THE SPECIFICITY OF THYROID PEROXIDASE TOWARD TYROSINE PEPTIDES

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Thyroid peroxidase exhibits specificity in the iodination of the tyrosine residues in peptide linkage. Among the peptides tested in this investigation, the enzyme showed preference for those having the sequence Glu-Tyr, suggesting that the enzyme-catalyzed iodination reaction involves specific interaction of the protein with the peptide substrate. Similar specificity was not noted with lactoperoxidase or horseradish peroxidase.

Recent studies have provided evidence for the view that the iodination of the tyrosine residues of thyroglobulin, and their conversion to iodothyronine residues, is effected in the thyroid by a peroxidase (1-4). This enzyme appears to be associated with particulate matter, but has been released by treatment with deoxycholate and trypsin, and has been subjected to partial purification (1, 5, 6). In the presence of H_2O_2, or of a peroxide-generating system, such enzyme preparations have been shown to effect the iodination not only of thyroglobulin or other proteins, but also of L-tyrosine. The peroxidatic iodination of free tyrosine has been effected with other known peroxidases, notably horseradish peroxidase (7, 8), chloroperoxidase (9, 10), and lactoperoxidase (11, 12); the last of these has been found to be especially active in this regard.

Since the physiological substrate of thyroid peroxidase appears to be a protein of high molecular weight (ca. 660, 000), and not free tyrosine (1, 9), it may be expected that in the iodination of proteins this peroxidase participates in cooperative secondary interactions with groups other than the phenolic groups of tyrosine residues. It may also be expected that the other

peroxidases that catalyze the iodination of free tyrosine will differ significantly from thyroid peroxidase with respect to such secondary interactions. Studies in this laboratory and elsewhere have shown that, in the action of proteinases on oligopeptides, secondary enzyme-substrate interactions at a distance from the site of catalytic action may play a major role in determining the specificity of these enzymes (13). The evidence for such interactions has come largely from systematic studies of the effect of structural changes in synthetic peptides on the kinetics of their enzymic cleavage. In the case of the peroxidases, there have been few reports on the enzymic iodination of synthetic tyrosine peptides; for example, Gly-Tyr, Leu-Tyr, and Gly-Leu-Tyr were found to be iodinated by lactoperoxidase much more slowly than L-tyrosine (11, 12, 14). In the light of the above considerations, it seemed desirable to test the action of partially-purified thyroid peroxidase on an extensive series of tyrosine peptides, and also to examine lactoperoxidase and horseradish peroxidase with regard to their specificity toward such peptides.

MATERIALS AND METHODS: Thyroid peroxidase was purified from homogenized beef thyroids according to methods described previously (1, 5). The enzyme fraction solubilized with deoxycholate and trypsin was precipitated with ammonium sulfate, and was passed successively through Sephadex G-75 and DEAE-cellulose. The product (ca. 2 mg of protein from 600 g of tissue) had a 410 nm/280 nm absorbance ratio of 0.27, and a specific activity of 1090 guaiacol units (GU) per mg of protein; for a definition of these units, see (5). The specific activity in the triiodide assay, performed as described below, was 1640 iodide units (IU) per mg of protein; one unit corresponds to an absorbance change of 1.0 per second. Protein determinations were performed by the method of Lowry et al. (15). Lactoperoxidase was generously provided by Dr. M. Morrison (via Dr. N. M. Alexander); its specific activity was 7500 GU or 15000 IU per mg of protein. Horseradish peroxidase was a Worthington preparation, and had

a specific activity of 8040 GU or 96 IU per mg of protein. The peptides used in these studies were largely from the collection in this laboratory, or had been purchased from Mann Research Chemicals; in all cases, their identity and purity were checked.

For the determination of the rate of iodination of tyrosine or of tyrosine derivatives by thyroid peroxidase, the following standard conditions were adopted: The reaction mixture (3 ml) contained 0.05 M potassium phosphate buffer (pH 7.4), 10 mM KI, the iodine acceptor (0-1.0 mM), and the enzyme (4.8 GU); the reaction (at 25°) was initiated by adding H_2O_2 to make its initial concentration 0.1 mM. The initial rate of the iodination reaction was determined by means of a Gilford recording spectrophotometer, and was followed for 5 min after the addition of H_2O_2; in all cases, the absorbance changes were linear during the first minute. In the case of lactoperoxidase, the enzyme concentration was 3.8 GU, and the initial concentrations of both KI and H_2O_2 were 0.1 mM. With horseradish peroxidase, the enzyme concentration was 40 GU, and the tests were conducted at 10 mM KI and 0.013 mM H_2O_2. The conversion of tyrosine to 3-iodotyrosine was followed by measuring the change in absorbance at 290 nm, and correcting for the absorbance of I_3^- (11). The oxidation of I^- to I_3^- was followed by measuring the change in absorbance at 353 nm and at 290 nm; all measurements were corrected for the nonenzymic production of triiodide (16).

