Catalysis of Iodination by Lactoperoxidase*

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ABSTRACT: The kinetics of iodination reactions catalyzed by lactoperoxidase have been studied using both potentiometric and spectrophotometric methods. The rate of iodination could be determined by use of the iodide sensor to detect changes in iodide concentrations. Since the difference in the spectrum of tyrosine and L-3-ITyr is maximal at 290 nm, the rate of L-3-ITyr formation could be followed at this wavelength. The iodination reaction involves three substrates: peroxide, iodide, and the phenolic compound which is iodinated. Kinetic studies indicate that a quaternary complex is not formed, but that a Ping-Pong-type mechanism is involved. Optimal concentrations of peroxide, iodide, and tyrosine for the initial rates were established. The $K_{m,app}$ values for L-Tyr at pH 7.4 and 5.0 were determined, at optimal concentrations of peroxide and iodide, to be 1.3×10^{-4} and 3.3×10^{-4} M. The effect of pH on these parameters was studied in detail. The rate of iodination varied considerably with pH, with maximal rates being obtained at pH 5.0. At this pH value, 1.5×10^{5} moles of L-Tyr was converted into L-3-ITyr per min per mole of lactoperoxidase. Most interesting was the fact that D-Tyr was iodinated at a faster rate than L-Tyr. The greatest difference in the relative rates of D- and L-Tyr occurred at the higher pH values. D-Tyr was iodinated 1.5 times more readily than L-Tyr at pH 8.2. These data suggest that the catalysis of iodination involves a complex with lactoperoxidase not only of the peroxide and iodide, but also of the phenolic compound. Lactoperoxidase catalyzed the iodination of L-3-ITyr to L-3,5-I_2Tyr. Under the experimental conditions employed, no changes could be observed when L-3,5-I_2Tyr was used as a substrate.

In order to study iodination or halogenation reactions catalyzed by peroxidase, a number of procedures have been used (Roche *et al.*, 1954; Covelli and Wolff, 1966; Hager *et al.*, 1966; Thomas and Hager, 1969). The most widely applied of these procedures is one in which iodination is assayed by isolating the iodinated derivatives and determining the amount of iodide incorporated into the organic compounds. In order to increase the sensitivity of this procedure, radioactive isotopes are usually employed. Thus, a measure of the iodination would be the radioactivity incorporated into a compound. A second method of following halogenation reactions involves the determination of a change in spectral properties of a molecule as a result of the halogenation (Covelli and Wolff, 1966; Hager *et al.*, 1966). Two modifications of this procedure are employed. A static method takes advantage of changes in ionization of the phenolic group and spectral properties to follow the iodination of tyrosine residues in proteins. Hager and his coworkers (1966) have followed halogenation kinetically by the change in absorbance due to halogenation of monochlorodimedon. More recently Thomas and Hager (1969) have taken advantage of the differences in fluorescence between tyrosine and its iodinated forms to follow the kinetics of halogenation.

In order to evaluate the ability of lactoperoxidase to catalyze the iodination of tyrosine and its derivatives, we have employed two methods. One is a spectrophotometric procedure based on the difference in spectral properties of tyrosine and monoiodotyrosine, while the second is a procedure using the iodide specific electrode (Morrison, 1968). With this latter type of apparatus, we are able to follow the kinetics of iodination catalyzed by peroxidases employing a wide variety of substrates.

Experimental Section

Reagent grade chemicals were used whenever possible. The following amino acids were used: L-tyrosine (Calbiochem), D-tyrosine (Calbiochem, Sigma Chemical Co.), and L-3,5-I_2Tyr (Calbiochem). Chromatographic purity of these compounds was assessed by thin-layer chromatography. A Cary Model 14 spectrophotometer was employed in obtaining spectra. The enzyme lactoperoxidase was isolated and purified as previously described (Morrison and Hultquist, 1963; Rombauts *et al.*, 1967). Concentration of the enzyme was based on a millimolar extinction coefficient of 114 at 412 nm. Hydrogen peroxide concentration was determined using a molar extinction coefficient of 72.4 at 230 nm (George, 1953). Purity of the amino acids and identification of the products of enzymatic iodination were assessed by thin-layer chromatography.

