THE PEROXIDATION OF MOLECULAR IODINE TO IODATE BY CHLOROPEROXIDASE[1, 2]

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Peroxidases catalyze a bewildering number of oxidation reactions with an apparent lack of substrate specificity for either the oxidant or the electron donor molecule. Thus, hydrogen peroxide, substituted hydrogen peroxides, molecular oxygen, hypochlorous acid and various inorganic oxidants can serve with varying degrees of success as the oxidizing agent, while phenols, aromatic amines, ene-diols, various leuco dyes, cytochrome c, and iodide ion can serve as electron donors in peroxidase reactions. Chloroperoxidase extends this donor list in the halide series to include bromide and chloride ions as well as iodide ion. Hence, this enzyme can catalyze the formation of molecular chlorine, bromine, and iodine from the respective halides and hydrogen peroxide, as reported by Hager, et al. (1967). In this report, we present evidence that chloroperoxidase can catalyze the oxidation of iodide ion beyond the molecular halogen level to an oxidation state of +5. This is the first report appearing in the literature of such a reaction being catalyzed by a peroxidase.

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[1]This is the fifth paper of a series dealing with chloroperoxidase. The preceding paper in this series is listed as reference (1).

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Methods and Materials. Chloroperoxidase was isolated from the growth medium of Caldariomyces fumago and purified as described by Morris and Hager (1966). Enzyme samples used in these experiments had a purity index of 1.1 (A_{403}:A_{280}), indicating an enzyme purity of about 80%. Iodine solutions were prepared by saturating glass distilled water with reagent grade iodine (Baker). Iodine concentrations were determined by the method of Hosoya (1963). Potassium iodide was added to the iodine solutions to a final concentration of 0.1M. The concentration of iodine was then determined from the absorbance at 350 mμ due to triiodide ion. Spectra were measured in a Cary 15 spectrophotometer against a buffer blank.

Experimental and Results. During the course of spectral studies on the mechanism of the chloroperoxidase-catalyzed iodination of tyrosine, it was noted that the presence of iodine rapidly accelerated the decomposition of the initial hydrogen peroxide complex of chloroperoxidase (analogous to complex I of horseradish peroxidase). Such behavior is typical for electron donors in peroxidase reactions. It thus appeared that iodine could serve as an electron donor in the peroxidase reaction and be oxidized to some higher oxidation state. It is easily established that iodine disappears from solution under peroxidative conditions in the presence of chloroperoxidase. Katzin (1953) has reported that iodine solutions have three absorption peaks, located at 462 mμ, 353 mμ, and 287 mμ. The latter two peaks are characteristic of triiodide ion. The 462 peak arises from solvated iodine. Figure 1, curve A shows the spectrum obtained when 1 mM iodine and 10 mM hydrogen peroxide were present together in 0.1M potassium phosphate buffer, pH 2.8. No change was observed in this spectrum after incubation for twenty minutes. However, thirty seconds after the addition of chloroperoxidase (0.75 μM), the absorption peaks due to the presence of iodine had completely disappeared (Figure 1, curve B). This reaction required both the presence of hydrogen peroxide and chloroperoxidase. During the course of iodine disappearance, the reaction mixture rapidly changed from the brown color of iodine to a clear solution. When iodide ion was added

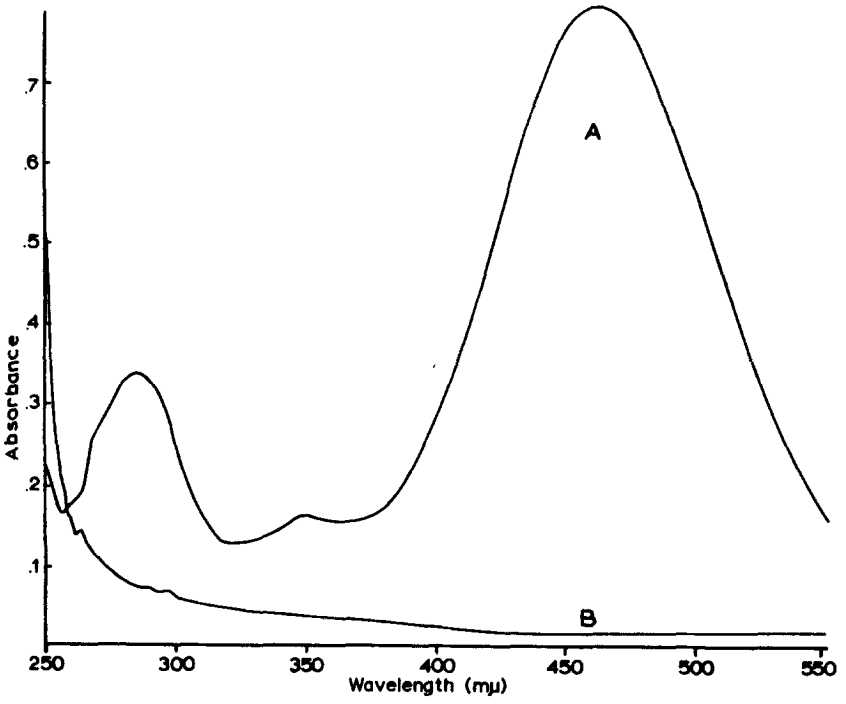

Figure 1. Oxidation of Iodine by Chloroperoxidase. The oxidation of iodine by chloroperoxidase was followed spectrally. The initial reaction mixture contained 1 mM iodine, 0.1M potassium phosphate buffer, pH 2.8, and 10 mM H_2O_2 in a total volume of 1 ml. The spectrum was taken in a Cary 15 spectrophotometer versus a buffer blank (curve A). Thirty seconds after the addition of chloroperoxidase (30 μg) the spectrum was repeated (curve B).

to this clear solution, molecular iodine reformed instantaneously. The amount of iodine that reformed depended on the original concentration of iodine present.

