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EVIDENCE FOR A THYROID PEROXIDASE ASSOCIATED "ACTIVE IODINE" SPECIES

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Summary

The role of thyroid peroxidase (donor:hydrogen-peroxide oxidoreductase, EC 1.11.1.7) in tyrosyl iodination has been investigated using a tripeptide substrate, α-L-glutamyl-L-tyrosyl-L-glutamic acid (Glu-Tyr-Glu).

Competition experiments performed with thyroid peroxidase and several iodide acceptors indicate that there is a site for these substrates on the enzyme. The rates of three reactions, peroxidase catalyzed iodination of Glu-Tyr-Glu, chemical iodination of the same tripeptide and peroxidase catalyzed production of iodine were measured as a function of pH, and were found to be different from one another, indicating that the enzymic iodinating species is not free iodine.

An iodinating species was demonstrated to be peroxidase-associated: This species was generated by incubation of the enzyme with iodide-125 and hydrogen peroxide. The labeled intermediate was separated from low molecular weight reaction components by gel filtration and was shown capable of iodinating Glu-Tyr-Glu in the absence of added iodide or peroxide. The iodinated product, Glu-Tyr(I)-Glu was identified by thin layer chromatography and radioautography.

These results support the idea that thyroid peroxidase plays a direct catalytic role in iodination. Iodination does not occur by way of free iodine generated by the enzyme and released into the medium, but rather via an iodinating species associated with the enzyme.

Abbreviations: (peroxidase-I_{oxid}), thyroid peroxidase-oxidized iodide; Glu-Tyr-Glu, α-L-glutamyl-L-tyrosyl-L-glutamic acid; Glu-Tyr(I)-Glu, α-L-glutamyl-L-monoiodotyrosyl-L-glutamic acid; I_3^-, triiodide.

Introduction

Thyroid tissue contains a multisubstrate, integral membrane peroxidase (donor:hydrogen-peroxide oxidoreductase, EC 1.11.1.7) which plays a central role in the biosynthesis of thyroxine (tetraiodothyronine). This involves oxidation of iodide [2—4] and a catalytic function in tyrosyl iodination [5—8].

The mechanism of iodination, however, remains unclear. Thyroid peroxidase structure and function has been reviewed by Maloof et al. [9,10], Taurog [11] and is included in a review of enzymic halogenation, by Morrison and Schonbaum [12].

This study will consider the role of thyroid peroxidase in iodination. We will present evidence that iodide, oxidized by peroxidase, is transferred directly from the enzyme to a tyrosyl acceptor, without intervening formation of unbound molecular iodine. The acceptor chosen for this work is the tripeptide, Glu-Tyr-Glu, reported by Krinsky and Fruton [13] to be an excellent substrate in thyroid peroxidase catalyzed iodination.

Experimental Procedures

Glu-Tyr-Glu was obtained from Schwartz-Mann, Orangeburg, N.Y. and carrier-free iodide-125 from New England Nuclear, Boston, Mass. $^{125}I_2$ was prepared as described by Jirousek and Pritchard [14]. All reagents were of the highest purity commercially obtainable. Radioactivity was measured with a Packard automatic gamma scintillation spectrometer. A Cary-15 spectrophotometer was used for spectral and kinetic measurements. Paper electrophoresis was performed using a Beckman model R, Series D apparatus, at 15 mA, 1.5 h, 0.05 M sodium bicarbonate buffer, pH 10.6.

The thyroid peroxidase preparation. Thyroid peroxidase was prepared using a modification of the procedure of Coval and Taurog [15,16]. The final specific activity of these preparations was typically around 90 guaiacol units per mg protein (1 unit = 1 μmol guaiacol oxidized per min based on an extinction coefficient of 5570 $cm^{-1} \cdot M^{-1}$, at 470 nm). Protein concentration was estimated by the method of Lowry et al. [17]. The absorbance ratio 410 nm/280 nm was 0.1.

Estimated purity of the enzyme. In polyacrylamide gel electrophoresis, at pH 8.3 and 9.0, peroxidase activity co-migrated with a broad band, containing both protein and carbohydrate. More than half of the applied protein coincided with the guaiacol reactive band. A faster moving, minor protein component was also detectable. Dodecyl sulfate polyacrylamide gel electrophoresis dissociated the preparation into 4 major and 2 or 3 minor components. In density gradient centrifugation, (5—20% sucrose) peroxidase activity co-sedimented with a protein band which comprised approximately 50% of the total applied protein. Based on the above data, the peroxidase preparation is estimated to be between 20 and 50% pure.

