Mechanisms that have been proposed for peroxidase-catalyzed iodination require the utilization of 1 mol of H$_2$O$_2$ for organic binding of 1 mol of iodide. When we measured the stoichiometry of this reaction using thyroid peroxidase or lactoperoxidase at pH 7.0, we consistently obtained a ratio less than 1.0. This was shown to be attributable to catalase-like activity of these enzymes, resulting in unproductive cleavage of H$_2$O$_2$. This catalatic activity was completely iodide-dependent.

To elucidate the mechanism of the iodide-dependent catalatic activity, the effects of various agents were investigated. The major observations may be summarized as follows: 1) The catalatic activity was inhibited in the presence of an iodine acceptor such as tyrosine. 2) The pseudohalide, SCN$^-$, could not replace I$^-$ as a promoter of catalatic activity. 3) The inhibitory effects of the thioureylene drugs, methimazole and carbimazole, on the iodide-dependent catalatic activity were very similar to those reported previously for thyroid peroxidase-catalyzed iodination. 4) High concentrations of I$^-$ inhibited the catalatic activity of thyroid peroxidase and lactoperoxidase in a manner similar to that described previously for peroxidase-catalyzed iodination.

On the basis of these observations and other findings, we have proposed a scheme which offers a possible explanation for iodide-dependent catalatic activity of thyroid peroxidase and lactoperoxidase. Compound 1 of the peroxidases is represented as EO, and oxidation of I$^-$ by EO is postulated to form enzyme-bound hypoiodite, represented in our scheme as [EOI]+. We suggest that the latter can react with H$_2$O$_2$ in a catalase-like reaction, with evolution of O$_2$. We postulate further that the same form of oxidized iodine is also involved in iodination of tyrosine, oxidation of thioureylene drugs, and oxidation of I$^-$, and that inhibition of catalatic activity by these agents occurs through competition with H$_2$O$_2$ for oxidized iodine.

In the course of experiments in which we measured the stoichiometry between H$_2$O$_2$ utilization and organic iodine formation in thyroid peroxidase- and lactoperoxidase-catalyzed iodination, we observed nonproductive disappearance of H$_2$O$_2$. This raised the possibility that these enzymes possess catalatic activity. Evidence for iodide-dependent catalatic activity of thyroid peroxidase and lactoperoxidase was recently presented in a brief report from this laboratory (1).

In the present communication we describe the stoichiometry experiments that led us to investigate the catalatic activity of thyroid peroxidase and lactoperoxidase. These are described in the Miniprint. The main text of the paper presents further studies on the mechanism of the iodide-dependent catalatic activity of these peroxidases. A scheme is proposed to explain this activity and to show its relationship to various peroxidative functions of these enzymes.

**MATERIALS AND METHODS**

**Measurement of O$_2$ Evolution—**Oxygen evolution was measured polarographically with a Clark-type oxygen electrode. The water-jacketed cell and the oxygen electrode were purchased from Gilson Medical Electronics, Middleton, WI. The input and output electronic components were built in our Bioengineering Department from schematic diagrams kindly provided by Gilson. Stirring was accomplished with a TRI-R model MS-7 micro-submersible stirrer (TRI-R Instruments, Rockville Center, NY), and recording was performed on a Gilford model 242 recorder (Gilford Instruments, Oberlin, OH).

Incubations were performed in 0.067 M phosphate, pH 7.0, at 24°C. All of the components of the incubation mixture, minus the enzyme, were added to the reaction cell in a total volume of 1.85 ml. After the cell was sealed with the glass, capillary bore stopper, the baseline was allowed to stabilize (1-2 min). The reaction was started by adding thyroid peroxidase, lactoperoxidase, or catalase in a small volume (<10 µl) through the capillary bore of the stopper. A Drummond microlitragal dispenser (Drummond Scientific Co, Broomall, PA) was very convenient for this purpose. Instrument response was determined in each experiment by addition of catalase (1 µg/ml) to a solution containing 100 µM H$_2$O$_2$ (equivalent to 50 µM O$_2$). The linearity of the response was established by adding small aliquots of H$_2$O$_2$ in 20 µM increments to 1 µg/ml of catalase in the reaction chamber. Electrode membranes were replaced daily.

When incubations were performed with lactoperoxidase, it was necessary to wash the cell extensively to remove residual enzyme, presumably adsorbed to the membrane or to the glass wall of the cell. This was accomplished by rinsing the reaction cell successively with several rinses each of deionized water, 0.7 M NH$_4$OH, 95% ethanol, and finally deionized water again. Rinsing with water alone sufficed to remove residual thyroid peroxidase or catalase from the reaction cell after these had been used in the incubation system.

