltyrosylamide). Since 2 molecules of *N*-acetyltyrosylamide are condensed to yield bis-(3,3'-*N*-acetyltyrosylamide), formally this reaction should be a third order process. This reaction has been previously reported to be second order (10). We have observed results similar to those of Bayse *et al.* (10) by a fluorimetric assay in the concentration range of *N*-acetyltyrosylamide employed in this paper.² There are several mechanisms which can explain the second order kinetics; two possibilities involve either strongly positive or negative cooperative binding of *N*-acetyltyrosylamide. This aspect of the kinetics has not been investigated further at this time. Consequently, we have modeled the formation of bis-(3,3'-*N*-acetyltyrosylamide) as a process which is second order, *i.e.* first order in *N*-acetyltyrosylamide and first order in enzyme, in accord with the published kinetic observations of Bayse *et al.* (10). Consequently,



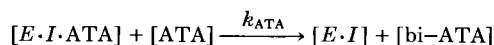
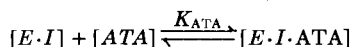
At pH 8.8, iodide is not oxidized and has no influence on the rate of *N*-acetyltyrosylamide oxidation in the iodide concentration range of 10^{-5} to 10^{-3} M. However, iodide has a profound influence on 1) the rate of inactivation of lactoperoxidase by MMI or methylthiouracil and 2) the rate of oxidation of MMI and methylthiouracil by lactoperoxidase. The effect of iodide is most easily understood in terms of formation of an enzyme-iodide complex, without specification of the oxidation state of the bound iodide.



where

$$K_{\text{iodide}} = [E][I^-]/[E \cdot I]$$

The lack of influence of iodide on the rate of oxidation of *N*-acetyltyrosylamide can be explained by several mechanisms. One possible way is that neither E nor $E \cdot I$ is involved in the rate-determining step, but this seems unlikely since the reaction is first order in enzyme concentration (10). Another possibility is that the iodide is not bound to the enzyme; however, this explanation is also unlikely since iodide strongly modifies the rates of enzyme inactivation by MMI and methylthiouracil and also the rates of enzymatic oxidation of these two goitrogens. Assuming that the $E \cdot I$ complex is formed, we must assume that *N*-acetyltyrosylamide has the same affinity to $[E]$ and $[E \cdot I]$ and that the two complexes $[E \cdot (\text{N-acetyltyrosylamide})]$ and $[E \cdot I \cdot (\text{N-acetyltyrosylamide})]$ are oxidized at the same rate. If these constants were not the same, any shift in the distribution of enzymatic species by the addition of iodide would affect the rate of formation of the product. We can therefore write



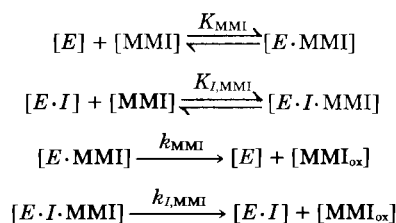
The fluorescence assay (ΔF) measures the formation of bis-(3,3'-*N*-acetyltyrosylamide) from *N*-acetyltyrosylamide. Therefore, the second differential equation is:

$$\frac{d\Delta F}{dt} = \frac{d[\text{bi-ATA}]}{dt} = k_{\text{ATA}} [E \cdot I \cdot \text{ATA}][\text{ATA}] + k_{\text{ATA}} [E \cdot \text{ATA}][\text{ATA}] = \frac{k_{\text{ATA}}}{K_{\text{ATA}}} [E(t)][\text{ATA}]^2 (1 + [I^-]/K_{\text{iodide}}) \quad (2)$$

Since the concentration of *N*-acetyltyrosylamide is in large excess and remains essentially constant during the experiment, it is treated as a constant.