Ascending thin-layer chromatography was carried out on MN silica gel N-HR (Macherey-Nagel) on precoated plastic sheets in benzene–xylene–95% acetic acid (1:1:3) or on MN cellulose powder 300 (Macherey-Nagel) precoated plastic sheets in 1-butanol–acetic acid–water (78:5:17) (Roche *et al.*, 1954). All plates were obtained from Brinkmann Instruments, Inc. The products of enzymatic iodination were obtained from lyophilized samples. The dry material was extracted in small volumes of methanol or 1-butanol and centrifuged to remove excess salt. The concentrated extract was then spotted on plates. Compounds were detected by one or more of the following sprays: 0.5% ninhydrin in butanol (heated for 10 min at 90°) for α-amino groups, diazotized sulfanilic acid for phenols (Daly and Guroff, 1968), and ferrichloride–ferricyanide–arsenious acid spray for iodinated compounds (Gmelin and

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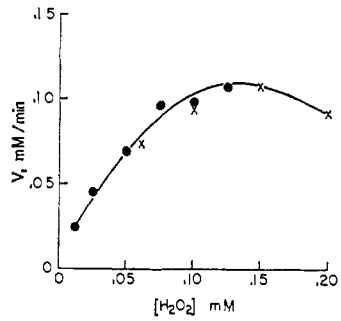

FIGURE 1: H_2O_2 optimum at pH 7.4. The conditions are those given under Experimental Section except for the variations in H_2O_2 concentrations. (●—●) L-3-ITyr production, (×—×) I^- disappearance.

Virtanen, 1959). The ferrichloride–ferricyanide–arsenous acid spray is not specific for iodinated compounds (Zappi and Bublitz, 1968) and can be used to detect tyrosine. It is, however, much more sensitive for the iodinated compounds. As little as 3×10^{-10} mole of tyrosine can be detected, in a spot 0.5 cm in diameter, while 3×10^{-11} mole of L-3-ITyr and 3×10^{-12} mole of L-3,5-I_2Tyr can be observed under the same conditions.

All of the commercial samples of L-3-ITyr checked chromatographically were contaminated with tyrosine, L-3,5-I_2Tyr, or both. Therefore, commercial L-3-ITyr was purified by a modification of the method of Pitt-Rivers and Sacks (1962). Dowex AG 1 (Cl^- form, X8, Bio-Rad Laboratories) was washed with 0.1 N NaOH until chloride free. This was followed by water washes until the effluent had reached neutrality. A wash of saturated sodium acetate was used to convert the resin into the acetate form; excess acetate was removed by further water washes. To a column 2.5 × 30 a solution of 60 mg of L-3-ITyr was applied. The L-3-ITyr was eluted with 0.5% acetic acid. The column was run at 4° in the dark. L-3-ITyr-containing fractions were pooled and concentrated by lyophilization. The fractions were repeatedly lyophilized from water to remove all traces of acetic acid. Such samples were chromatographically homogeneous in the two solvent systems. The purified L-3-ITyr was stored in the freezer.

Iodide sensors, which were obtained from Orion Research, Inc., Cambride, Mass., or National Instrument Laboratories, Inc., Washington, D. C., were employed for the determination of iodide concentrations. The electrodes were connected to a millivolt meter and recorder and were calibrated with standard iodide solutions. The calibration was checked by reading in the concentration range employed and was not markedly affected in the pH range 4–9. A constant temperature of 22–25° was maintained in a jacketed reaction flask, and the solution was stirred with a magnetic stirrer. With this type of apparatus concentrations of iodide from 1×10^{-1} to 1×10^{-7} M were readily determined. The response of the apparatus to iodide was unaffected by concentrations of peroxide as high as 1×10^{-2} M. Oxidized forms of iodide do not affect the potentiometric determination of iodide except when by reaction with iodide they change its concentration.