Polyacrylamide gel electrophoresis was performed at room temperature [18], using 7.5% and 10% acrylamide, 2.5 mA per gel, 1.5 h, 0.05 M Tris, 0.38 M glycine buffers and 100 μg of protein per gel. Protein was detected by Coomassie Blue, carbohydrate by periodic acid-Schiff reagent [19] and peroxi-

dase activity located by immersing the gels in the standard guaiacol assay mixture. Dodecyl sulfate polyacrylamide gel electrophoresis was conducted according to the procedures of Fairbanks et al. [19]. Density gradient centrifugation was carried out for 18 h, 30 000 rev./min at 4°C, using a Beckman model L3-50 ultracentrifuge and an SW-41 rotor.

Assays of thyroid peroxidase. Peroxidase activity was assayed by guaiacol peroxidation [20], and iodide peroxidation to tri-iodide [4]. Iodination of monoiodotyrosine was measured as previously described [21].

Iodination of Glu-Tyr-Glu was measured spectrophotometrically, as an initial rate of formation of Glu-Tyr(I)-Glu (λ_{max}, 290 nm; E_M 2430 $cm^{-1} \cdot M^{-1}$ for the monoiodotyrosyl residue) [8].

The pH dependence of chemical iodination of Glu-Tyr-Glu, thyroid peroxidase-catalyzed iodination of Glu-Tyr-Glu and thyroid peroxidase catalyzed triiodide generation. Phosphate/citrate buffers were adjusted to pH values, ranging from 4.75 to 9.1, and used as shown in Fig. 1.

Generation and gel separation of (peroxidase-I_{oxid}). Thyroid peroxidase was incubated with approximately equimolar $K^{125}I$ and H_2O_2. Estimation of peroxidase was based on a molecular weight of 100 000 and an estimated maximal purity of 50%. The incubation mixture was assayed, then immediately applied to a 0.9 × 15 cm column of Sephadex G-25, equilibrated with deionized water. Each fraction collector tube contained 5 nmol Glu-Tyr-Glu. Emergent fractions were assayed for peroxidase activity (guaiacol), protein and radioactivity.

Iodination of Glu-Tyr-Glu by (peroxidase-$^{125}I_{oxid}$). Excluded fractions were analyzed for iodinated Glu-Tyr-Glu by thin layer chromatography and radioautography as described in Fig. 3.

Results

Thyroid peroxidase generates molecular iodine from iodide in the absence of alternate acceptor substrates and, under certain conditions, during the enzymic iodination of monoiodo to diiodotyrosine. Enzymic I_2 formation is not detectable during the iodination of Glu-Tyr-Glu to Glu-Tyr(I)-Glu. These observations are related to the apparent K_m values of those acceptors, as shown in Table I. Glu-Tyr-Glu has the lowest apparent K_m, implying the greatest affinity for thyroid peroxidase. Monoiodotyrosine has an intermediate K_m value, and under previously described conditions, can be iodinated in the presence of Glu-

TABLE I

THE RELATIVE AFFINITIES OF THREE ACCEPTORS FOR (PEROXIDASE I_{oxid})

The concentration ranges used for determination of apparent Michaelis constants were: Glu-Tyr-Glu, 5—400 μM; monoiodotyrosine, 0.05—3 mM; I^-, 0.4—5.0 mM.

Peroxidase catalyzed reaction	Apparent K_m, acceptor substrate	Simultaneous production of I_3^-
Glu-Tyr-Glu→Gly-Tyr(I)-Glu	$3 \cdot 10^{-6}$ M	No
Monoiodotyrosine → diiodotyrosine	10^{-4} M	Yes
$I^- \rightarrow I_3^-$	$5 \cdot 10^{-3}$ M	—

Tyr-Glu [8]. Iodide, with the highest apparent K_m value, can compete with monoiodotyrosine but not with the tripeptide.

pH dependence. The pH dependencies of the rates of the 3 reactions shown in Fig. 1 were compared and found to be unrelated. Iodination of Glu-Tyr-Glu