Maguire and Dunford have investigated the kinetics of iodide oxidation as a function of pH (13). At pH 8.8, they observed a slow rate of disappearance of Compound II and concluded that it was a measure of iodide oxidation. It should be pointed out that they used 200-fold more enzyme in their experiments than we have used. Using their rate constants obtained at pH 8.81, our enzyme concentration (4.8×10^{-9} M) and approximately the highest iodide concentration used in our experiments (10^{-4} M), the calculated concentration of iodide oxidized by Compound II using their rate equation would be 2×10^{-10} M after 100 s of incubation. This calculation was made by assuming that all of the enzyme is present as Compound II. In addition, we have measured directly the disappearance of iodide from its absorption peak at 225 nm. At 7×10^{-5} M iodide and 4×10^{-9} M lactoperoxidase, we observed almost complete oxidation of the iodide after approximately 3 min at pH 6.15. However, when the pH was raised to 8.8 we were unable to detect any decrease in absorbance, *i.e.* any disappearance of iodide. Iodide is consequently treated as a constant since it is present in a large excess and is essentially not oxidized under these conditions.

Thioureylene compounds can, in principle, be oxidized by either the enzyme-iodide complex or by the free enzyme. Therefore, the following reactions apply:



where $[\text{MMI}_{\text{ox}}]$ is the concentration of oxidized MMI at any time. It is known, however, that very little or no MMI (or methylthiouracil) is oxidized in the absence of iodide (9). Consequently, the processes in the absence of iodide need not be included in the third differential equation:

$$\frac{d[\text{MMI}_{\text{ox}}(t)]}{dt} = \frac{k_{I, \text{MMI}}[E(t)][I^-][\text{MMI}(t)]}{K_{I, \text{MMI}}K_{\text{iodide}}} \quad (3)$$

Finally, by conservation of mass, an equation can be written which includes all the viable enzymatic species:

$$[E_t] = [E] + [E \cdot I] + [E \cdot \text{ATA}] + [E \cdot I \cdot \text{ATA}] + [E \cdot I \cdot \text{MMI}] + [E \cdot \text{MMI}] \quad (4)$$

Each term of Equation 4 is a function of time.

The experimental data, which have been analyzed by a least square fit to Equations 1 to 4, provide the values of some of the different equilibria and rate constants. In this report, we have principally investigated the effects of iodide in order to obtain its binding constant. We will report subsequently on the effects of iodide on the individual inactivation, binding, and oxidation rate constants in more detail.

RESULTS

Kinetic Analysis

Oxidation of *N*-Acetyltyrosylamide

The rate of oxidation of *N*-acetyltyrosylamide was measured fluorimetrically by the rate of formation of its oxidation

² Unpublished experiments of the authors; manuscript in preparation.

product, bis-(3,3'-*N*-acetyltyrosylamide), at pH 8.8. At this pH, there is no oxidation of iodide; furthermore, we have not observed any effect of iodide between 10^{-5} and 10^{-3} M on the rate of *N*-acetyltyrosylamide oxidation at pH 8.8. This implies that there is little or no competition between *N*-acetyltyrosylamide and iodide for the enzyme.

Effect of Methylthiouracil on *N*-Acetyltyrosylamide Oxidation

Absence of Iodide—An inhibition of the oxidation of *N*-acetyltyrosylamide by lactoperoxidase at pH 8.8 was observed when 1.5×10^{-5} M methylthiouracil was added to the enzyme assay mixture. When lactoperoxidase was preincubated with methylthiouracil for 2 min, the enzyme activity was almost completely inhibited (Fig. 1, Curve 3). On the other hand, when the enzyme was the last component added (Fig. 1, Curve 2), there was only a very small inhibition by the same concentration of methylthiouracil. The strong inhibition in the case of the preincubation experiment does not depend on the depletion of H_2O_2 since adding the same quantity of fresh lactoperoxidase to the inhibited system resulted in the normal rate of oxidation of *N*-acetyltyrosylamide (Fig. 1, Curve 4).