Unless otherwise indicated, the following conditions were employed for assay of the lactoperoxidase-catalyzed iodination of tyrosine at pH 7.4. The final concentrations were 8.1×10^{-4} M L-tyrosine, 1.0×10^{-4} M H_2O_2, 9.0×10^{-5} M KI, 1.0×10^{-3} M EDTA, 7.4×10^{-9} M lactoperoxidase, in 0.05 M phosphate buffer. The final volume of the reaction mixture was 5.0 ml. The rate of change of iodide concentration was determined at 25° with the apparatus described above. The reaction was initiated by the addition of the enzyme.

TABLE I: Optimal Conditions for Iodination of Tyrosine as a Function of pH.[a]

pH	H_2O_2 (M × 10^{-4})	I^- (M × 10^{-4})	Tyr (M × 10^{-4})	Sp Act. (moles of L-3-ITyr/min mole of Lactoperoxidase
8.2	0.5	1.0	8.1	0.27×10^4
7.4	1.0	1.0	8.1	1.05×10^4
7.0	1.0	1.0	16.2	2.16×10^4
6.5	4.0	2.0	30.0	3.65×10^4
5.0	6.0	3.5	8.1	15.80×10^4
4.2	4.0	2.0	16.2	8.65×10^4

[a] 7.4×10^{-9} M lactoperoxidase, 1×10^{-3} M EDTA, 0.05 M buffers of glycylglycine at pH 8.2, phosphate at pH 7.4–6.5, and acetate at pH 5.0, 4.2. Rates were measured as I^- disappearance at pH 8.2, 7.4; L-3-ITyr production at pH 7.4 and below.

L-3-ITyr formation was assayed spectrophotometrically at 290 nm on a Gilford recording spectrophotometer at 25°. Final concentrations used were the same as those for the procedure described above in a final volume of 3.0 ml. The reaction was initiated with either enzyme or peroxide. The initial rate of increase in absorbance at 290 nm, due to formation of L-3-ITyr, was determined. The difference in absorbance between L-3-ITyr and tyrosine gives a maximum at 290 nm and has a δ molar extinction coefficient of 2340 at pH 7.4 and below. This value was used in calculations of L-3-ITyr production.

In order to determine whether any oxidized forms of iodide were present in our reaction mixture, excess neutralized 0.05 M sodium arsenite or 0.05 M sodium thiosulfate was added. The arsenite was prepared according to the method of Dyer (1956). Under these conditions, I_2 or I_3^- is converted into iodide. Arsenite was used at neutral or higher pH, and thiosulfate at lower pH values.

Results

Taking advantage of the ability of the iodide sensor to detect changes in iodide concentrations, the rate of iodination could be determined from the change in concentration of iodide (Morrison, 1968). The various parameters for the variables in the iodination reaction catalyzed by lactoperoxidase were investigated. The iodination catalyzed by lactoperoxidase involves three substrates: peroxide, iodide, and tyrosine.

A broad optimum for peroxide concentration was found in the region of 1×10^{-4} M at pH 7.4. As with most peroxidases, the enzyme is sensitive to high concentrations of peroxide, and has the optimum shown in Figure 1. As shown in Table I, the peroxide optimum varied with reaction conditions.

Iodide does not give a simple saturation curve. Figure 2

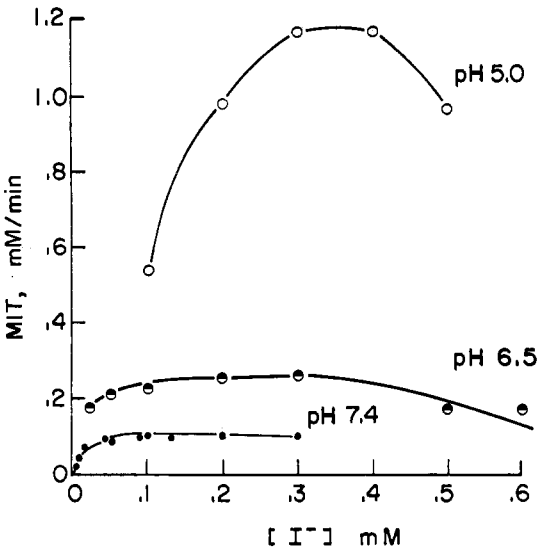

FIGURE 2: Effect of iodide concentration on rate of iodination. Optimal concentrations of H_2O_2 and L-Tyr (see Table I) were employed at each pH value indicated.

shows that high concentrations inhibit iodination. This is most apparent at pH 5.0. The optimum iodide concentration varies with pH values as can also be seen in Figure 2. However, saturation occurs at relatively low concentrations throughout the pH range studied (see Table I).

