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LACTOPEROXIDASE-CATALYZED IODINATION OF TYROSINE PEPTIDES

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SUMMARY

1. The rate of lactoperoxidase (donor:H_2O_2 oxidoreductase, EC 1.11.1.7)-catalyzed iodination of tyrosine derivatives and tyrosine containing peptides was studied at neutral pH. Lactoperoxidase catalyzes the iodination of tyrosine, its ester and amide at comparable rates while derivatives of the amino group, such as *N*-acetyltyrosine, are iodinated at significantly lower rates. Neutral amino acid containing dipeptides in which tyrosine is N-terminal are more readily iodinated than those in which tyrosine is C-terminal. Tyr > Tyr–Gly = Tyr–Ala ≥ Tyr–Val = Tyr–Leu = Tyr–Phe = Phe–Tyr > Leu–Tyr = Val–Tyr = Ala–Tyr ≥ Gly–Tyr. Lysine containing dipeptides also are iodinated in the same manner. Tyr > Tyr–Lys > Lys–Tyr. The converse appears to be true in the case of glutamic acid containing dipeptides. Tyr > Glu–Tyr ≥ Tyr–Glu. At pH 3.6 the rate of iodination is the same for tyrosine and all the peptides.

2. At pH 7.4 horseradish peroxidase does not catalyze the direct iodination of tyrosine or any of the tyrosine derivatives studied. It is pointed out that spectrophotometric assays for iodination of tyrosine may not distinguish between oxidative coupling and iodination.

INTRODUCTION

It has long been recognized that the ability of peroxidases (donor:H_2O_2 oxidoreductase, EC 1.11.1.7) to oxidize iodide to iodine is almost ubiquitous to hemoprotein peroxidases. All peroxidases are consequently capable of catalyzing the iodination of tyrosine or tyrosine containing proteins, since I_2 or the I_3^- generated by this oxidation rapidly reacts with the phenolic compounds to yield the iodinated derivatives. Recent work has clearly shown that lactoperoxidase catalyzes the direct iodination of tyrosine and does not necessarily catalyze the iodination of tyrosine *via* I_2[1–4]. These studies have further indicated that lactoperoxidase is relatively selective. The D- and L-isomers of tyrosine are iodinated at significantly different rates and tyrosine peptides appeared to be iodinated slower than tyrosine. It was of interest, therefore, to investigate tyrosine-containing peptides as substrates in the lactoperoxidase-catalyzed iodination reaction.

EXPERIMENTAL METHODS AND RESULTS

Lactoperoxidase was isolated and purified as previously reported[5]. Concentration was determined at 412 nm using a millimolar extinction coefficient of 114[6]. Samples used in this study had an absorbance ratio at 412 nm/280 nm of 0.7 or higher. Horseradish peroxidase was obtained either from Sigma Chemical Company (Type VII-L) or from Worthington. Concentration was determined at 403 nm using a millimolar extinction coefficient of 91[7].

Tyrosine peptides were obtained from commercial sources and were checked for purity by thin-layer chromatography. Solvent systems employed were *n*-butanol–acetic acid–water (12:1:4, by vol.)[8] and *n*-butanol–pyridine–water (1:1:1, by vol.)[9]. The thin-layer plates were MN cellulose powder 300 (Machery-Nagel) precoated on plastic sheets 25 cm × 25 cm. The plates were obtained from Brinkmann Instruments, Inc. Detection sprays employed were ninhydrin and diazotized sulfanilic acid for phenols[10].

Kinetics of iodination were measured as previously reported[1,2] by following with time either decrease in iodide concentration or increase in 3-monoiodotyrosine concentration. Iodide concentration was monitored by use of an iodide electrode. Absorbance of 3-monoiodotyrosine was followed at 290 nm on a Gilford recording spectrophotometer. A Δ millimolar extinction coefficient of 2.34 was used for calculation of concentration. Unless otherwise noted, reaction mixtures contained final concentrations of 0.1 mM H_2O_2, 0.1 mM iodide, 0.9 mM tyrosine or tyrosine derivatives, and 6.2 nM lactoperoxidase in 0.05 M phosphate buffer, pH 7.4. H_2O_2 and iodide concentrations of 0.1 mM were optimal for the iodination of tyrosine at pH 7.4[2].

The influence of variation in lactoperoxidase concentration on the iodination rate of glycyl-L-tyrosine was studied and found to be a linear function of lactoperoxidase concentration. In Table I are recorded the rates of iodination of a number of tyrosine derivatives. Values are expressed relative to the rate of L-tyrosine. Dipeptides in which tyrosine is N-terminal were better substrates for iodination catalyzed by lactoperoxidase than those where tyrosine is C-terminal.

The rate differentials noted in structurally similar substrates may be a reflection of differences in affinity of binding to the enzyme. Therefore, experiments were performed using L-tyrosylglycine and glycyl-L-tyrosine to determine their K_m values in the iodination reaction. Conventional Lineweaver–Burk plots[11] revealed within experimental error identical K_m values for both peptides with that of L-tyrosine, and of the order of 0.1 mM.