**Catalase—**Bovine liver catalase (2 x crystallized, suspension in water containing 0.1% thymol, 30,000-40,000 units/mg) was purchased from Sigma (catalog No. C-100). An aliquot of the well mixed

1 Portions of this paper dealing with the stoichiometry between H$_2$O utilization and organic iodine formation (including Figs. 1-6, and Table I) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-1466, cite the authors, and include a check or money order for $4.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Fig. 7. Effect of I\(^{-}\) concentration on degradation of H\(_2\)O\(_2\) by thyroid peroxidase (TPO) and lactoperoxidase (LPO). The incubation mixtures contained 100 \(\mu\)M H\(_2\)O\(_2\) and either 1.3 mg/ml of thyroid peroxidase (left panel) or 1.0 mg/ml of lactoperoxidase (right panel) in 0.067 M phosphate, pH 7.0. The concentration of I\(^{-}\) was varied as indicated. The reaction was initiated at 37°C by addition of the enzymes, and aliquots (2.5 \(\mu\)l) were removed at intervals for measurement of the H\(_2\)O\(_2\) concentration.

| Time (min) | TPO | LPO |
|------------|-----|-----|
| 0          | 100 | 100 |
| 1          | 80  | 80  |
| 2          | 60  | 60  |
| 3          | 40  | 40  |
| 4          | 20  | 20  |

**RESULTS**

Effect of Iodide Concentration on Degradation of H\(_2\)O\(_2\) by Thyroid Peroxidase and Lactoperoxidase—Fig. 7 shows that the disappearance of 100 \(\mu\)M H\(_2\)O\(_2\) from an incubation mixture containing thyroid peroxidase or lactoperoxidase was highly dependent on the I\(^{-}\) concentration. Very little, if any, H\(_2\)O\(_2\) was degraded in the complete absence of I\(^{-}\). However, as little as 1 \(\mu\)M I\(^{-}\) had a definite stimulatory effect, especially with thyroid peroxidase. Increasing concentrations of I\(^{-}\) resulted in progressive increases both in the rate and the extent of H\(_2\)O\(_2\) disappearance. At the highest I\(^{-}\) concentration (100 \(\mu\)M) all of the H\(_2\)O\(_2\) was degraded within 1 min in the presence of lactoperoxidase, and more than 90% was degraded in a similar period in the presence of thyroid peroxidase. Low concentrations of I\(^{-}\) were more effective with latter, but high concentrations of I\(^{-}\) were more effective with the former. Presumably, therefore, (K\(_{m}\) for I\(^{-}\)) is greater for thyroid peroxidase but V\(_{max}\) is greater for lactoperoxidase.

Only a small fraction of the H\(_2\)O\(_2\) that disappeared from the incubation mixture could be accounted for by oxidation of I\(^{-}\) to I\(^{2-}\). This was most apparent in the samples containing 1 \(\mu\)M I\(^{-}\). Under these conditions 25 \(\mu\)M H\(_2\)O\(_2\) was degraded by thyroid peroxidase and 15 \(\mu\)M H\(_2\)O\(_2\) by lactoperoxidase. In samples containing 100 \(\mu\)M I\(^{-}\) only about 5 \(\mu\)M I\(^{-}\) was formed (data not shown), compared to the 80–100 \(\mu\)M H\(_2\)O\(_2\) that disappeared from the reaction mixture. Utilization of H\(_2\)O\(_2\) for I\(^{-}\) oxidation, therefore, could not account for the disappearance of H\(_2\)O\(_2\) observed in Fig. 7. Rather, it appeared that thyroid peroxidase and lactoperoxidase display catalytic activity in the presence of iodide. Further evidence for this was obtained in experiments measuring O\(_2\) evolution, described in the following section.

O\(_2\) Evolution from H\(_2\)O\(_2\) Catalyzed by Thyroid Peroxidase, Lactoperoxidase, and Catalase: Effect of Iodide and Iodine Acceptor—Fig. 8 shows the results of experiments in which O\(_2\) evolution was measured in incubation mixtures containing H\(_2\)O\(_2\) and thyroid peroxidase, lactoperoxidase, or catalase. Fig. 8a shows results obtained with thyroid peroxidase in the absence of iodide. Under these conditions very little evolution of O\(_2\) was detected. However, in the presence of 10 \(\mu\)M I\(^{-}\) (Fig. 8b), a rapid and marked evolution of O\(_2\) was observed. Addition of catalase after the reaction had plateaued showed no further evolution of O\(_2\), indicating that all the H\(_2\)O\(_2\) had been degraded. Fig. 8c shows results obtained when the incubation mixture included an iodine acceptor (150 \(\mu\)M tyrosine). Under these conditions O\(_2\) evolution was greatly reduced, both in rate and in extent. Only about 8 nmol/ml of O\(_2\) were evolved, compared to about 50 nmol/ml observed in the absence of tyrosine (Fig. 8d). The much reduced O\(_2\) evolution cannot be attributed to lack of H\(_2\)O\(_2\), since addition of catalase after the reaction had plateaued resulted in release of an additional 36 nmol of O\(_2\). It seems more likely that utilization of the iodide for tyrosine iodination was responsible for the greatly reduced catalatic activity, since in other experiments (results not shown) it was observed that under the conditions of Fig. 8c almost all the iodide was utilized for tyrosine iodination within 1 min after the initiation of the reaction.