To quantitate this reaction more carefully, a known amount of iodide ion was substituted for the iodine solution. It has been previously documented by Hager et al. (1966) that chloroperoxidase catalyzes the rapid peroxidation of iodide ion to form molecular iodine, as shown in the following equation.

$$2\ I^- + H_2O_2 \rightarrow I_2 + 2\ OH^- \qquad (1)$$

Thus, a fixed amount of iodide ion can be used to generate a known amount of iodine quantitatively. In separate experiments, different initial concentrations of iodide ion were incubated with chloroperoxidase and excess hydrogen peroxide. During the incubation period, iodide ion was oxidized to the molecular species,

which then disappeared as it was oxidized to a higher oxidation state. A ten minute incubation under the conditions shown in Table 1 was found to be ample for the formation and further oxidation of molecular iodine. Also, this incubation period was sufficient for the decomposition of the excess hydrogen peroxide through the catalatic activity of chloroperoxidase. After the incubation period, 1 M potassium iodide was added to a final concentration of 0.1M, and the resulting formation of triiodide ion was measured spectrophotometrically. The stoichiometry shows that 3 molecules of iodine were formed for each molecule of iodide ion originally present (Table 1). The amount of hydrogen peroxide used was immaterial as long as it was well in excess (10 or more times greater than the initial iodide ion concentration). If iodide ion was initially absent from the reaction mixture, trivial amounts of iodine were formed (see Table 1, lines 1c and 2b).

Table 1. Number of Oxidizing Equivalents in the Product of Iodine Oxidation.

Reaction Mixture	Equivalents of Iodine in Product (mμmoles)	Relative Equivalents of Iodine in Product per Original I^-
1. 1 μmole H_2O_2 plus...		
a. 10 mμmoles KI	30	3.0
b. 100 mμmoles KI	273	2.7
	269	2.7
c. Minus KI	6	-
2. 10 μmoles H_2O_2 plus...		
a. 1000 mμmoles KI	3050	3.1
	3120	3.1
b. Minus KI	230	

All reactions were carried out in 0.9 ml of solution, containing, in addition to the listed components, 100 μmoles of potassium phosphate buffer, pH 2.8, and 30 μg of chloroperoxidase. After a 10 minute incubation period 100 μmoles of potassium iodide (0.1 ml) was added. The amount of iodine present after the addition of potassium iodide was determined from the absorption at 350 mμ (see Methods and Materials). For samples 1b and 2a, dilutions of 1 to 10 and 1 to 100 respectively were made in 0.1M potassium iodide, 0.1M potassium phosphate buffer, pH 2.8, to adjust the absorbance values to a reasonable range.

The stoichiometry is consistent with iodide ion being oxidized all the way to iodate ion, as can be seen from the following equations. Equation 2 shows the overall reaction that takes place during the incubation period. For each iodide ion originally present in the incubation mixture, one molecule of iodate is formed via the oxidation of iodide to iodine and the subsequent oxidation of the molecular species.

$$I^- + 3\ H_2O_2 \rightarrow IO_3^- + 3\ H_2O \qquad (2)$$

The addition of iodide ion after the incubation period results in the reduction of the iodate ion to form molecular iodine (equation 3). Thus, for each iodide

$$IO_3^- + 5\ I^- + 6\ H^+ \rightarrow 3\ I_2 + 3\ H_2O \qquad (3)$$

ion originally present, 3 molecules of iodine would eventually be formed.

To further confirm the identity of the reaction product, it was isolated from a reaction mixture and used in a colorimetric test for the presence of iodate ion. The complete reaction mixture, containing 1 mM potassium iodide, 10 mM hydrogen peroxide, 0.1M potassium phosphate buffer, pH 2.8, and 15 µg chloroperoxidase in a total volume of 3 ml, was incubated for 10 minutes. Similar incubation mixtures were run lacking either chloroperoxidase, iodide ion, or hydrogen peroxide. After the incubation period, 0.1 ml of 0.1M silver nitrate was added to each mixture. Yellow precipitates (Ag I) developed in the sample lacking enzyme and the sample lacking hydrogen peroxide. No precipitate developed in the sample without iodide. In the complete reaction mixture, a white crystalline precipitate developed.

Each of the precipitates was tested colorimetrically for the presence of iodate ion, as described by Charlot (1954). This test is based on the reduction of iodate ion by thiocyanate ion in acid solution to form molecular iodine. All the precipitates were washed thoroughly with water, and then taken up in 1 ml of 0.5 N HCl. To each solution was added 1 ml of CCl_4 and 0.1 ml of 1% KCNS. After shaking, the purple color of iodine developed only from the precipitate that originated in the complete reaction mixture. No color developed from the other precipitates.

Summary and Conclusions. Chloroperoxidase can catalyze the peroxidation of molecular iodine to a higher oxidation state. A colorimetric test indicates that the reaction product is iodate ion. Stoichiometric measurements on the number of oxidizing equivalents in the product agree with this conclusion. This is the first demonstration of the enzymatic oxidation of a molecular halogen species.

It is rather doubtful that this reaction has any physiological significance for the mold from which chloroperoxidase is obtained, since this mold will not grow in the presence of iodide ion. However, such a reaction might provide the halogenating system of the thyroid gland with a storage system for the iodinating equivalents of iodine.

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