Under optimal conditions of iodination, the K_m values for tyrosine vary with pH. The $K_{m,app}$ values for L-tyrosine were determined to be 1.3×10^{-4} and 3.3×10^{-4} M at pH 7.4 and 5.0, respectively. Since tyrosine is relatively insoluble, it is fortunate that the saturation levels are low.

Employing the optimal conditions obtained at pH 7.4, the rate of the reaction is proportional to lactoperoxidase concentration as shown in Figure 3. At high dilutions of the enzyme, however, the rate is no longer proportional to enzyme concentration. This may be due to the instability of lactoperoxidase in dilute solution (Morrison *et al.*, 1957; Steele and Morrison, 1969).

Figure 4 shows a comparison of the difference spectrum obtained at pH 5.0 with equimolar concentrations of L-3-ITyr *vs.* Tyr and the difference spectrum of the iodination reaction mixture versus the same solution without added peroxide. Since for each mole of tyrosine iodinated 1 mole of L-3-ITyr is produced, the spectra in both cases should be the same. The results in Figure 3 show that they are identical, within experimental error.

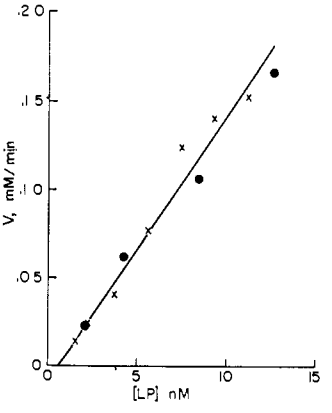

FIGURE 3: Rate of iodination at pH 7.4 as a function of lactoperoxidase concentration. The conditions are those given under Experimental Section except that 6.5×10^{-5} M H_2O_2 was employed. (●—●) L-3-ITyr production, (×—×) I^- disappearance.

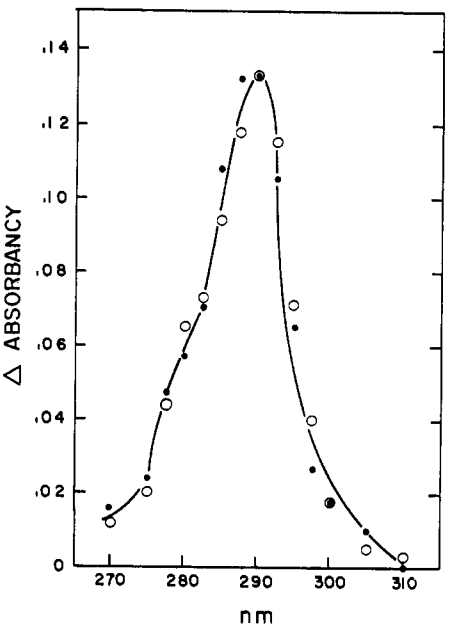

FIGURE 4: Difference spectrum of L-3-ITyr and L-tyrosine at pH 5.0. O—O was obtained with 0.6×10^{-4} M L-3-ITyr in one cuvet and the same concentration of L-tyrosine in the other. ●—● was observed when each cuvet contained 4.58×10^{-4} M L-tyrosine, 1.0×10^{-4} M KI, 1.0×10^{-3} M EDTA, and 7.4×10^{-9} M lactoperoxidase in 0.05 M acetate buffer (pH 5.0), and to one cuvet 0.6×10^{-4} M H_2O_2 was added. After 2 min the difference spectrum was recorded. The cuvet had a 1-cm light path.