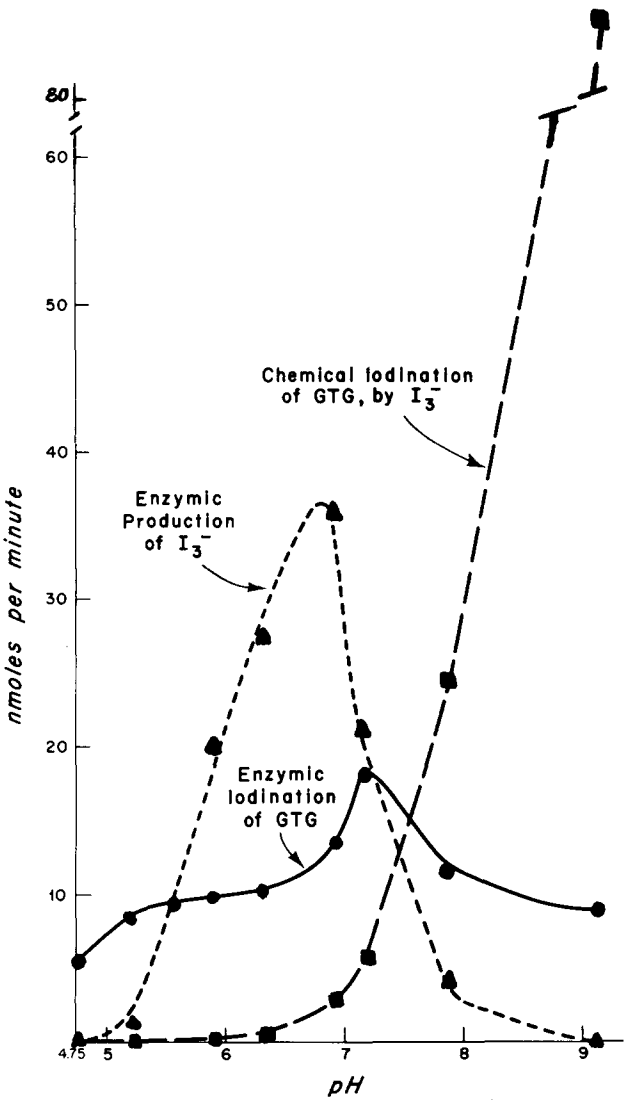

Fig. 1. The pH-dependence of 3 reactions: (a) chemical iodination of Glu-Tyr-Glu (■- - - - -■), (b) enzymic iodination of Glu-Tyr-Glu (●———●). (c) enzymic generation of I_3^- (▲- - - - -▲). In (a), 100 μmol KI, 75 nmol I_3^-. 50 μmol phosphate/citrate buffer at the indicated pH, and 0.5 μmol Glu-Tyr-Glu were mixed in a total volume of 1.0 ml, and the rate of disappearance of I_3^- was measured at 353 nm (E_M = 22 900 $cm^{-1} \cdot M^{-1}$). The reference cuvette contained 19 nmol I_3^- and no Glu-Tyr-Glu. (b) The assay system contained 6.6 μg peroxidase, 0.1 μmol KI, 0.5 μmol Glu-Tyr-Glu, 0.088 μmol H_2O_2 and 50 μmols phosphate/citrate buffer, in 1.0 ml. (c) Initial rates of I_3^- formation were measured at 353 nm, (expanded absorbance scale). The 1.0 ml system, contained 1.0 μmol KI, 0.088 μmol H_2O_2 and 50 μmol phosphate/citrate buffer. The total iodide oxidized by thyroid peroxidase (I_2 + I_3^-) was estimated from the measured I_3^-, the initial I^- concentration and the known [22] equilibrium constant for formation of I_3^- from I^- and I_2.

by triiodide is undetectable below pH 6.5; above this value, the rate increases exponentially, in accord with the known pH dependence of tyrosine iodination by I_3^- [22—24]. In contrast, enzymic iodination of Glu-Tyr-Glu occurs over the entire pH range tested, including pH 4.75—6.5, where no iodination by triiodide is detectable. Finally, enzymic triiodide formation shows a pH dependence which is unrelated to enzymic tripeptide iodination: pH 5—6.5 marks the range of greatest change in triiodide formation but least change in Glu-Tyr-Glu iodination. Thin layer chromatography and radioautography of the enzymic iodination system at each pH confirmed that Glu-Tyr(I)-Glu was formed over the entire pH range. No iodo tripeptide was formed in a control incubation. (pH 7.1, complete system except for hydrogen peroxide).