Fig. 8, d, e, and f, shows results of analogous experiments performed with lactoperoxidase. As in the case of thyroid peroxidase, very little O\(_2\) was evolved in the absence of I\(^{-}\) (Fig. 8d). However, in the presence of 25 \(\mu\)M I\(^{-}\), O\(_2\) evolution was very evident (Fig. 8e). The reaction was slower with lactoperoxidase than with thyroid peroxidase, in agreement with the results shown in Fig. 7. When tyrosine was present in the incubation mixture (Fig. 8f), O\(_2\) evolution was greatly diminished, although not quite to the low level observed with thyroid peroxidase (Fig. 8c). The somewhat higher level obtained with lactoperoxidase is probably attributable to the higher iodide concentration that was used with this enzyme.

Results obtained with catalase are shown in Fig. 8, g, h, and i. Degradation of H\(_2\)O\(_2\) by catalase was unaffected by 100 \(\mu\)M iodide (Fig. 8h), or by the presence of 100 \(\mu\)M I\(^{-}\) + 150 \(\mu\)M tyrosine (Fig. 8i). In other experiments (results not shown) we observed that catalase does not catalyze iodination of tyrosine.

Effect of KSCN on Thyroid Peroxidase Degradation of...
H$_2$O$_2$. Thiocyanate anion (SCN$^-$) is classified as a pseudohalide and bears many resemblances to iodide in its chemical behavior. It was of interest in the present study, therefore, to determine whether thiocyanate, like iodide, stimulates catalatic degradation of H$_2$O$_2$ by thyroid peroxidase and lactoperoxidase. Experiments were also performed with perchlorate (ClO$_4^-$), a potent inhibitor of iodide transport in the thyroid gland (30). Fig. 9A shows the disappearance of H$_2$O$_2$ from incubation mixtures containing H$_2$O$_2$, thyroid peroxidase, and either SCN$^-$ or ClO$_4^-$. No effect on H$_2$O$_2$ degradation was observed with ClO$_4^-$. On the other hand, a progressive increase in both the rate and extent of H$_2$O$_2$ degradation was observed with SCN$^-$ as its concentration was raised from 0 to 250 $\mu M$. Although the results with SCN$^-$ resemble those observed with iodide, SCN$^-$ was much weaker than I$^-$ in promoting H$_2$O$_2$ degradation. As seen in Fig. 9A, 100 $\mu M$ SCN$^-$ was less effective than 10 $\mu M$ I$^-$. It seemed possible, therefore, that the mechanism of H$_2$O$_2$ degradation observed with SCN$^-$ is different from that seen with I$^-$.

That this is indeed the case was demonstrated in experiments measuring O$_2$ evolution. As shown in Fig. 9B, no catalatic activity of thyroid peroxidase was detected in an incubation mixture containing 250 $\mu M$ SCN$^-$ (Curve a), in contrast to the results obtained in the presence of 100 $\mu M$ I$^-$ (Curve b). Thus, the disappearance of H$_2$O$_2$ observed with 250 $\mu M$ SCN$^-$ in Fig. 9A cannot be attributed to catalatic activity. Since SCN$^-$ has been reported (31) to be oxidized by lactoperoxidase + H$_2$O$_2$, it seemed likely that the observed disappearance of H$_2$O$_2$ could be explained by thyroid peroxidase-catalyzed oxidation of SCN$^-$.

Oxidation of SCN$^-$ by H$_2$O$_2$ in the presence of thyroid peroxidase was measured both spectrophotometrically and by examination of the reaction products of $^3$S-labeled SCN$^-$ (results not shown). By following the absorbance ($A_{500}$) of SCN$^-$ on addition of successive increments of H$_2$O$_2$, we observed that 2.7 mol of H$_2$O$_2$ were required for complete oxidation of 1 mol of SCN$^-$, a result in good agreement with the calculated value of 3.0 required for the reaction previously reported for lactoperoxidase-catalyzed oxidation of SCN$^-$ (31): 3H$_2$O$_2$ + SCN$^-$ + HSO$_3^-$ + 2H$_2$O + HCN. Results of the labeling experiments showed that $[^{35}S]$sulfate was the only labeled product of the peroxidase-catalyzed oxidation of SCN$^-$ (identified by filter paper chromatography in ethanol/ammonium acetate).

Effect of Methimazole on Iodide-dependent Catalatic Activity of Thyroid Peroxidase—In a previous study in this laboratory (32) we showed that the thioureylene drug, MMI, inhibits thyroid peroxidase-catalyzed iodination reversibly or irreversibly. The type of inhibition is determined largely by the ratio of iodide to drug. When this ratio is high, enzyme-catalyzed oxidation of the drug is favored and inhibition of iodination is reversible. However, at lower iodide to drug ratios, thyroid peroxidase is rapidly inactivated, and inhibition of iodination is irreversible. It was of interest to examine the effect of MMI on the iodide-dependent catalatic activity of thyroid peroxidase.