The difference in the spectrum of tyrosine and L-3-ITyr is maximal at 290 nm. The rate of the iodination of tyrosine can, therefore, be followed spectrophotometrically at this wavelength. As shown in Figures 1 and 3, the same kinetics are obtained using the spectrophotometric assay for L-3-ITyr production or the potentiometric assay for iodide disappearance. Using L-3,5-I_2Tyr as substrate, no spectral or potentiometric changes can be observed.

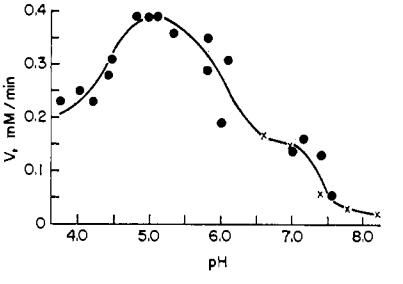

FIGURE 5: Rate of iodination as a function of pH. The reaction conditions are those given under Experimental Section except for the variation in pH values. Phosphate (0.05 M) was employed at pH values of 6 and above, while 0.05 M acetate was employed at lower pH values. (●—●) L-3-ITyr production, (×—×) I^- disappearance.

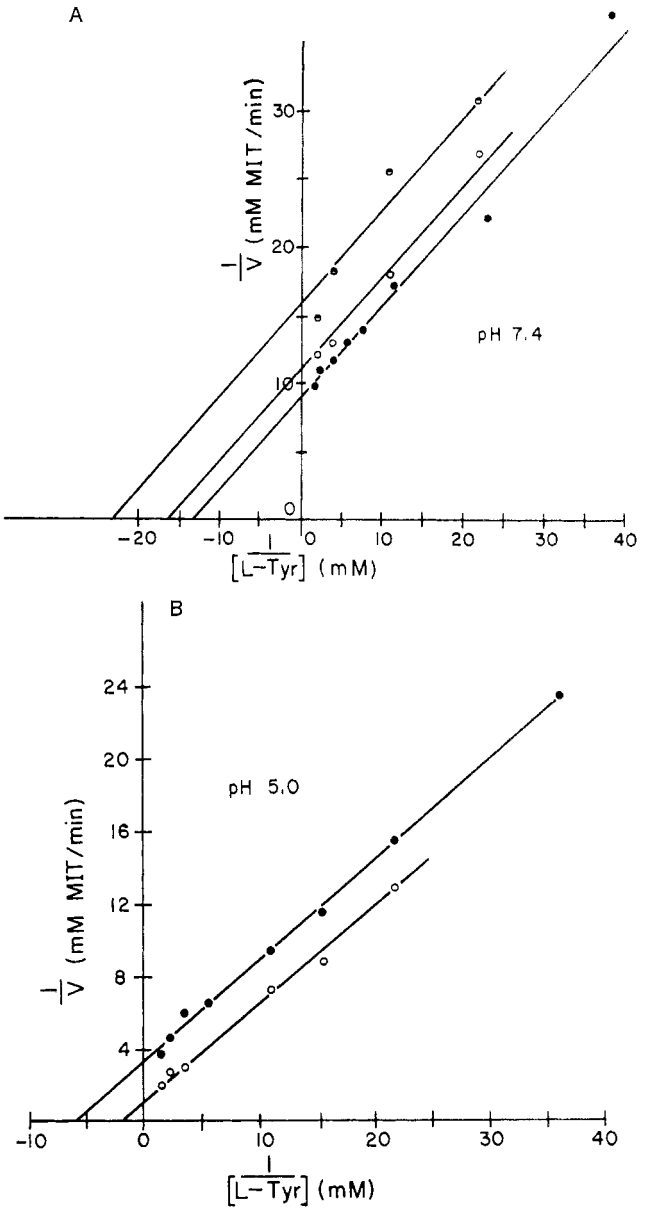

FIGURE 6: Reciprocal velocity *vs.* reciprocal L-tyrosine concentration. (A) The data were obtained at pH 7.4, using various concentrations of H_2O_2 and KI in a fixed ratio. (●—●) 0.67×10^{-4} M H_2O_2 and KI, (○—○) 0.50×10^{-4} M H_2O_2 and KI, and (◓—◓) 0.25×10^{-4} M H_2O_2 and KI. Other conditions are described in Experimental Section. (B) The data were obtained at pH 5.0, using different concentrations of H_2O_2 and KI in a fixed ratio. (○—○) 2.0×10^{-4} M H_2O_2 and 1.0×10^{-4} M KI, (●—●) 1.0×10^{-4} M H_2O_2, and 0.5×10^{-4} M KI. Other conditions are as described in Experimental Section except that 0.05 M acetate, pH 5.0, was used.