The effect of four concentrations of methylthiouracil on the rate of lactoperoxidase inactivation is presented in Fig. 2. The

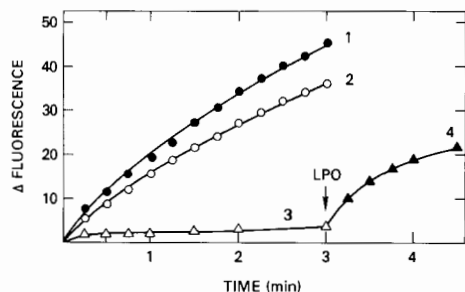


FIG. 1. Effect of methylthiouracil on *N*-acetyltyrosylamide oxidation. The experiment was performed with 4.20×10^{-4} M *N*-acetyltyrosylamide, 4.50×10^{-9} M lactoperoxidase (LPO) and 2.94×10^{-5} M H_2O_2 . ●—●, control without methylthiouracil; △—△, lactoperoxidase with H_2O_2 was preincubated with methylthiouracil (1.5×10^{-5} M) for 2 min, before the addition of *N*-acetyltyrosylamide; ▲—▲, the effect of a second addition of lactoperoxidase (4.50×10^{-9} M) 3 min after the preceding experiment; ○—○, methylthiouracil (1.50×10^{-5} M) was added to enzyme solution immediately after addition of *N*-acetyltyrosylamide.

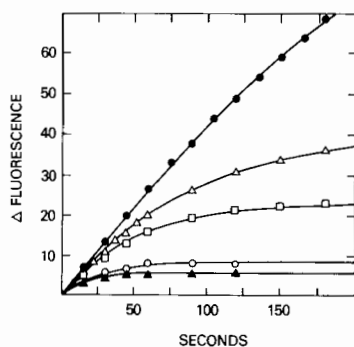


FIG. 2 (left). Effect of methylthiouracil concentration on the oxidation of *N*-acetyltyrosylamide. The experiment was performed with 4.20×10^{-4} M *N*-acetyltyrosylamide, 4.50×10^{-9} M lactoperoxidase, 2.94×10^{-5} M H_2O_2 , and different concentrations of methylthiouracil ●—●, control without methylthiouracil; △—△, 0.75×10^{-5} M; □—□, 1.5×10^{-5} M; ○—○, 3.0×10^{-5} M; and ▲—▲, 4.5×10^{-5} M. K_{ATA} was assumed to be 2×10^{-3} M.

FIG. 3 (center). Effect of iodide on the inhibition by methylthiouracil of *N*-acetyltyrosylamide oxidation. The reaction conditions are

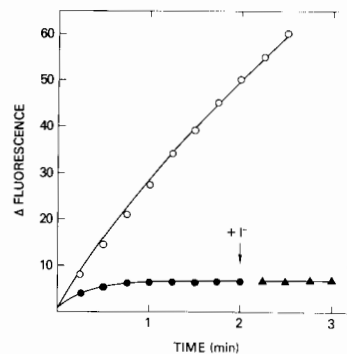
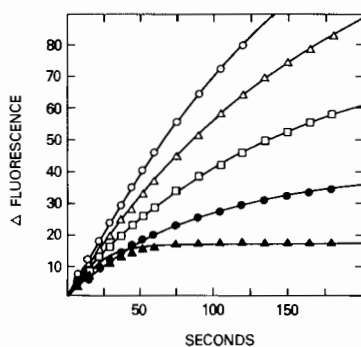
lines are theoretical based on solution of Equations 1 to 4. A least square fit of the points at the lowest concentrations (0.75 and 1.50×10^{-5} M methylthiouracil) gave inactivation rate constants (k_2) of 1446 ± 22 M $^{-1}$ s $^{-1}$ and 1435 ± 30 M $^{-1}$ s $^{-1}$, respectively. At the two higher methylthiouracil concentrations, the rates of inactivation were larger but the k_2 values had much larger standard errors (2420 ± 160 M $^{-1}$ s $^{-1}$; 2260 ± 115 M $^{-1}$ s $^{-1}$). Since the initial rate of formation of bis-(3,3'-*N*-acetyltyrosylamide) decreases in the presence of increasing amounts of methylthiouracil (Fig. 2), it must be concluded that methylthiouracil not only inactivates the enzyme but is also bound and competes with *N*-acetyltyrosylamide for the free enzyme.