Results obtained with MMI are illustrated in Fig. 10. Effects of 10, 25, and 50 $\mu M$ MMI were determined on H$_2$O$_2$ disappearance (Fig. 10A) and on O$_2$ evolution (Fig. 10B) in incubation mixtures containing 100 $\mu M$ I$^-$ and 100 $\mu M$ H$_2$O$_2$. At the lower concentrations of MMI (10 and 25 $\mu M$), all the H$_2$O$_2$ disappeared from the reaction mixture. The rate was slower with 25 $\mu M$ than with 10 $\mu M$ MMI. Both drug concentrations gave slower rates than the control sample containing no MMI. At the highest MMI concentration (50 $\mu M$), both the rate and the extent of the H$_2$O$_2$ disappearance were markedly inhibited. Under these conditions only about 25% of the H$_2$O$_2$ disappeared from the reaction mixture.

Investigation of the effect of MMI on the catalatic activity of thyroid peroxidase, illustrated in Fig. 10B, provided information about the mechanism of the H$_2$O$_2$ disappearance depicted in Fig. 10A. The observation that no O$_2$ was evolved in the presence of 50 $\mu M$ MMI (Curve d) indicates that the disappearance of about 25% of the H$_2$O$_2$ from the reaction mixture (Fig. 10A) cannot be attributed to catalatic activity of thyroid peroxidase. It appeared likely, therefore, that H$_2$O$_2$ was utilized to oxidize MMI. This was shown to be the case by performing the reaction in a cuvette and following the peak absorbance of MMI (A$_{250}$) in the recording spectrophotometer (results not shown). The decrease in A$_{250}$ corresponded to the disappearance of approximately 40 nmol/ml of MMI. Assuming that the sole oxidation product was the disulfide of MMI, this reaction would consume 20 nmol/ml of H$_2$O$_2$. Within the limits of the experimental error of the measurements, this could readily explain the observed disappearance of H$_2$O$_2$ from the reaction mixture. Parallel experiments in which enzyme activity was followed by guaiacol...
Iodide-dependent Catalatic Activity of Peroxidases

**Fig. 9.** Effect of KSCN and NaNClO₄ on H₂O₂ degradation and on O₂ evolution by thyroid peroxidase (TPO). The incubation mixtures contained 100 μM H₂O₂ and 1.3 μg/ml of thyroid peroxidase in 0.067 M phosphate, pH 7.0, at 24 °C. A shows effect of 250 μM ClO₄⁻ and of varying concentrations of SCN⁻ on H₂O₂ degradation. Aliquots (2.5 μl) were removed at intervals for measurement of H₂O₂ concentration. B shows results of oxygraph experiments comparing effects of 250 μM SCN⁻, 250 μM ClO₄⁻, and 100 μM I⁻ on O₂ evolution. Additions were made as indicated on the figure.

**Fig. 10.** Effect of MMI on iodide-dependent degradation of H₂O₂ by thyroid peroxidase (TPO); correlation with MMI effect on O₂ evolution. The incubation mixtures contained 100 μM H₂O₂, 100 μM I⁻, and 1.3 μg/ml of thyroid peroxidase in 0.067 M phosphate, pH 7.0, at 24 °C. A shows effect of various concentrations of MMI on H₂O₂ degradation after initiation of the reaction with thyroid peroxidase. B shows effect of the same concentrations of MMI on O₂ evolution as determined in the oxygraph. Additions were made as indicated on the figure.

As shown in our previous study (32), demonstrated that the thyroid peroxidase was completely inactivated during the course of the MMI oxidation and that failure to oxidize the MMI to higher oxidation products could be attributed to enzyme inactivation.

In contrast to the results with 50 μM MMI, there was extensive catalatic activity of thyroid peroxidase in the presence of 25 μM MMI (Fig. 10B, Curve c). Under the latter conditions, the O₂ evolved on addition of the enzyme was equivalent to 75 μM H₂O₂, showing that the disappearance of H₂O₂ in the presence of 25 μM MMI in Fig. 10A was largely attributable to catalatic activity of thyroid peroxidase. The remainder of the H₂O₂ (25 μM) was presumably utilized for oxidation of the 25 μM MMI in the reaction mixture. The stoichiometry of the reaction (1 mol of H₂O₂/mol of MMI) indicates that the MMI was oxidized beyond the disulfide stage. As shown in our previous study (32), under conditions where MMI was oxidized to higher oxidation products, inactivation of thyroid peroxidase was not a major factor in limiting enzyme activity.

Results obtained with 10 μM MMI were comparable to those observed with 25 μM MMI. When the reaction mixture contained 10 μM MMI, the O₂ evolved on addition of thyroid peroxidase was equivalent to 89 μM H₂O₂ (Fig. 10B, Curve b). The remainder of the H₂O₂ was presumably utilized for oxidation of the 10 μM MMI in the incubation mixture.

