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IODIDE-DEPENDENT CATALATIC ACTIVITY OF THYROID PEROXIDASE AND LACTOPEROXIDASE

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Thyroid peroxidase (TPO) and lactoperoxidase (LPO) display significant catalatic activity at pH 7.0 in the presence of low concentrations of iodide, based both on measurements of H_2O_2 disappearance and O_2 evolution. In the absence of iodide only minor catalatic activity was detected. The stimulatory effect of iodide could not be explained by protection of the enzymes against inactivation by H_2O_2. A mechanism is suggested involving an enzyme-hypoiodite complex as an intermediate.

In the course of experiments in which we attempted to measure the stoichiometry between H_2O_2 utilization and organic iodine formation in peroxidase catalyzed iodination, we observed non-productive disappearance of H_2O_2. This raised the possibility that these enzymes possess catalatic activity. In the present communication we provide evidence that both TPO and LPO display significant catalatic activity in the presence of a low concentration of iodide.

Materials and Methods

Catalatic activity was followed by two different procedures: 1) Disappearance of H_2O_2, and 2) O_2 evolution.

Measurement of H_2O_2 - The concentration of H_2O_2 was determined by the cytochrome c peroxidase procedure, essentially as described by Boveris et al. (1). Cytochrome c peroxidase was prepared from baker's yeast by the method of Nelson et al. (2). One ml of 1 μM cytochrome c peroxidase was placed in both the sample and reference cuvettes of a Varian-Cary Model 219 double beam spectrophotometer. An aliquot of the incubation mixture (2.5 μl) was removed at intervals after the initiation of the reaction and added to the sample cuvette. The spectrum between 435 and 385 nm was recorded, and the value for $A_{425-395}$ was taken as a measure of the H_2O_2 concentration. A standard curve with known amounts of H_2O_2 was run with each experiment. The H_2O_2 standards were prepared freshly from a 30% stock solution of H_2O_2, which had been standardized by iodimetric titration (3).

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Abbreviations: TPO, thyroid peroxidase; LPO, lactoperoxidase; CPO, chloroperoxidase

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Measurement of O_2 evolution - Oxygen evolution was followed with a Clark-type oxygen electrode, essentially as described by Estabrook (4). The instrument was calibrated using electron transport particles from beef heart and a standardized solution of NADH (4).

Thyroid peroxidase - Hog thyroid peroxidase was prepared as previously described (5). The preparation used in this study had a value for A_{410}/A_{280} of 0.50, and it was estimated to be about 80 percent pure (6).

Lactoperoxidase - This was purchased from PL Biochemicals and was dialyzed against 0.067 M phosphate, pH 7.0 before use. The value for A_{412}/A_{280} was 0.89.

Catalase - Bovine liver catalase (2X crystalline suspension in water containing 0.1% thymol) was purchased from Sigma Chemical Co. The stated activity was 30,000-40,000 Sigma units per mg protein.

Enzyme concentration - The concentration of both TPO and LPO was expressed in terms of protein, measured by the method of Lowry et al. (7) with crystalline BSA standards. Based on a M.W. of 78,500 for LPO (8) and a M.W. of 90,000 for TPO (5), we have assumed that 1 µg/ml LPO is equivalent in molar concentration to 1.3 µg/ml TPO. For catalase the protein concentration (mg/ml) was calculated as A_{280} X 0.667.

Measurement of I_2 and iodate - The concentration of I_2 was determined by diluting an aliquot of the incubation mixture with an equal volume of 0.02 N KI and measuring the absorbance of I_3^- at 353 nmeters. A standard curve with known concentrations of I_2 in 0.01 N KI was prepared simultaneously. The possibility of formation of iodate was examined by paper chromatography, using an incubation mixture containing ^{131}I labeled iodide (9).

Results

Effect of iodide on degradation of H_2O_2 by TPO or LPO - Fig. 1 shows the effect of iodide on the disappearance of 100 µM H_2O_2 from incubation mixtures containing TPO (Fig. 1A) or LPO (Fig. 1B). In the absence of iodide the enzymes produced very little decrease in the concentration of H_2O_2. However, in the presence of 10 µM I^- there was a marked disappearance of H_2O_2 from the solution. The initial rate of the reaction was more rapid with TPO than with LPO, but by 10 min the H_2O_2 had almost completely disappeared with both enzymes. When the I^- concentration was raised to 100 µM the rate of H_2O_2 degradation was markedly increased. Under these conditions about 97% of the H_2O_2 was degraded in one min in the presence of LPO and about 84% in the case of TPO.