Generation of (peroxidase-I_{oxid}) and iodination of Glu-Tyr-Glu by (peroxidase-I_{oxid}). Approximate equimolar quantities of thyroid peroxidase, $^{125}I^-$, and H_2O_2 were incubated together, then subjected to Sephadex G-25 filtration (Fig. 2). Effluent fractions, exposed to Glu-Tyr-Glu present in each fraction collector tube, were then tested for the presence of iodinated tripeptide by thin layer chromatography and radioautography (Fig. 3).

In Fig. 2, the void volume (V_o) fractions contained all of the protein and enzyme activity, compared to a control chromatographed without prior incubation with $^{125}I^-$ and H_2O_2. Recovery of applied peroxidase was 50%; typical

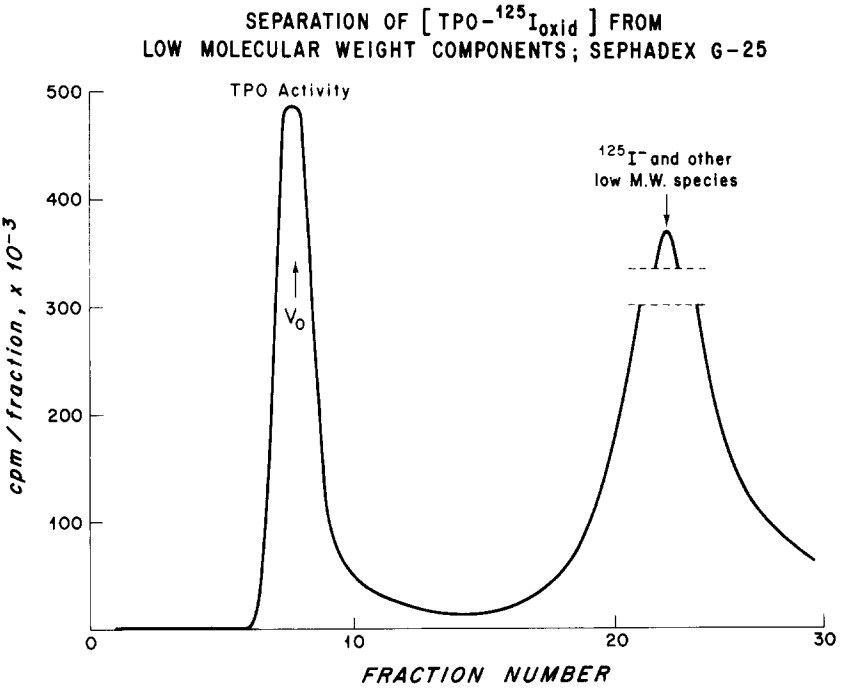

Fig. 2. Gel separation of (peroxidase · $^{125}I_{oxid}$) ([TPO-$^{125}I_{oxid}$]) from low molecular weight components. The experimental conditions are described in the Experimental Procedures section. The incubation system, containing approximately 1.4 nmol peroxidase, 1.5 nmol of $K^{125}I$ and 1.5 nmol of H_2O_2, in 0.5 ml of 0.025 M citrate buffer, pH 5.6 was applied to the column. $5 \cdot 10^6$ cpm were applied to the column, 10^6 cpm emerged in the void volume, corresponding to 0.4 nmol ^{125}I per nmol peroxidase. An additional $3.8 \cdot 10^6$ cpm was recovered in the retarded peak.

for these experiments. 20% of the total radioactivity was recovered in the void volume, representing approximately 0.4 nmol ^{125}I associated with each nmol of thyroid peroxidase. The retarded fractions contained small molecules; unreacted $^{125}I^-$ and H_2O_2.

No low molecular weight iodinating species (e.g., HOI, I_3^-) was generated by the peroxidase during the initial incubation period, since no iodinated Glu-Tyr-Glu was detectable in the retarded fractions.

The thin-layer chromatography and radioautogram of the V_o fractions are shown in Fig. 3. The tripeptide and its iodinated derivatives, visualized with ninhydrin, migrate as shown. Protein remains at the origin. During the preliminary incubation, some iodination of the endogenous protein occurs. The remainder of the label is transferred to Glu-Tyr-Glu, forming the iodinated tripeptide. In this experiment, radioactivity was distributed between Glu-Tyr(I)-Glu, and protein in the ratio 1.8 : 1, as determined from radioactivities of silica gel segments. Paper electrophoresis confirmed the results of Fig. 3; iodoprotein and Glu-Tyr(I)-Glu, were detected by ninhydrin and radioactivity assay, in the above ratio.