Correlation between the Effect of MMI on O₂ Evolution and on Iodination—It may be seen in Fig. 10B that there was a time lag between the addition of thyroid peroxidase and the start of O₂ evolution in samples containing 10 and 25 μM MMI. We reported (32) a similar (though longer) lag in previous studies in which we investigated the inhibitory effects of MMI on thyroid peroxidase-catalyzed iodination in reaction mixtures containing glucose-glucose oxidase as the source of H₂O₂. It was of interest in this connection to determine whether, under the conditions of the present ex-
periment, we would observe a lag in iodination comparable to that seen in Fig. 10B. Accordingly, experiments were performed under the conditions used in Fig. 10B, except that an iodine acceptor (bovine serum albumin) was added to the reaction mixture. The results are shown in Fig. 11. A lag in iodination was observed, which compared very well with that seen in the oxygraph experiments. With 25 μM MMI, iodination was completely inhibited for 30 s, agreeing very closely with the lag in catalatic activity shown in Fig. 10B. With 10 μM MMI, iodination was greatly inhibited at 15 s, corresponding closely to the approximately 10-s lag observed for catalatic activity. Both iodination and catalatic activity were irreversibly inhibited by 50 μM MMI, presumably because the thyroid peroxidase was inactivated under these conditions (32).

Effect of Carbimazole on H₂O₂ Degradation and Catalatic Activity—Carbimazole is a derivative of MMI, which, unlike MMI, does not readily inactivate thyroid peroxidase. As described in a previous communication (32), it acts as a purely reversible inhibitor of thyroid peroxidase-catalyzed iodination. It was of interest, therefore, to compare its effects with those of MMI described in the preceding section. Fig. 12A shows the effect of 50 μM CBZ on H₂O₂ degradation in a reaction mixture containing 100 μM H₂O₂ and either 0, 25, or 100 μM I⁻. All of the H₂O₂ was degraded in the presence of both concentrations of iodide, but the rate was much faster with 100 μM I⁻. Under the latter conditions all of the H₂O₂ disappeared within 3 min. These results contrast with those for MMI, which, under similar conditions, showed the disappearance of only 25% of the H₂O₂ (Fig. 10A). The effects of 50 μM CBZ on catalatic activity are shown in Fig. 12B. In the absence of I⁻, no catalatic activity was observed, and subsequent addition of catalase resulted in evolution of 50 μM O₂, indicating that none of the H₂O₂ had been degraded (in agreement with the results in Fig. 12A). In the presence of 100 μM I⁻ there was likewise no catalatic activity of thyroid peroxidase (Fig. 12B). However, in this case there was no O₂ evolution on subsequent addition of catalase, indicating that all the H₂O₂ had been utilized to oxidize the CBZ. These results also contrast with those observed with MMI under similar conditions (Fig. 10B).

Further evidence for a difference in mechanism between CBZ and MMI was obtained in the following experiment (results not shown). In the presence of 100 μM H₂O₂ and 100 μM I⁻, both drugs at 50 μM completely inhibited O₂ evolution on addition of thyroid peroxidase. However, addition of 100 μM H₂O₂ after 5 min to the sample containing CBZ resulted in a marked evolution of O₂, indicating that the thyroid peroxidase was still active. A similar addition of H₂O₂ to the MMI sample resulted in no evolution of O₂, indicating that enzyme inactivation had occurred with this drug.

Iodination studies performed as in Fig. 11 indicated that 50 μM CBZ inhibited iodination more than 95% (results not shown). In this case the result resembled that obtained with MMI (Fig. 11). However, the mechanism of inhibition of iodination was different. CBZ inhibited iodination competitively by utilizing the H₂O₂ for its own oxidation. However, MMI inhibited iodination primarily through inactivation of thyroid peroxidase.

Effect of Excess I⁻ on Catalatic Effect of Thyroid Peroxidase—High concentrations of iodide inhibit thyroid peroxidase-catalyzed iodination (6, 8), presumably by utilizing the I⁻ for I₂ formation rather than for iodination. It was of interest, therefore, to test the effect of increased concentrations of I⁻ on catalatic activity. Experiments were performed both with thyroid peroxidase and with lactoperoxidase, and the results are shown in Fig. 13. Catalatic activity of both enzymes was progressively inhibited as the concentration of I⁻ was raised from 30 μM to 10 mM. A slight inhibitory effect was observed at 100 μM I⁻, the standard concentration used in most of our studies. The inhibitory effect of I⁻ was greater for lactoperoxidase than for thyroid peroxidase. This correlates with observations made in this laboratory indicating that lactoperoxidase is much more active than thyroid peroxidase in oxidizing I⁻ to I₂ (data not shown). As the concentration of I⁻ was increased in the incubation mixtures, a progressive increase in the formation of I₃⁻ was observed, evident by its characteristic color. This suggested that oxidation of I⁻ to I₂ was a competing reaction and that the inhibition of catalatic activity observed in Fig. 13 involves competition between H₂O₂ and I⁻ for some common intermediate.
The mechanism of thyroid peroxidase- and lactoperoxidase-catalyzed iodination of tyrosine and tyrosyl residues in protein has been discussed by various investigators. Mechanisms involving an iodine free radical (2, 8, 9), iodinium ion, (13-15, 34, 35), or hypiodiate (1, 10) have been proposed. According to all these schemes it would be expected that 1 mol of H$_2$O$_2$ is required for organic binding of 1 mol of iodide. In the present study we examined the stoichiometric relationship where H$_2$O$_2$ was limiting. The reaction was initiated at 24 °C with either 1.3 µg/ml of thyroid peroxidase or 1.0 µg/ml of lactoperoxidase and was continued until all the H$_2$O$_2$ was utilized.