Presence of Iodide—The irreversible inactivation of peroxidase activity by methylthiouracil could be strongly inhibited by iodide provided that it was added before lactoperoxidase was exposed to methylthiouracil (Fig. 3). At the pH of these experiments, *i.e.* 8.8, *N*-acetyltyrosylamide is not iodinated. The stabilization of peroxidase activity depends on the iodide concentration. If the iodide is added after methylthiouracil, when the rate of *N*-acetyltyrosylamide oxidation is negligible, no recovery of lactoperoxidase activity is found (Fig. 4). Iodide, therefore, prevents methylthiouracil inactivation of the enzyme only if it is added before methylthiouracil.

The effect of iodide on the inhibition of methylthiouracil inactivation of lactoperoxidase is shown in Fig. 3. The points are experimental and the lines are a least square fit based on the differential Equations 1 to 4. For the three levels of iodide, the average value of K_{iodide} is $2.2 \pm 0.2 \times 10^{-5}$ M and that for the inactivation rate, *i.e.* k_2 is 3640 ± 80 M $^{-1}$ s $^{-1}$. The average value of K_{LMTU} for the three experiments was $1.7 \pm 1.3 \times 10^{-5}$ M.

Effect of MMI on *N*-Acetyltyrosylamide Oxidation

Absence of Iodide—Another antithyroid drug, MMI, also inhibited the oxidation of *N*-acetyltyrosylamide by lactoperoxidase under the same conditions but only about one-tenth as much MMI as methylthiouracil was needed in order to produce the same degree of inhibition. The kinetics of inactivation were similar to that of methylthiouracil since the enzyme was also irreversibly inactivated. The theoretical curves (points are experimental) are shown in Fig. 5 using the average rate constant (k_2), *i.e.* $11.0 \pm 2.7 \times 10^{+3}$ M $^{-1}$ s $^{-1}$ obtained from the four curves when analyzed independently.



those given in Fig. 2. The upper curve ○—○ is without methylthiouracil; the four lower curves had 1.5×10^{-5} M methylthiouracil. Iodide concentrations were: △—△, 1.5×10^{-4} M; □—□, 3.0×10^{-5} M; ●—●, 1.5×10^{-5} M; and ▲—▲, 0.0 M.

FIG. 4 (right). Inability of iodide to restore lactoperoxidase activity after inactivation by methylthiouracil. Lactoperoxidase was incubated in the presence of H_2O_2 (2.94×10^{-5} M), methylthiouracil (4.50×10^{-5} M), and *N*-acetyltyrosylamide (4.20×10^{-4} M). Addition of iodide (6.00×10^{-5} M) 3 min, later was ineffective in restoring enzyme activity.

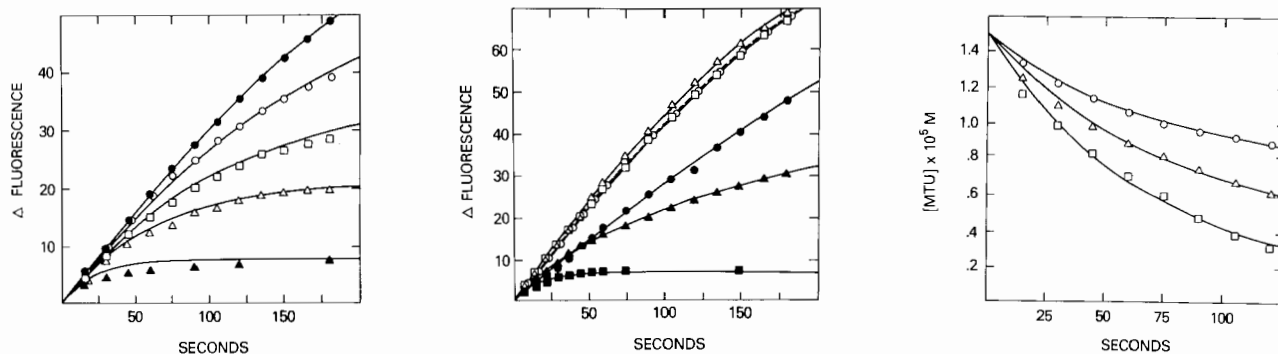


FIG. 5 (left). Effect of MMI concentration on the oxidation of *N*-acetyltyrosylamide. To lactoperoxidase, H₂O₂, and *N*-acetyltyrosylamide (conditions of Fig. 2) different concentrations of MMI were added: ●—●, control (no MMI); ○—○, 4.5 × 10⁻⁷ M; □—□, 7.5 × 10⁻⁷ M; △—△, 1.5 × 10⁻⁶ M; ▲—▲, 4.5 × 10⁻⁶ M.