The disappearance of H_2O_2 from the incubation mixture was accompanied by very little oxidation of I^- to I_2 (data not shown). Even in the samples containing 100 µM I^- only about 5 µM I_2 was formed. This could account for the disappearance of only about 5 µM H_2O_2 of the initially present 100 µM H_2O_2. Formation of iodate under these conditions was negligible. It seemed unlikely, therefore, that utilization of H_2O_2 for I^- oxidation could account for the observed disappearance of H_2O_2. Rather, it appeared that in the presence of I^-

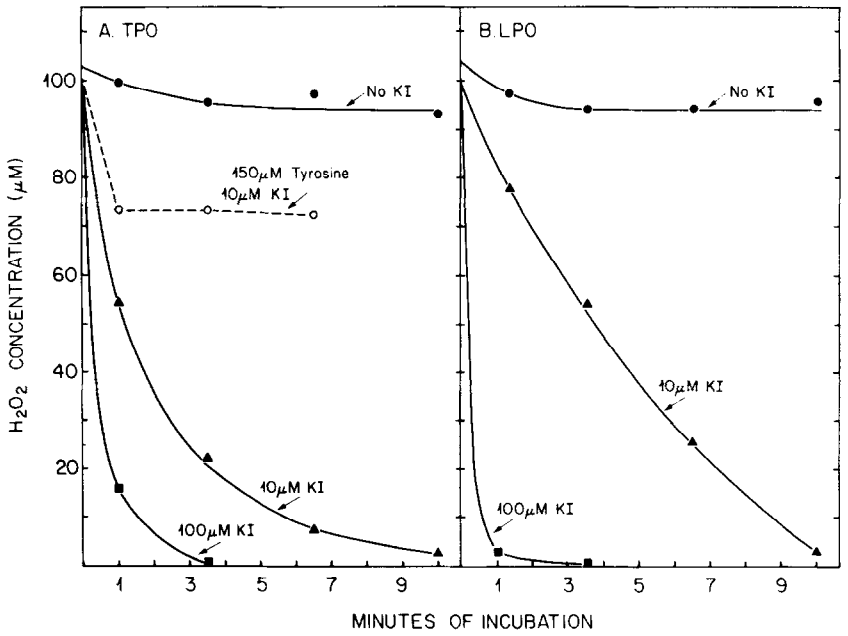

Fig. 1 Effect of iodide on degradation of H_2O_2 by TPO or LPO. Incubation tubes were prepared containing 100 μM H_2O_2 in 0.067 M phosphate, pH 7.0 at 37C, with or without iodide. At zero time a small volume of TPO or LPO was added to provide a final concentration of 1.3 or 1.0 μg/ml, respectively. At intervals small aliquots (2.5 μl) of the incubation mixture were removed for measurement of H_2O_2 concentration, as described under Methods.

both TPO and LPO displayed catalatic activity. Further evidence for this was obtained in experiments measuring O_2 evolution, as described below.

H_2O_2 degradation by TPO + iodide in the presence of tyrosine - When 150 μM tyrosine was present in the incubation mixture containing 100 μM H_2O_2 and 10 μM I^-, the catalatic action of TPO was greatly suppressed (Fig. 1A). Under these conditions H_2O_2 was utilized for iodination of tyrosine. However, the disappearance of H_2O_2 exceeded that required for iodination (assuming 1 molecule of H_2O_2 required to bind 1 atom of I). Presumably, therefore, some catalatic activity of TPO occurred even in the presence of the iodine acceptor. This was also observed in experiments in which O_2 evolution was measured (see below).

Inactivation of TPO and LPO by H_2O_2; effect of iodide - Conversion of TPO and LPO to a less active or inactive form (compound III) by excess H_2O_2 has been described previously (10,11). It was necessary, therefore, to consider the possibility that the iodide-dependence of the catalatic activity of TPO and

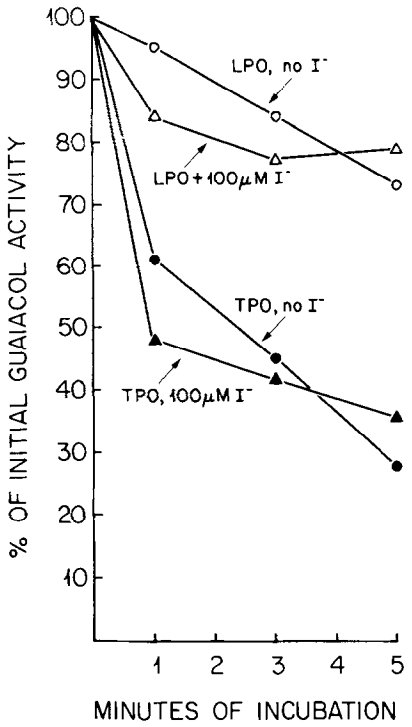

Fig. 2 Inactivation of TPO and LPO by H_2O_2. Incubation tubes were prepared containing 100 μM H_2O_2 in 0.067 M phosphate, pH 7.0 at 37C, with or without iodide. At zero time a small volume of TPO or LPO was added to provide a final concentration of 1.3 or 2.0 μg/ml, respectively. At intervals, 200 μl aliquots were removed for guaiacol assay as previously described (6).

LPO might reflect a protective action of iodide against inactivation by H_2O_2. Figure 2 shows the time course of inactivation of TPO and LPO by 100 μM H_2O_2 in the presence and absence of iodide. A very significant inactivation of TPO was observed in the case of TPO, but this was not diminished, and indeed appeared to be accelerated, in the presence of 100 μM I^-. These results indicate that the iodide-dependence of the catalatic action of TPO and LPO cannot be attributed to protection against inactivation by H_2O_2.