**DISCUSSION**

The distinction between peroxidases and catalase is generally based on the manner in which they utilize H$_2$O$_2$. Catalase catalyzes the rapid degradation of H$_2$O$_2$ to form oxygen. Peroxidases catalyze the utilization of H$_2$O$_2$ for the oxidation of a donor compound. Thomas et al. (36) showed that chloroperoxidase, a mold enzyme, displays significant catalatic activity and that it bridges some of the classical differences between enzymes of the peroxidase and catalase type. The catalatic activity of chloroperoxidase was observed both in the presence and in the absence of Cl$^-$. The pH optimum for the halide-independent reaction (4.5) was greater than that for the halide-dependent reaction (2.75). Subsequent studies by Manthy and Hager (37) demonstrated that bromoperoxidase, isolated from a marine alga, also catalyzed the evolution of O$_2$ from H$_2$O$_2$. Significant activity was observed at pH 6.8 in the absence of halide, but the activity was greatly enhanced in the presence of 100 mM Br$^-$. In the present study we have shown that thyroid peroxidase and lactoperoxidase display significant catalatic activity. This activity, in contrast to that of chloroperoxidase and bromoperoxidase, is completely iodide-dependent. A possible catalatic activity of thyroid peroxidase was briefly reported by Ohtaki et al. (13, 14), but no mention was made of an iodide requirement.

To elucidate the mechanism of the iodide-dependent catalatic activity, we have made use of previously reported (32, 38) inhibitors of thyroid peroxidase- and lactoperoxidase-catalyzed iodination. Following is a summary of observations made in this and in our previous study (1), which led to the development of a general scheme showing how the iodide-dependent catalatic activity may relate to the various peroxidative functions of these enzymes (see below).

1) Thyroid peroxidase and lactoperoxidase exhibit marked catalatic activity in the presence of iodide, based both on measurements of H$_2$O$_2$ disappearance and O$_2$ evolution. In the absence of iodide, the enzymes catalyze neither H$_2$O$_2$ disappearance nor O$_2$ evolution.

2) The presence of only 1 µM I$^-$ in an incubation mixture containing 100 µM H$_2$O$_2$ and 1.3 µg/ml of thyroid peroxidase resulted in the degradation of 25 µM H$_2$O$_2$ (Fig. 7). Clearly, therefore, oxidation of I$^-$ to I$_2$ could not stoichiometrically account for the disappearance of the H$_2$O$_2$, and it appeared the I$^-$ must be catalytically involved in the enzymatic reaction leading to H$_2$O$_2$ disappearance. In samples containing 100 µM I$^-$ it was possible to measure I$_2$ formed in the reaction, and again it was evident that oxidation of I$^-$ to I$_2$ was much too small to account for the measured disappearance of H$_2$O$_2$. Oxidation of iodide to iodate was negligible, as shown in our previous study (1).

3) The pseudohalogen, SCN$^-$, cannot replace I$^-$ as a promoter of catalatic activity, even though SCN$^-$ is readily oxidized by H$_2$O$_2$ + thyroid peroxidase or lactoperoxidase. As shown in Fig. 9, H$_2$O$_2$ was degraded in the presence of SCN$^-$, but no evolution of O$_2$ accompanied the reaction. In this case H$_2$O$_2$ was utilized solely for the oxidation of the anion, in contrast to the catalatic cleavage of H$_2$O$_2$ that occurred in the presence of I$^-$. Another anion, CI0$_4^-$, which competes with I$^-$ for a transport system in the thyroid, also had no stimulatory effect on the catalatic activity of thyroid peroxidase and lactoperoxidase (Fig. 9). Unlike SCN$^-$, CI0$_4^-$ is not oxidized by the peroxidase system.

4) The thioureylene drug, MMI, was shown in previous studies in this laboratory to act both as a reversible and irreversible inhibitor of thyroid peroxidase- and lactoperoxidase-catalyzed iodination, and a scheme was proposed to explain its mechanism of action (32). In the present study we examined the effects of MMI on the catalatic activity of thyroid peroxidase, and we observed that this reaction may also be inhibited reversibly or irreversibly by MMI, depending on the concentration of drug (Fig. 10). Moreover, in the presence of concentrations of MMI leading to reversible inhibition, a time lag was observed which corresponded closely in extent to the time lag seen in iodination experiments performed under similar conditions (Figs. 10 and 11).