FIG. 6 (center). The effect of iodide on *N*-acetyltyrosylamide oxidation in the presence of MMI (conditions of Fig. 2). All curves except the control had 4.5 × 10⁻⁶ M MMI. Iodide concentrations were: △—△, 1.5 × 10⁻⁴ M; □—□, 6.0 × 10⁻⁵ M; ●—●, 3.0 × 10⁻⁵ M; ▲—▲, 1.5 × 10⁻⁵ M; and ■—■, 4.5 × 10⁻⁶ M. The control curve

(○—○) is essentially identical with the curve indicated by □—□ and is drawn as a *dashed line*.

FIG. 7 (right). Effect of iodide on methylthiouracil (MTU) oxidation measured by the change in absorption at 300 nm. Lactoperoxidase was present at 4.50 × 10⁻⁹ M, H₂O₂ at 2.94 × 10⁻⁵ M, and methylthiouracil at 1.50 × 10⁻⁵ M, pH 8.8. Different concentrations of I⁻ were added at time zero: ○—○, 1.50 × 10⁻⁵ M; △—△, 3.0 × 10⁻⁵ M; □—□, 9 × 10⁻⁵ M. The ordinate scale has been converted to methylthiouracil concentration.

TABLE I

Equilibrium constants for lactoperoxidase complex with iodide
The experimental conditions are given in the various figures.

<i>N</i> -Acetyltyrosylamide	Substrate	$K_{\text{iodide}} \times 10^{+5}$
+	MTU ^a	2.2 ± 0.2
+	MMI	2.0 ± 0.5
-	MTU	1.9 ± 0.1

^a MTU, methylthiouracil.

The kinetics of lactoperoxidase inactivation fit Equations 1 to 4 for both goitrogens although the rate with MMI is almost an order of magnitude greater.

Presence of Iodide—Iodide also stabilized lactoperoxidase activity to inactivation by MMI at levels comparable to those observed with methylthiouracil. The effect of several concentrations of iodide on the oxidation of *N*-acetyltyrosylamide in the presence of 4.5 × 10⁻⁶ M MMI is shown in Fig. 6. The data at two concentrations (*i.e.* 1.6 and 4.5 × 10⁻⁵ M) of iodide gave K_{iodide} values of 1.5 ± 0.3 × 10⁻⁵ M and 2.6 ± 0.1 × 10⁻⁵ M, respectively. The k_2 value was 17 ± 8 × 10⁺³ M⁻¹ s⁻¹. Thus, the dissociation constants of the enzyme·iodide complex are comparable although the inactivation rate constants for MMI and methylthiouracil are very different. The values for $K_{I, \text{MMI}}$ for 1.50 and 4.50 × 10⁻⁵ M iodide concentrations were also determined by the above analysis and are, respectively, 1.1 ± 0.4 × 10⁻⁵ M and 0.22 ± 0.02 × 10⁻⁵ M.

Oxidation of Methylthiouracil

A direct method of following the oxidation of methylthiouracil was also used. The absorption changes of methylthiouracil in the near ultraviolet could be followed spectrophotometrically which allowed direct kinetic observation of its disappearance during oxidation. Methylthiouracil is oxidized only in the presence of iodide. The rate of disappearance of methylthiouracil was measured, therefore, as a function of iodide concentration (Fig. 7).

The data were analyzed by Equations 1, 3, and 4. The curves in Fig. 7 were calculated from a best fit value of $K_{\text{iodide}} = 1.90 \pm 0.10 \times 10^{-5}$ M. It is evident that the same value of K_{iodide} is obtained either when oxidation of methylthiouracil is observed directly or indirectly from the rate of *N*-acetyltyrosylamide oxidation (Table I).