Employing the conditions found to be optimal at pH 7.4, the rate of iodination was studied as a function of pH as shown in Figure 5. Table I compares all the conditions for optimum iodination of tyrosine at various pH values. The highest specific activity under optimal conditions is at pH 5.0. It should be noted that the conditions employed to obtain Figure 5 were not optimal at all pH values. The differences at the various pH values are even more marked than those shown in the figure (compare Table I and Figure 5).

In a three-substrate system such as the one under study, it is possible to establish whether a quaternary complex is formed (Fromm, 1967). In the data shown in Figure 6, two substrates, peroxide and iodide, are held constant while the third, tyrosine, is varied. The same experiment is repeated but at different concentrations of the fixed substrates, peroxide and iodide. The ratio of peroxide to iodide is kept constant. Double-reciprocal plots of the initial rates obtained under these conditions yield parallel lines over a wide concentration range. This suggests that a quaternary complex is not formed, but that a type of Ping-Pong mechanism is involved (Fromm, 1967). The same situation was observed at both pH 5.0 and 7.4.

TABLE II: Relative Rates of Iodination at pH 7.4.[a]

Substrate	Rel Rate
L-Tyr	1.00
D-Tyr	1.22
L-3-ITyr	0.34
L-3,5-I_2Tyr	0.00

[a] The conditions of assay are as described under Experimental Section. The rate is expressed as the change in iodide concentration.

TABLE III: Comparison of Iodination of Tyrosine Isomers at Various pH Values.[a]

pH	Sp Act. (moles/min mole of Lactoperoxidase) D-Tyr	Sp Act. (moles/min mole of Lactoperoxidase) L-Tyr	Rel Act., Ratio D:L-Tyr
4.6	4.24×10^4	3.82×10^4	1.11
7.4	1.20×10^4	0.99×10^4	1.22
8.2	0.29×10^4	0.19×10^4	1.47

[a] The kinetics of the reaction at pH 4.6 were measured spectrophotometrically, while the rates at pH 7.4 and 8.2 were measured using the iodide sensor. Assay methods are described under Experimental Section, except that the tyrosine isomers were present in final concentrations of 1.65×10^{-3} M.

Table II compares the rate of iodination of tyrosine and iodinated tyrosine derivatives. Tyrosine is iodinated more readily than any of the derivatives. L-3-ITyr is iodinated at a considerably lower rate than tyrosine, and no change in iodide can be observed in the case of L-3,5-I_2Tyr. Most interesting is the finding that D-tyrosine is iodinated more rapidly than L-tyrosine. In Table III, the rate of iodination of D- and L-tyrosine is compared at different pH values. At low pH values, the rate of iodination is maximal, but there is little difference between the rate of iodination of D and L isomers. At pH 8.2 the D isomer is iodinated at 1.5 times the rate of L-tyrosine.

An attempt was made to determine whether the enzyme lactoperoxidase was capable of catalyzing the iodination of

tyrosine by functioning as an iodinase. In these experiments, both pH 5.0 and 7.4 were investigated, and in place of peroxide and iodide, oxidized forms of iodide were employed. Solutions of I_3^- or I_2 were added to the reaction mixture containing tyrosine and the enzyme, but there was no evidence that the enzyme was capable of catalyzing the iodination of tyrosine in the absence of peroxide.

The possibility suggested by the work of Thomas and Hager (1969) that I_2 is the substrate for peroxidase-catalyzed iodination was also investigated. At low pH values where I_2 is stable and the spontaneous reaction between phenols and I_2 is very slow, lactoperoxidase in the presence of peroxide does catalyze iodination. The rate of iodination in this pH region is nearly identical whether I_2 or I^- is employed as substrate.