5) The thioureylene drug, CBZ, is a derivative of MMI, but, unlike MMI, it does not readily inactivate thyroid peroxidase.
For this reason, as shown in a previous study (32), it acts only as a reversible inhibitor of thyroid peroxidase-catalyzed iodination. In the present study we have shown that CBZ inhibits the catalytic activity of thyroid peroxidase, and that in this case also, the inhibition is reversible (Fig. 12). Thus, the inhibitory effects of CBZ on iodination and on catalatic activity suggest that this drug acts as a competitive inhibitor in both reactions.

6) High concentrations of \( I^- \) were shown to inhibit the catalytic activity of thyroid peroxidase and lactoperoxidase (Fig. 13). A similar inhibition of thyroid peroxidase-catalyzed iodination by excess \( I^- \) was reported previously (6, 8), and in both instances oxidation of \( I^- \) to \( I^- \) appeared to be correlated with the degree of inhibition.

7) Conversion of thyroid peroxidase and lactoperoxidase to a less active or inactive form (Compound I11) by excess \( H_2O_2 \) has been described by other investigators (13, 39), and it was necessary to consider the possibility that the iodide dependence of the catalatic activity of these peroxidases might reflect a protective action of iodide against inactivation by \( H_2O_2 \). We examined this possibility in our previous study (1), and we observed that although thyroid peroxidase, and to a lesser extent lactoperoxidase, were significantly inactivated on addition of 100 \( \mu \)M \( H_2O_2 \), the inactivation was not prevented by iodide. Thus, the role of iodide in promoting catalatic activity cannot be attributed to a protective effect on the enzymes.

Discussion of a possible mechanism for iodide-dependent catalatic activity must also be based on an understanding of the mechanism of thyroid peroxidase- and lactoperoxidase-catalyzed iodination. The complete details of the iodination mechanism have not been elucidated, but the most recent evidence favors a 2-electron rather than a free radical mechanism (13-15). In their extensive studies on the mechanism of chloroperoxidase-catalyzed chlorination, Hager and co-workers (40, 41) postulated that the chlorinating intermediate may be represented as an enzyme-bound halogen ion (Cl\(^-\)), or as a hypohalite ion. Morrison and Schonbaum (10) proposed a scheme for peroxidase-catalyzed iodination involving an enzyme-hypohaloid acid complex. The postulation of a hypohaloid intermediate in the iodination reaction offers a possible explanation for iodide-dependent catalatic activity observed in the present study. Of special interest in this connection is the following reaction, reported by Liebhafsky (42):

\[
10^- + H_2O_2 \rightarrow O_2 + H_2O + I^- \\
\]

This reaction provides a chemical basis for \( O_2 \) evolution, the essential element of catalatic activity.

In Fig. 14 we propose a scheme to explain iodide-dependent catalatic activity of thyroid peroxidase and lactoperoxidase, and to show the relationship of this reaction to various peroxidative functions of these enzymes. A discussion of the various reactions in Fig. 14 is provided in the following comments.

(a) Reaction I shows the formation of Compound I, represented in the scheme as EO. The exact nature of Compound I of peroxidase enzymes is not fully elucidated. Dolphin et al. (43) have presented evidence that Compound I is best represented as an Fe(IV)-porphyrin \( \pi \)-cation radical species. Hager and co-workers (40, 41) have accepted this formulation and, in addition, that Compound I of chloroperoxidase contains only a single oxygen atom derived from substrate peroxyde. Morrison and Schonbaum (10) have used the representation, EO, to denote Compound I of other peroxidases.

(b) Reactions 2 and 3 are independent of \( I^- \) and show that EO reacts directly with donors such as SCN\(^-\) and guaiacol, thus catalyzing oxidation of these compounds by \( H_2O_2 \).

(c) Reaction 4 shows that EO may be involved in catalyzing the coupling of 2 molecules of diiodotyrosine (DIT) contained within the protein, thyroglobulin, to form the thyroid hormone, thyroxine (\( T_4 \)). We have shown previously (24, 44) that thyroid peroxidase catalyzes this coupling reaction in the absence of \( I^- \). Courtin et al. (39) have recently proposed the existence of two different forms of lactoperoxidase Compound I, one of which catalyzes iodination and the other coupling. This would imply that there may be two different forms of EO. If this proposal is confirmed, the scheme in Fig. 14 would require some revision, but this would not affect the major conclusions of the present study.

(d) Reactions 5 and 6 are also independent of \( I^- \) and show that an oxidized form of the enzyme may be inactivated by 6-propyl-2-thiouracil (PTU) and MMI (33) or by \( H_2O_2 \) (13, 39). Although for the sake of simplicity the scheme shows this inactivation to occur with Compound I, Compound II may also be involved in these reactions.