As shown in Table II, formation of (peroxidase-I_{oxid}) requires the complete

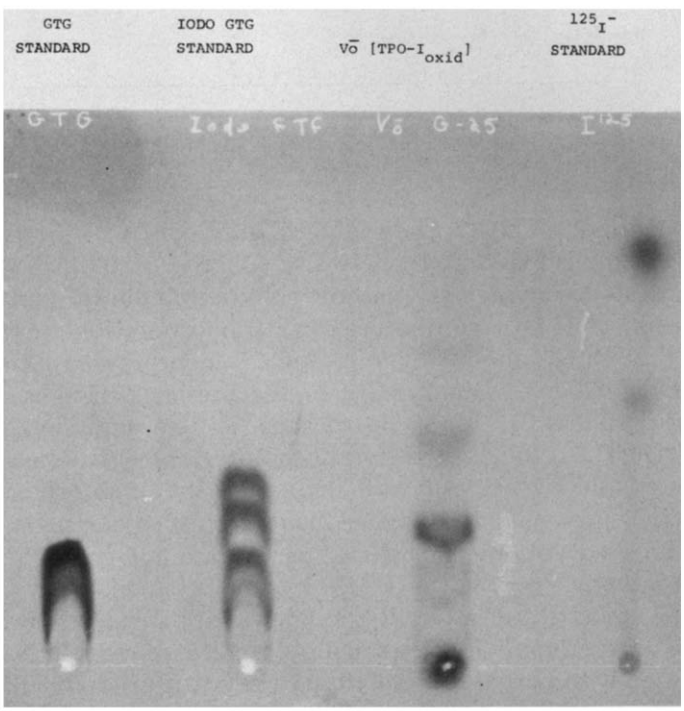

Fig. 3. Iodination of Glu-Tyr-Glu by (peroxidase · I_{oxid}); Chromatographic identification of Glu-[^{125}I] Tyr-Glu. The V_0 fraction of Fig. 2 was lyophilized, applied to a thin-layer plate coated with Silica gel G, and developed, using a 1-butanol/acetic acid/water (12 : 1 : 4) solvent [13]. The left-hand lanes have been visualized with ninhydrin; the two right-hand lanes are visualized in the superimposed radioautograph. Exposure of the thin-layer plate to X-ray film was for 24 h. Glu-Tyr-Glu was iodinated for use as a chromatographic standard, by reaction with freshly dissolved equimolar *N*-iodosuccinimide. The iodo-products were also identified by ultraviolet spectroscopy [8].

TABLE II

REQUIREMENTS FOR INCORPORATION OF TOTAL AND TRANSFERABLE ^{125}I-INTO THYROID PEROXIDASE G-25 FRACTIONS

Generation and gel separation of (peroxidase-$^{125}I_{oxid}$), and detection of iodinated Glu-Tyr-Glu were carried out as described in Experimental Procedures. The V_0 fractions of the complete system (Exp. 1) contained 1 μCi of iodide-125, representing 0.35 nmol iodine associated with each nmol enzyme. In 3, 240 μg peroxidase was inactivated by incubation in 1 mM aminotriazole, 0.5 mM EDTA, 10 μM H_2O_2, 10 μM KI and 0.1 M potassium phosphate, pH 7.4, 1 h, 4°C. In 4, 250 μg porcine thyroglobulin replaced 240 μg thyroid peroxidase in the standard incubation system. In 5, 5 nmol Glu-Tyr-Glu and 3 nmol H_2O_2 were added to each V_0 fraction prior to thin layer chromatography. The same procedure was followed in 6, except that 3 nmol $^{125}I^-$ (1 μCi) replaced H_2O_2. In exp. 7, $^{125}I_2$ (0.06 μCi), equimolar with peroxidase, replaced H_2O_2 and $^{125}I^-$ in the incubation system.