The products obtained under initial rate conditions were identified by chromatography. The reaction mixture with both D- and L-tyrosine contained two components: the original tyrosine, and a new component, corresponding in mobility in both solvent systems to that of authentic L-3-ITyr. Further, the material was identifiable with spray reagents as being a phenolic amino acid containing iodide. No L-3,5-I_2Tyr was observed in any of the samples investigated. Control experiments in which the enzyme or peroxide were omitted gave no evidence of L-3-ITyr. With L-3-ITyr as substrate a new compound with the mobility and color reactions of authentic L-3,5-I_2Tyr was found. Control incubations, in which lactoperoxidase or H_2O_2 was omitted, also showed no detectable product formation.

A variety of other amino acids and peptides were studied and will be the subject of another paper. It is important to note, however, that with the exception of histidine, none of the other naturally occurring amino acids was iodinated.

Discussion

Previous methods used to study the kinetics of iodination reactions catalyzed by peroxidases have not been well suited to the type of investigation presented here. Static procedures based on the isolation of iodinated derivatives and determination of the amount of iodide incorporated are not easily applied to kinetic study of iodination. The use of spectral procedures such as those employed by Hager *et al.* (1966) make it necessary to study the spectrum of each substrate and its iodinated derivatives. Only when the spectrum of the iodinated compound deviates sufficiently from that of the noniodinated compound can a spectral assay be used. Consequently, catalysis of the iodination was not generally applied to the study of a wide variety of substrates. The use of the iodide specific electrode makes it possible to overcome this obstacle, and to monitor reactions continuously, thus making it possible to study the kinetics of iodination of a wide variety of compounds.

The kinetics of peroxidase-catalyzed tyrosine iodination have not been comprehensively investigated. Lactoperoxidase, horseradish peroxidase, chloroperoxidase, and crude beef thyroid peroxidase (Coval and Taurog, 1967; Hager *et al.*, 1966; Klebanoff *et al.*, 1962; Ljunggren, 1966; Taurog and Howells, 1966; Thomas and Hager, 1969; Yip and Hadley, 1966) have all been shown to be capable of catalyzing the iodination of tyrosine. No comprehensive investigation of the effect of concentrations of various substrates or pH has been undertaken with any of these peroxidases. In none of these studies have the investigators calculated turnover numbers of the peroxidase in the catalysis of tyrosine iodination. It should be stressed that the kinetics of iodination with tyrosine as a substrate are not identical with those with proteins or peptides (Morrison, 1968; Morrison *et al.*, 1970).

The overall iodination reaction, involving at least three substrates, is unquestionably a multistep process. Hence, the kinetics of the peroxidase-catalyzed iodination are complex. A number of investigators (Fromm, 1967; Cleland, 1963; Frieden, 1959) have dealt with the problem of multisubstrate reactions and developed equations which make it possible to derive information concerning the mechanism of catalysis from initial rate studies. The Lineweaver–Burk plots shown in Figure 6 indicate that a mechanism is operative which does not require that all substrates, peroxide, iodide, and tyrosine, be present on the enzyme simultaneously. This is consistent with a mechanism in which the enzyme is first oxidized to the relatively stable forms of peroxidases produced by the interaction of peroxide and the enzyme.

Lactoperoxidase and other peroxidases can catalyze the oxidation of iodide to triiodide very efficiently (Hosoya and Morrison, 1967; Alexander, 1962; Hosoya, 1963). Triiodide would interfere with the spectrophotometric assay for L-3-ITyr since it would produce an increased absorbance at 290 nm. The formation of triiodide would also interfere with the potentiometric assay procedure. Oxidation of iodide to triiodide would result in a change of millivolts equivalent to the change in iodide concentration. To determine whether any triiodide was present during the course of the reactions, sodium arsenite or sodium thiosulfate was added. Since these reagents convert triiodide into iodide, a decrease in absorbance in the 290-nm region of the spectrum would occur. In a similar way, the potentiometric procedure would reflect the conversion of I_3^- into I^-. No evidence for triiodide interference in the assay procedure was obtained at any stage of the reaction.