RESULTS: Some of the data obtained on the initial rate of iodination of tyrosine derivatives by thyroid peroxidase are given in Table I for initial concentrations of iodine acceptor of 0.02-0.12 mM; experiments at higher concentrations in all cases gave evidence of inhibition of 3-iodotyrosine formation when the acceptor concentration exceeded 0.2 mM, but in some instances inhibition was noted at lower levels. Not listed in the Table are data for D-tyrosine, N-formyl-L-tyrosine, L-tyrosinamide, Gly-Tyr, and Tyr-Gly, which were iodinated, under the conditions of these experiments, at initial rates comparable to that for L-tyrosine.

Table I: Initial Rate of Iodination of Tyrosine Peptides by Thyroid Peroxidase*

Acceptor (mM)	0.02	0.04	0.08	0.12
L-Tyrosine	2	4	4	4
Z-Tyr+	2	7	5	2
Tyr-Tyr	4	5	5	7
Gly-Tyr-Gly	4	5	5	4
Glu-Tyr	14	16	12	12
Tyr-Glu	7	12	9	7
Gly-Glu-Tyr	7	31	76	45
Gly-Leu-Tyr	7	12	5	4
Z-Tyr-Glu+	5	7	8	5
Glu-Tyr-Glu	10	28	28	26

* μM 3-iodotyrosine formed per minute

+ Z = benzyloxycarbonyl

It will be noted that the two tripeptides containing the Glu-Tyr unit are exceptionally good substrates; thus, under optimal conditions, Gly-Glu-Tyr was iodinated to 95% within one minute. That the product of the reaction is the 3-iodotyrosine peptide was shown by its isolation from a large scale reaction mixture, by gel filtration through Sephadex G-10. Thin-layer chromatography with 1-butanol:acetic acid:water (12:1:4) as the solvent showed one major ninhydrin-reactive component of R_F 0.34 and a faint spot of R_F 0.20, the latter corresponding to Gly-Glu-Tyr. Treatment of the isolated peptide with carboxypeptidase A led to the disappearance of these components, and the formation of components of R_F 0.48 (corresponding to 3-iodotyrosine), R_F 0.20 (Gly-Glu), and R_F 0.15 (faint, tyrosine). No 3,5-diiodotyrosine (R_F 0.62) was found.

The kinetic parameters for the iodination of Glu-Tyr-Glu and Gly-Glu-Tyr by thyroid peroxidase were estimated for the range of acceptor concentration 0.004-0.032 mM, where no substrate inhibition was evident. Extrapolation of the Lineweaver-Burk plots gave apparent K_M values of approximately 0.02 mM, indicating binding of the peptides to the enzyme; further work is needed to determine the kinetic parameters more precisely.

No correlation was found between the rates at which the tyrosine derivatives were iodinated by thyroid peroxidase and their effect on the rate of formation of I_3^-. Although I_3^- was formed in the absence of the enzyme, no iodination of the tyrosine derivatives occurred under these conditions.

The data in Table II show that although lactoperoxidase is more effective in the iodination of L-tyrosine than thyroid peroxidase, under the conditions of these experiments, all the tyrosine derivatives tested (except the ethyl ester and amide of L-tyrosine) were less reactive than the free amino acid; this result agrees with earlier conclusions (12) based on the examination of a more limited series of tyrosine derivatives. It should be added that no detectable I_3^- was present in any of the reaction mixtures, and that no inhibition of iodination was found at high substrate concentrations (up to 8 mM). With horseradish peroxidase, which is a much less effective catalyst of the iodination reaction than the two other enzymes, a measurable iodination of L-tyrosine was not found at concentrations below 0.8 mM; however, several tyrosine derivatives containing two aromatic groups (e.g., Tyr-Tyr) were effectively iodinated at much lower acceptor levels (Table II). No inhibition of iodination was found with

Table II: Initial Rate of Iodination of Tyrosine Peptides by Lactoperoxidase and by Horseradish Peroxidase*

	Lactoperoxidase		Horseradish Peroxidase	
Acceptor (mM)	0.02	0.08	0.08	0.20
L-Tyrosine	7	18	0	0
D-Tyrosine	10	22	0	0
Formyl-Tyr	1	3	0	0
Z-Tyr+	3	8	5	19
Tyr-Tyr	5	7	5	43
Gly-Tyr-Gly	0	0	2	3
Gly-Glu-Tyr	2	5	0	4
Glu-Tyr-Glu	2	4	0	4

* μM 3-iodotyrosine formed per minute

+ Z = benzyloxycarbonyl

horseradish peroxidase at high concentrations (up to 8 mM) of these better substrates.

DISCUSSION: The finding that Gly-Glu-Tyr and Glu-Tyr-Glu are iodinated by thyroid peroxidase much more rapidly than the other tyrosine derivatives tested supports the view that the enzyme combines specifically with the iodine acceptor. The apparent preference of the enzyme for the Glu-Tyr sequence is consistent with the amino acid composition of thyroglobulin (17) and of other proteins that are extensively iodinated by the enzyme (1). Further synthetic work is in progress to modify systematically the structure of Gly-Glu-Tyr, in order to delineate more precisely the influence of secondary enzyme-substrate interactions on the iodination of oligopeptides by thyroid peroxidase. The availability of sensitive peptide substrates will also permit more incisive studies on the mechanism of the iodination reaction, and an approach to the problem of the mode of formation of the iodothyronine residues of thyroglobulin.

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