(e) Reaction 7 shows the oxidation of \( I^- \) by EO to form an intermediate which we have represented as [EOI]. This representation differs in charge from the [EOII] postulated by Holmberg et al. (40) as the intermediate in chloroperoxidase-catalyzed chlorination, because we thought it necessary to preserve charge balance in the reaction. In the hypothetical complex, [EOI], the I is present in an oxidation state equivalent to \( I^- \). It seems possible that [EOI] would give rise to \( E + IO^- \), where E represents the native (ferric) form of the enzyme. In such a case, the reactions with [EOI] in Fig. 14 may involve the free anion, \( IO^- \), rather than enzyme-bound hypohalide. It is of interest that an analogous intermediate, OSCN\(^-\), has been postulated in lactoperoxidase-catalyzed oxidation of the pseudohalide, SCN\(^-\) (31).

(f) Reaction 8 is the basis for the iodide-dependent catalatic activity observed in the present study. We propose that the intermediate, [EOI], may react with a second molecule of \( H_2O_2 \) in a catalase-like reaction, with liberation of \( O_2 \). The reduction of hypohaloid acid or hypohalide by \( H_2O_2 \) is a known reaction (42, 45).

(g) Reactions 9, 10, and 11 represent well described peroxidative actions of thyroid peroxidase and lactoperoxidase. The major difference between Fig. 14 and an earlier proposed scheme (32) is that the oxidized Intermediate is now represented as [EOII], instead of the less specific, E-I-. The new scheme in Fig. 14 explains the observed inhibitory effects of \( I^- \), thioureyene drugs, and tyrosine on the catalatic activity of thyroid peroxidase and lactoperoxidase by proposing that...
all these compounds compete with H$_2$O for a common form of oxidized iodine. We believe that the scheme presented in Fig. 14 is consistent with all the data presented in this paper.

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Supplementary Material to
MECHANISM OF IODIDE-DEPENDENT ACTIVITY IN THYROID PEROXIDASE AND LACTOPEROXIDASE

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The rate of tyrosine iodination was catalyzed by TPO, glucose, and glucose oxidase, and either A) 1.3 μM TPO + 0.5 μM BSA, B) 0.5 μM glucose, or C) 1.3 μM TPO + 0.5 μM BSA, 0.5 μM glucose, or D) 0.1 μM glucose + 1.0 μM BSA. The incubation mixture used for the measurement of iodination contained 100 μM BSA, 10 μM iodide, and either 150 μM tyrosine or 1 μM low molecular weight thyroglobulin in 0.1 M phosphate buffer, pH 7.0. The reaction was initiated with varying concentrations of H₂O₂ and was measured after 5 min. A similar increase further iodination occurred after this time.

As low concentrations of H₂O₂, the ratio, moles 1 bound/μmol tyrosine, was slightly lower than 1:1, resembling the ratios observed when H₂O₂ was generated with glucose-glucose oxidase. However, the ratio fell off significantly as the H₂O₂ concentration increased, declining to values below 2.0 at 1.0 μM H₂O₂.

Figure 6 shows the time course of H₂O₂ disappearance and organic iodine formation when 100 μM H₂O₂ was added to the incubation mixture. It is apparent that by 3 min all the H₂O₂ (100 μM H₂O₂) had disappeared, whereas only 10% of organic iodine had been formed. Resulting that only one mole of iodine is required for the formation of one mole of organic iodine. Most of the iodide H₂O₂ has disappeared through some other process than organic iodine formation. This observation is in contrast to the results of other investigators, who report a much greater departure from stoichiometry of organic iodine formation. This observation is in agreement with the observation that the catalytic activity of catalase relative to the peroxidase activity increased with increasing concentration of H₂O₂. This would explain the much greater deviation from stoichiometry for an inactive catalase than for the active catalase. A lower level of catalytic activity at reduced concentrations of H₂O₂, with H₂O₂ generated by glucose-glucose oxidase, would explain why we found consistently higher values at a stoichiometric ratio of 1:1.

Relationship between H₂O₂ concentration and organic iodine formation during TPO-catalyzed iodination of BSA. The incubation mixture contained 1.3 μM TPO, 100 μM BSA, and either 150 μM tyrosine or 1 μM low molecular weight thyroglobulin in 0.1 M phosphate buffer, pH 7.0. The reaction was initiated with varying concentrations of H₂O₂, and the reaction mixture was measured after 5 min.

The results in Table 1 show the relationship between the rate of organic iodine formation and H₂O₂ concentration in all respects except that the rate of iodination was proportional to the concentration of H₂O₂, whereas the rate of iodination was inversely proportional to the concentration of H₂O₂. The results in Table 1 also show that the rate of iodination was inversely dependent on the concentration of H₂O₂. The results in Table 1 also show that the rate of iodination was inversely dependent on the concentration of H₂O₂. The results in Table 1 also show that the rate of iodination was inversely dependent on the concentration of H₂O₂. The results in Table 1 also show that the rate of iodination was inversely dependent on the concentration of H₂O₂.
Mechanism of iodide-dependent catalatic activity of thyroid peroxidase and lactoperoxidase.
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