A number of investigators have employed the production of triiodide in an assay for peroxidase. It should be emphasized that this assay for triiodide is very dependent upon the concentration of iodide. With the low concentration of iodine employed in these studies such assays are impossible. As a matter of fact, in the absence of tyrosine no triiodide formation could be detected at neutral or higher pH values. At lower pH values, a transient change in absorbance, probably attributable to a very small amount of triiodide, could be observed. With the iodide sensor, there is a change in potential consistent with the disappearance of I^- and its conversion into an oxidized form. In either case, the observed changes are rapidly reversible with arsenite or thiosulfate. The potentiometric procedure was used with caution at low pH values. The reaction products were always checked to determine whether the observed changes were in any way attributable to oxidized iodide. In the presence of tyrosine, the spectrophotometric assay gave completely reliable results, and was the assay of choice below pH 7. Even though the enzyme is capable of oxidizing iodide to triiodide, the triiodide formed would not iodinate tyrosine at the rates observed. Further, if the enzyme catalysis only involved oxidation of iodide, no differences in the rate of iodination of D- and L-tyrosine should be observed. Thus, it is clear that iodination catalyzed by lactoperoxidase does not take place *via* triiodide.

It is clear from studies at low pH values that lactoper-

oxidase catalysis of iodination is not simply the result of oxidation of I^- to I_2 which then reacts spontaneously with tyrosine. At pH values below 5 this spontaneous reaction is very slow. Our results which indicate that lactoperoxidase can use either I^- or I_2 as substrate are similar to the results obtained by Thomas and Hager (1969). It is not necessary to assume that I_2 is an obligatory intermediate in the iodination reaction. It may simply represent a competitive side reaction. The mechanism of catalysis may involve an oxidized species of iodide which reacts to give either I_2 or iodination at low pH. The reaction leading to I_2 is very much faster than the reaction which results in iodination. The reactive oxidized iodide in this scheme would be catalytically generated from either I^- or I_2. Thus, a reactive oxidized iodide is common to I_2 and L-3-ITyr formation.

Both assay procedures give essentially identical results and only a single product, L-3-ITyr, can be identified chromatographically. Stoichiometry of the reaction dictates that 1 mole of tyrosine disappears for every mole of L-3-ITyr produced. Theoretically, then, the difference spectrum between equimolar solutions of L-3-ITyr and tyrosine should be obtained in the reaction. As can be seen in Figure 5, the difference spectrum obtained from the reaction mixture is, indeed, identical with the difference spectrum of L-3-ITyr *vs.* Tyr. If any other product such as L-3,5-I_2Tyr were formed, deviations in the spectrum would have resulted. Thus, the evidence is convincing that the procedures employed accurately reflect the anticipated iodination reaction.

The present investigation clearly indicates that, in the iodination reaction, tyrosine must form a complex with lactoperoxidase. This complex formation is stereospecific involving the α-amino group, and D-tyrosine is iodinated more readily than L-tyrosine. Although stereospecificity in peroxidase-catalyzed reactions has been shown (Saunders *et al.*, 1964; Yonetani and Ray, 1966), the substrates have always been hydrogen donors. These studies, then, represent a new type of specificity. The enzyme mechanism can involve two active sites: the oxidation site and the halogenation site. The halogenation site may be equivalent to a transiodination site in the enzyme. This would be comparable with the enzyme activity labeled "iodinase." Lactoperoxidase, however, in the absence of peroxide, does not catalyze the iodination of tyrosine in the presence of I_3^- or I_2, and the enzyme probably, therefore, does not have "iodinase" activity.

The effect of pH on the reaction was pronounced. The pH optimum was at 5.0. At this pH, however, little stereospecificity is observed. At the higher pH values the reaction is stereospecific, and appears to be independent of the buffer used. The differences in the stereospecificity also clearly suggest the possibility that ionizable groups of the enzyme are involved in the binding of the phenolic compounds. The most obvious suggestion would be that the amino group of the substrate, tyrosine, is involved. Results with derivatives of tyrosine modified at the carboxyl or amino groups confirm this speculation (Morrison *et al.*, 1970).

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