Incubation system (pre-G-25)	Comparative incorporation of $^{125}I^-$ into protein-containing V_0 fractions (%)	Transfer of ^{125}I into Gly-Tyr-Glu in V_0 fractions
1. Complete system; enzyme, K^{125}I, H_2O_2	100	Yes
2. Complete system, omit enzyme	0	No
3. Complete system, but peroxidase inactivated by pre-incubation with amino-triazole and H_2O_2 followed by dialysis	8	Not done
4. Complete system, thyroglobulin replaces enzyme	0	Not done
5. Complete system, omit H_2O_2	8	No
6. Complete system, omit iodide	—	No
7. Enzyme + molecular iodine-125; omit H_2O_2 and iodide	6	No

incubation system: Active peroxidase, H_2O_2 and iodide (Experiment 1). Formation of (peroxidase-I_{oxid}) is diminished more than 10-fold if active peroxidase is omitted, replaced by inactivated enzyme or replaced by thyroglobulin (Experiments 2, 3, and 4). Omission of H_2O_2 also diminishes ^{125}I incorporation more than 10-fold. Moreover, that small amount of ^{125}I which is incorporated into the V_0 fraction is not transferable to Glu-Tyr-Glu in the presence of subsequently added H_2O_2 (Experiment 5). In an incubation system containing enzyme and H_2O_2 (Experiment 6), the V_0 fraction does not carry out iodination of Glu-Tyr-Glu when supplemented with $^{125}I^-$, indicating that under these conditions, none of the H_2O_2 present in the original incubation system remains adsorbed to protein in the void volume; nor does "peroxidase compound I" persist during gel chromatography.

A mixture of thyroid peroxidase and molecular iodine cannot substitute for enzyme, iodide, and H_2O_2 (Experiment 7). The small proportion of radioactive I_2 which remained associated with peroxidase fractions, was not transferable to Glu-Tyr-Glu.

The control experiments summarized in Table II demonstrate that incorporation of transferable ^{125}I into thyroid peroxidase requires the simultaneous presence of active enzyme, iodide and H_2O_2. Non-specific adsorption of H_2O_2, $^{125}I^-$ or $^{125}I_2$ is minimal and cannot account for the radioactive labeling of the void volume fractions or the iodination of Glu-Tyr-Glu in these fractions.

Discussion

Peroxidases, including thyroid peroxidase, catalyze both peroxidation and halogenation. Enzymic halogenation has recently been reviewed [12]. One of the generally proposed reaction sequences may be outlined as follows:

Peroxidation: (a) Peroxidase + H_2O_2 ⇌ oxidized peroxidase (Compound I)

(b) oxidized peroxidase + I^- ⇌ (peroxidase-I_{oxid}) + H_2O (1)

Iodination: (Peroxidase-I_{oxid}) + acceptor ⇌ peroxidase + iodinated acceptor (2)

In Eqn. 1, the enzyme-bound halogen intermediate has been demonstrated for peroxidase by gel filtration (this report) and for horseradish peroxidase by spectroscopy [25—27] and gel filtration [28]. Transfer of enzyme-bound I_{oxid} to an acceptor (Eqn. 2) was shown for peroxidase (this report) and for horseradish peroxidase [28].

Iodination of proteins by I_2 is a well-known reaction [22], and in theory, could be the mechanism by which peroxidase mediated iodinations occur. However, a considerable body of evidence from this and other laboratories argues against this scheme.

We have shown (this report and ref. 8) that acceptor substrates differ in their apparent K_m values and in their abilities to compete for (peroxidase-I_{oxid}). The finding of saturation kinetics and mutual competition implies in itself that there is a site for these substrates on thyroid peroxidase. Our data support the conclusions of Taurog [5], Pommier et al. [6,7] and Dème et al. [29], who have shown that the two thyroid peroxidase-catalyzed reactions, generation of molecular iodine and iodination of protein, are separate processes, with different iodide dependencies.

Data on iodide concentration alone are insufficient for a firm conclusion about the iodinating species. Interpretation is complicated by uncertainty about the rate of iodination by I_2, relative to that for (peroxidase-I_{oxid}). Our pH data provide an alternate approach, leading to the same conclusion. We have shown that Glu-Tyr-Glu is iodinated by thyroid peroxidase with a different pH dependence than that seen for non-enzymic iodination of the same tripeptide. Moreover, the pH dependencies of the two enzymic reactions, generation of molecular iodine and iodination of Glu-Tyr-Glu, are also dissimilar. Apparently, free iodine is neither a necessary nor even a preferred intermediate in peroxidase-catalyzed iodination.

We demonstrated (Figs. 2 and 3) the existence of a peroxidase-associated intermediate, capable of iodinating Glu-Tyr-Glu in the absence of H_2O_2 and iodide. The amount of enzyme in the incubation system is approximate, because all of the presently described peroxidase preparations are of uncertain purity. Our analytical data indicate 20—50% purity for our preparation. The maximal purity, 50%, was chosen for calculations, to provide a minimum estimate for binding of I_{oxid} to thyroid peroxidase. The conclusions based on the binding and transfer experiments would remain unchanged, even at 20% enzymic purity.