The initial velocities of oxidation of methylthiouracil were

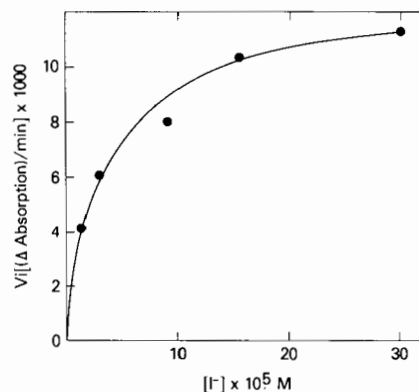


FIG. 8. Effect of iodide on initial rates (V_i) of methylthiouracil oxidation. Increasing concentrations of iodide (from 1.50 × 10⁻⁵ M to 3.00 × 10⁻⁴ M) were used with constant amounts of methylthiouracil (1.50 × 10⁻⁵ M), lactoperoxidase (4.50 × 10⁻⁹ M), and H₂O₂ (2.94 × 10⁻⁵ M). Initial rates of methylthiouracil oxidation were measured at 300 nm (as reported in Fig. 7) during the first 20 s of the absorbance measurements.

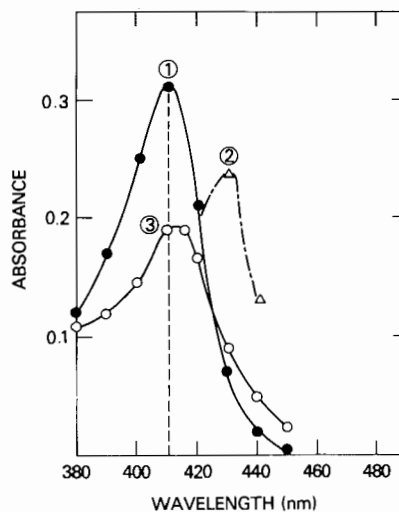


FIG. 9. Effect of methylthiouracil on the lactoperoxidase H₂O₂ complex at pH 8.8. The concentration of lactoperoxidase was 2.63 × 10⁻⁶ M. Curve 1: native enzyme, no H₂O₂; Curve 2: lactoperoxidase plus H₂O₂; Curve 3: after addition of methylthiouracil, 1.5 × 10⁻⁵ M, in the presence of H₂O₂.

also evaluated as an empirical measure of the effect of iodide and are shown in Fig. 8. The iodide concentration which gives half the maximum initial velocity is clearly similar to that obtained by the analysis of the effect of iodide on the inhibition of *N*-acetyltyrosylamide oxidation by methylthiouracil.

Effect of Methylthiouracil on the Soret Spectrum of Lactoperoxidase

The influence of iodide on the rate of inactivation of lactoperoxidase by methylthiouracil could also be shown directly since profound changes occur in heme absorption in the Soret region in the absence of iodide (Fig. 9). When H₂O₂ was added to the lactoperoxidase, the expected lactoperoxidase·H₂O₂ complex (Fig. 9, *Curve 2*) was found. When methylthiouracil was added to the lactoperoxidase·H₂O₂ complex, a significant decrease in absorption and a large blue shift in the Soret absorption band (Fig. 9, *Curve 3*) was observed. We were unable to recover the original spectrum (Fig. 9, *Curve 2*) by the further addition of H₂O₂. Dialysis in the absence of peroxide for 15 h also did not change the spectrum of the inactivated enzyme. No effect was observed when methylthiouracil (1.5×10^{-4} M) was added to lactoperoxidase in the absence of H₂O₂ (Fig. 9, *Curve 1*). When iodide is present before methylthiouracil is added, the Soret spectrum of lactoperoxidase remains unchanged during the oxidation of methylthiouracil.

DISCUSSION

The rate of inactivation of lactoperoxidase by two antithyroid drugs, methylthiouracil and MMI, has been measured. The effect of iodide in preventing the inactivation of lactoperoxidase by MMI and methylthiouracil could be evaluated because it is not consumed, *i.e.* oxidized, at pH 8.8 and it has no influence on the rate of *N*-acetyltyrosylamide oxidation.