DISCUSSION

The lactoperoxidase-catalyzed iodination of tyrosine has been shown to proceed by a ping-pong mechanism[2]. The rate-limiting step is unknown although the oxidation of iodide is clearly more rapid than the overall iodination reaction[2,4]. Under the conditions employed in this study, the overall reaction is first order with respect to the enzyme and independent of the substrates concentrations; thus, it is possible to evaluate the variation in the rate of the reaction with various tyrosine substrates. As previously shown[3] the tyrosine ester and amide are iodinated at similar rates to tyrosine. Derivatives of tyrosine involving the amino group are iodinated at signifi-

TABLE I

COMPARATIVE RATES OF LACTOPEROXIDASE CATALYZED IODINATION OF TYROSINE DERIVATIVES

Concentrations and components of the reaction mixtures are given under Experimental Methods and Results.

Substrate	*Rate relative to* L-*tyrosine*
L-Tyrosine	1.00*
L-Tyrosine methyl ester	1.16
N-Acetyl-L-tyrosine	0.22
L-Tyrosylglycine	0.37
L-Tyrosyl-L-alanine	0.40
L-Tyrosyl-L-valine	0.25
L-Tyrosyl-L-leucine	0.21
L-Tyrosyl-L-phenylalanine	0.26
Glycyl-L-tyrosine	0.10
L-Alanyl-L-tyrosine	0.15
L-Valyl-L-tyrosine	0.15
L-Leucyl-L-tyrosine	0.13
L-Phenylalanyl-L-tyrosine	0.23
L-Lysyl-L-tyrosine	0.09
L-Tyrosyl-L-lysine	0.26
L-Glutamyl-L-tyrosine	0.25
L-Tyrosyl-L-glutamate	0.16
L-Tyrosyl-L-tyrosine	0.26
L-Tyrosyl-L-tyrosyl-L-tyrosine	0.26
L-Valyl-L-tyrosyl-L-valine	0.17

* Under these conditions, lactoperoxidase catalyzed the production of $1 \cdot 10^4$ moles monoiodotyrosine/min per mole of enzyme.

cantly lower rates as shown in Table I. Even *N*-acetyl-L-tyrosine is iodinated at a lower rate than tyrosine. All peptides have a significantly lower rate of iodination than tyrosine. The aromatic and aliphatic amino acid-containing peptides in which tyrosine is N-terminal are more readily iodinated than those where the tyrosine is C-terminal. This is most dramatically illustrated in the case of tyrosylglycine and glycyltyrosine. In contrast to the enzyme catalyzed iodination, Mayberry *et al.*[12,13] have shown that glycyltyrosine is chemically iodinated more readily than tyrosine.

In the enzyme catalyzed iodination, tyrosine containing dipeptides of the basic amino acid lysine act about the same as the dipeptides containing aliphatic amino acids. In the case of the acidic amino acid glutamic acid, glutamyltyrosine and tyrosylglutamate do not react in the same manner. In this case where tyrosine is in the C-terminal position it is iodinated more readily.

At a pH value of 3.6 the rate of iodination is the same for tyrosine and the peptides, although glutamyltyrosine may be iodinated somewhat more readily. It is interesting to note that Krinsky and Fruton[8] found that the sequence glutamyltyrosine was iodinated most readily by the thyroid peroxidase, but also noted that the peptide is iodinated at about the same rate as other dipeptides with lactoperoxidase.

Takeda *et al.*[14] studied the effect of adjacent amino acid groups on the rate of chemical iodination of tyrosine. They found that the peptide glutamyltyrosylglutamate was iodinated somewhat more readily than similar tripeptides in which tyrosine was sandwiched between lysine, leucine, or glycine residues. However, the

differences in the rate of the tyrosine iodination in these four peptides were relatively minor.

Horseradish peroxidase, on the other hand, at pH 7.4 does not catalyze the direct iodination of tyrosine or any derivatives listed in Table I. These results are in contrast to the findings of Krinsky and Fruton[8], who claim to have observed an appreciable iodination of tyrosyltyrosine catalyzed by horseradish peroxidase. The discrepancy in these findings may be in the assay conditions or in the methods employed to assay for iodination.

The two reactions, oxidative coupling and iodination, produce absorbance changes in the same region of the spectrum. The oxidative coupling results in increased absorbance at 290 nm and 315 nm. The formation of monoiodotyrosine also produces absorbance changes in this region of the spectrum and its formation is commonly followed spectrophotometrically at 290 nm. It is, therefore, possible to mistake oxidation of tyrosine for iodination of tyrosine unless due care is taken to distinguish these reactions. Under our conditions horseradish peroxidase does not iodinate tyrosine, any of the peptides listed or the benzyloxycarbonyltyrosine derivative. Horseradish peroxidase does, however, catalyze the peroxide-dependent oxidation of tyrosine and tyrosine peptides.

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