Generation of (peroxidase-I_{oxid}) requires active enzyme, iodide, and peroxide. Molecular iodine cannot substitute for iodide and H_2O_2; it is not associated

with the peroxidase fraction to any significant extent, nor is the small amount of incorporated label transferable to Glu-Tyr-Glu. This finding eliminates molecular iodine, formed in the initial incubation and adsorbed to the enzyme, as the iodinating species. A low molecular weight iodinating species other than unbound I_2 has also been ruled out since iodinated tripeptide was demonstrable only in the void volume fractions. The data of Table II eliminate the possibility that the substrates, iodide and H_2O_2, are adsorbed to protein, emerge with peroxidase in the void volume and iodinate the tripeptide.

The incubation and gel filtration procedures of the binding and transfer experiments and the general conclusions are similar to those of Nunez and Pommier in their study of horseradish peroxidase [28].

The enzyme-associated transferable "I_{oxid}" has yet to be characterized, both with respect to its chemical and oxidation state and its association with the enzyme. This work is presently in progress in the laboratory.

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References

1 Neary, J.T., Davidson, B., Armstrong, A., Strout, H.V., Soodak, M. and Maloof, F. (1976) J. Biol. Chem. 251, 2525–2529
2 Serif, G.S. and Kirkwood, S. (1958) J. Biol. Chem. 233, 109–115
3 Alexander, N.M. (1959) J. Biol. Chem. 234, 1530–1533
4 Alexander, N.M. (1962) Anal. Biochem. 4, 341–345
5 Taurog, A. (1970) Arch. Biochem. Biophys. 139, 212–220
6 Pommier, J., de Prailaune, S. and Nunez, J. (1972) Biochemie 54, 483–492
7 Pommier, J., Dême, D. and Nunez, J. (1973) Eur. J. Biochem. 37, 406–414
8 Davidson, B., Neary, J.T., Strout, H.V., Maloof, F. and Soodak, M. (1976) Proc. VII Int. Thyroid Conf. 1975, Excerpta Medica, Series 378, 143–146
9 Maloof, F. and Soodak, M. (1963) Pharmacol. Rev. 15, 43–95
10 Maloof, F., Smith, S. and Soodak, M. (1969) Mechanism of Reactions of Sulfur Compounds 4, 61–67
11 Taurog, A. (1970) Rec. Prog. Horm. Res. 26, 189–247
12 Morrison, M. and Schonbaum, G.R. (1976) Annu. Rev. Biochem. 45, 861–888
13 Krinsky, M.M. and Fruton, J.S. (1971) Biochem. Biophys. Res. Commun. 43, 935–940
14 Jirousek, L. and Pritchard, E.T. (1970) Biochim. Biophys. Acta 208, 275–284
15 Coval, M. and Taurog, A. (1967) J. Biol. Chem. 242, 5510–5523
16 Neary, J.T., Koepsell, D., Davidson, B., Armstrong, A., Strout, H.V., Soodak, M. and Maloof, F. (1977) J. Biol. Chem. 252, 1264–1271
17 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
18 Ornstein, L. (1964) Ann. N.Y. Acad. Sci. 121, 321–349
19 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606–2617
20 Hosoya, T., Kondo, Y. and Ui, N. (1962) J. Biochem. (Tokyo) 52, 180–189
21 Neary, J.T., Davidson, B., Maloof, F. and Soodak, M. (1973) Ann. N.Y. Acad. Sci. 212, 183–194
22 Hughes, W.L. (1957) Ann. N.Y. Acad. Sci. 70, 3–17
23 Li, C.H. (1942) J. Am. Chem. Soc. 64, 1147–1152
24 Mayberry, W.E. and Bertoli, D.A. (1965) J. Org. Chem. 30, 2029–2034
25 Björksten, F. (1970) Biochim. Biophys. Acta 212, 396–406
26 Björksten, F. (1970) Biochim. Biophys. Acta 212, 407–416
27 Chiang, R., Rand-Meir, T., Makino, R. and Hager, L.P. (1976) J. Biol. Chem. 251, 6340–6346
28 Nunez, J. and Pommier, J. (1968) Eur. J. Biochem. 5, 114–118
29 Dême, D., Fimiani, E., Pommier, J. and Nunez, J. (1975) Eur. J. Biochem. 51, 329–336