Measurement of O_2 evolution; effect of iodide - Fig. 3 shows the rate of formation of O_2 in solutions containing H_2O_2 and either TPO or LPO. In the absence of I^-, both peroxidases displayed only very minor catalatic activity. However, catalatic activity was greatly potentiated in the presence of I^-. The rate of evolution of O_2 was greater with 100 μM I^- than with 10 μM I^-, in

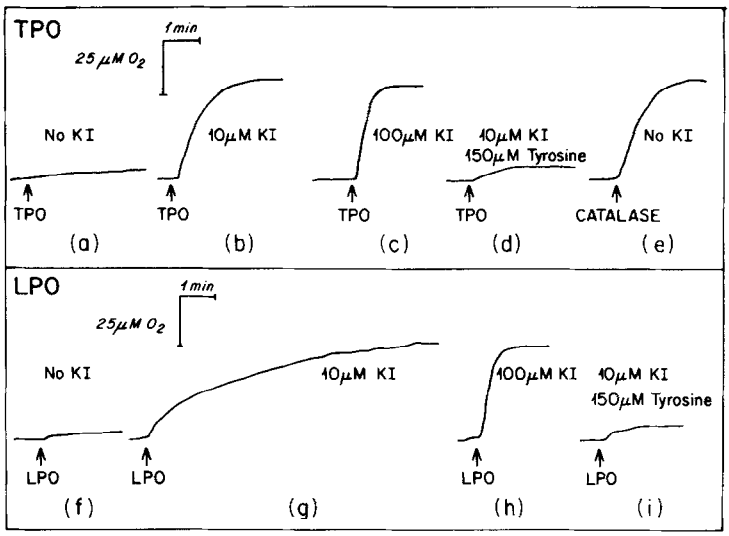

Fig. 3 Oxygen evolution from H_2O_2 incubated with TPO or LPO. Incubation was at 25C in a reaction chamber containing 2.6 ml of 100 μM H_2O_2 in 0.067 M phosphate, pH 7.0. TPO was added at 1.3 μg/ml, LPO at 1.0 μg/ml, and catalase at 0.37 μg/ml.

agreement with the H_2O_2 degradation data shown in Fig. 1. In the presence of 100 μM I^- and 100 μM H_2O_2 the catalatic activity of TPO and LPO per mg of protein was comparable to that of catalase. Catalase itself was not affected by iodide (not shown). In the presence of 150 μM tyrosine (Figs. 3d and 3i), O_2 evolution was greatly reduced, but not completely suppressed. Under these conditions, as indicated above (Fig. 1A), iodide was used primarily for iodination. However, some catalatic action occurred concurrently with iodination.

Discussion

Although a distinction is generally made between peroxidases and catalase based on their reactivity toward H_2O_2, it was shown by Thomas et al. (12) that chloroperoxidase (CPO) bridges some of the classical differences between enzymes of the peroxidase and catalase type. These studies demonstrated that CPO displays significant catalase activity, both in the presence of Cl^- (pH optimum 2.75) and in the absence of Cl^- (pH optimum 4.5). Ohtaki et al. (11) referred briefly to a catalase-like activity of TPO but did not specify an iodide requirement.

In the present study we have shown that TPO and LPO display significant catalatic activity. In contrast to CPO, however, this activity was observed at pH 7.0 and was almost completely iodide-dependent.

Discussion of possible mechanisms for the iodide-dependent catalatic action of TPO and LPO presupposes an understanding of the mechanism of TPO- and LPO-catalyzed iodination. This is a subject of continuing investigation. The most extensive studies on the mechanism of peroxidase-catalyzed halogenation are those of Hager et al. (13,14) on CPO-catalyzed chlorination. They suggested that a probable structure for the halogenating intermediate is an -OCl ligand on the ferric heme of the enzyme. Morrison and Schonbaum (15) extended this concept to peroxidase-catalyzed iodination and proposed that the reaction between compound I (of TPO and LPO) and iodide yields effectively an enzyme-hypoiodous acid complex, which may be represented as EOI. In the context of this formulation, we suggest that, in the absence of an iodine acceptor, this intermediate reacts with a second molecule of H_2O_2 in a catalase-like reaction. Reduction of hypoiodite by H_2O_2 has been studied by Liebhafsky (16,17), who reported the following reaction: $HIO + H_2O_2 \rightarrow H^+ + I^- + H_2O + O_2$. This reaction provides a chemical basis for the O_2 evolution observed in the present study. Such a reaction not only provides an explanation for the iodide-dependent catalatic activity of TPO and LPO, but it offers support for EOI as an intermediate in peroxidase-catalyzed iodination, as proposed by Morrison and Schonbaum. Further studies on the mechanism of the iodide-dependent catalatic activity of TPO and LPO will be reported in a subsequent communication.

Acknowledgements

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