The effects of iodide on the properties of lactoperoxidase can be understood if iodide forms an equilibrium complex with a specific site on lactoperoxidase. We have been able to determine the binding constant for this complex using the two antithyroid drugs as substrates. The same constant was obtained for both goitrogens and with one goitrogen by two different procedures (Table I).

Another mechanism which can be postulated to explain the effect of iodide might be one in which iodide ion reacts chemically with an oxidized species of goitrogen to form a nontoxic product. This possibility is unlikely since this model would not predict the saturation by iodide observed in Fig. 8. A limit to the stimulatory effect of iodide on methylthiouracil oxidation is better explained if one assumes that iodide specifically binds to the enzyme and that this enzyme-iodide complex is able to catalyze methylthiouracil oxidation. Also, the binding constant for iodide calculated from the kinetic analysis (Table I) is in accord with the value obtained for the half-maximal effect of iodide on methylthiouracil oxidation (Fig. 8).

The stabilization of lactoperoxidase activity towards methylthiouracil inactivation was evident by the preservation of the Soret band of the enzyme when iodide was present. In the absence of iodide, the Soret band of lactoperoxidase is drastically modified by methylthiouracil with a rapid loss of enzyme activity. The loss in activity is irreversible since when iodide was added after methylthiouracil inactivation, no recovery was found. The change in heme absorption in the Soret region could result from either a modification in protein structure which altered the apoprotein-heme interaction or by direct reaction with the heme group.

We are not certain of the way in which methylthiouracil inactivates lactoperoxidase. In the case of 3-aminotriazole, another type of antithyroid drug, it has been shown that the triazole combines with 2 residues of histidine and the rate of combination agrees with the rate of inactivation (14). The inactivation of lactoperoxidase by 3-aminotriazole involves a similar change in the Soret band to that observed with methylthiouracil. However, it should be pointed out that iodide does not protect lactoperoxidase towards 3-aminotriazole inactivation.²

The role we have suggested for iodide is quite different from that proposed in other reports although the observed effects of iodide are similar (8, 9). Iodide has been portrayed as an oxidizable intermediate (*E·I*⁺ or *E·I*⁰) in the oxidation of the antithyroid drugs. It should be noted that our analysis does not specify the oxidation state of the iodide while it is bound to the enzyme. We have shown, however, that iodide is not a substrate, *i.e.* is not consumed, and that it acts as an "effector" regulating the relative rates of enzyme inactivation and substrate oxidation.²

REFERENCES

1. Coval, M. L., and Taurog, A. (1967) *J. Biol. Chem.* **242**, 5510-5523
2. Pommier, J., De Prailaune, S., and Nunez, J. (1972) *Biochimie (Paris)* **54**, 483-492
3. Deme, D., Fimiani, E., Pommier, J., and Nunez, J. (1975) *Eur. J. Biochem.* **51**, 329-336
4. Taurog, A., Lothrop, M. L., and Estabrook, R. W. (1970) *Arch. Biochem. Biophys.* **139**, 221-229
5. Astwood, E. B. (1943) *J. Pharmacol.* **78**, 79-89
6. Astwood, E. B., Sullivan, J., Bissel, A., and Tyslovitz, R. (1943) *Endocrinology* **32**, 210-225
7. Maloof, F., and Soodak, M. (1965) in *Current Topics in Thyroid Research* (Cassano, C., and Andreoli, M., eds) pp. 227-290, Academic Press, New York
8. Morris, D. R., and Hager, L. P. (1966) *J. Biol. Chem.* **241**, 3582-3589
9. Taurog, A. (1976) *Endocrinology* **98**, 1031-1046
10. Bayse, G. S., Michaels, A. W., and Morrison, M. (1972) *Biochim. Biophys. Acta* **284**, 34-42
11. Andersen, S. O. (1966) *Acta Physiol. Scand.* **66**, Suppl. 263, 9-81
12. Knott, G. D., and Schrager, R. I. (1972) *ACM Sigraph Notices* **6**, 1704-1710
13. Maguire, R., and Dunford, H. (1972) *Biochemistry* **11**, 937-941
14. Chang, J. Y., and Schroeder, W. A. (1973) *Arch. Biochem. Biophys.* **156**, 475-479