

Spatial requirement for coupling of iodotyrosine residues to form thyroid hormones

(thyroglobulin models/enzymatic iodination of tyrosine-containing peptides and polymers/models for thyroxine biosynthesis)

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ABSTRACT A linear random copolymer of tyrosine and lysine and two synthetic oligopeptides containing two tyrosine residues in addition to lysine residues give thyroid hormone (thyroxine and triiodothyronine) residues in good yield upon enzymatic iodination with thyroid peroxidase. These synthetic peptides may serve as simple models for thyroglobulin, the protein in which biosynthesis of the thyroid hormone takes place. For the formation of significant amounts of hormone, such model compounds must contain at least two properly spaced tyrosine residues.

Thyroid hormone biosynthesis takes place in thyroglobulin, a protein of high molecular weight (660,000). Certain tyrosine residues in this protein are iodinated to form monoiodo- and diiodotyrosine residues, some of which then react with each other ("coupling reaction") to form hormone (thyroxine and 3,5,3'-triiodothyronine) residues. Both iodination and coupling are catalyzed by thyroid peroxidase (1, 2). The more easily available lactoperoxidase can replace thyroid peroxidase in experiments *in vitro*.

Because only a small fraction of the iodotyrosine residues in thyroglobulin couple, it is evident that the spatial alignment of the interacting residues is of utmost importance. The nature of the amino acid residues in the vicinity of the coupling sites and the distance between the two iodotyrosine residues are certainly among the most important factors influencing the spatial alignment.

Thyroglobulin, whose primary sequence is unknown, is much too complex a molecule (3, 4) for a study of the role of these parameters. We have, therefore, searched for simple models for thyroglobulin that contain, besides tyrosine, only one or a few other amino acids. We have investigated a number of tyrosine-containing synthetic di- and tripeptides, oligopeptides, and copolymers for their ability to produce thyroid hormone. Treatment of some of these with iodide in the presence of thyroid peroxidase and a hydrogen peroxide generating system caused not only iodination of tyrosine residues, but also coupling of iodotyrosine residues with the formation of thyroid hormones in good yield.

MATERIALS AND METHODS

Thyroid peroxidase [specific activity, 230 units/mg; one unit of peroxidase causes an increase of the absorbance at 353 nm (I_{3}^{-}) by 1 absorbance unit per min at 20° in the following system: 10 mM iodide, 6 mM glucose, 5 μ g of glucose oxidase per ml, in 50 mM phosphate buffer, pH 7.2] was prepared from hog thyroids as described previously (5). Lactoperoxidase was from Calbiochem and glucose oxidase was from Boehringer

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Tyrosyl- N^{α} -lysine was synthesized by A. Nishinaga, Kyoto University. Other tyrosine-containing di- and tripeptides were purchased from various commercial sources. The first two copolymers of tyrosine listed in Table 1 were a gift of J. Ramachandran, Hormone Research Laboratory of the University of California; all other copolymers were kindly supplied by M. Sela and R. Arnon, The Weizmann Institute of Science. Multichain copolymers had a polylysine backbone with side chains coupled through their free ϵ -NH₂ groups to the COOH groups of poly(DLAla) chains or of poly(LTyr) chains whose NH₂ groups were then coupled to the α -COOH groups of poly(LGlu) chains or to the COOH groups of poly(DLAla) chains. The NH₂ groups of the resulting branched copolymers were then coupled to the COOH groups of poly(LTyr) chains or to the α -COOH groups of poly(LGlu) chains. (See Table 1.)

Iodination of these substrates with $^{125}\text{I}^{-}$ (0.25 mM) was carried out at pH 7.2 as described previously for thyroglobulin (6), using 0.4–0.8 unit of peroxidase per ml for kinetic studies and a 5- to 8-times higher concentration for exhaustive iodination, in which case the reaction was permitted to proceed for 1 hr.

Iodoamino acid distribution was determined by paper chromatography (2) or by thin-layer chromatography (7), after hydrolysis with Pronase.

RESULTS AND DISCUSSION

Free tyrosine is iodinated by iodide in the presence of thyroid peroxidase, glucose, and glucose-oxidase, but the diiodotyrosine formed does not undergo self-coupling (J.-L. Michot, H. Edelhoeh, and J. Nunez, unpublished data), nor does it couple with diiodotyrosine residues in thyroglobulin (6). None of 10 di- and tripeptides of tyrosine yielded detectable amounts of thyroxine upon enzymatic iodination. Another dipeptide, tyrosyl- N^{α} -lysine, formed only traces of thyroxine.

Results obtained with synthetic copolymers of tyrosine are shown in Table 1. All of these copolymers could be iodinated with thyroid peroxidase, although to a lesser extent than with lactoperoxidase. Only in one of the polymers, a linear random copolymer of tyrosine and lysine, were all tyrosine residues converted to diiodotyrosine residues with either one of the two enzymes.

This polymer had an average molecular weight of 29,500 and contained 2.1 mol % tyrosine. This is slightly less than the tyrosine content of thyroglobulin (3–4%). The same polymer was the only one in which coupling proceeded with an appreciable thyroxine yield, which was 11% with lactoperoxidase and 15% with thyroid peroxidase. This means that 15 out of 100 diiodotyrosine residues coupled to form thyroxine. The kinetics of a typical iodination experiment with thyroid peroxidase are shown in Fig. 1. It is of interest to note that these kinetics closely

Table 1. Enzymatic iodination of copolymers with lactoperoxidase (LPO) and with thyroid peroxidase (TPO)

Copolymer	Type of copolymer*	Iodination, iodine incorporation (% of maximal incorporation)		Coupling, amount of thyroxine formed (% of incorporated iodine)	
		LPO	TPO	LPO	TPO
		(LTyr,LAla,LGlu) _n (33 mol % Tyr)	LR	27	0.74
(LTyr-LAla-LGlu) _n (33 mol % Tyr)	LO	33	2.7	2	0
(LTyr,LGlu) _n (2.5 mol % Tyr)	LR	100	37	2	1
(LTyr,LLys) _n (2.1 mol % Tyr)	LR	100	100	11	15
poly(LLys) poly(LTyr)-poly(LGlu)-poly(DLAla) (15 wt % Tyr)	M	44	3.1	0	0
poly(LLys) poly(LTyr)-poly(LGlu)-poly(DLAla) (21 wt % Tyr)	M	37	3.4	0	0
poly(LLys) poly(LGlu)-poly(DLAla)-poly(LTyr) (10 wt % Tyr)	M	27	8.6	0	0

* LR, linear random; LO, linear ordered; M, multichain.

resemble those of the iodination of thyroglobulin (2).

Because the tyrosine-lysine copolymer gave a good coupling yield while di- and tripeptides containing a single tyrosine residue, including tyrosine-lysine, gave only insignificant amounts of thyroxine, if any, it can be concluded that efficient coupling requires interaction of *two* diiodotyrosine residues within the same peptide chain (intramolecular reaction). The polymer must be able to assume a conformation in which the two interacting diiodotyrosine residues come close together and align themselves in such a manner that coupling can proceed.

The distance between the two diiodotyrosine residues along the polypeptide chain is therefore of utmost importance. Because the polymer that gave a good coupling yield has a random sequence, this distance cannot be determined. For this reason, we have synthesized, using the rapid solid-phase method of Corley *et al.* (8), two oligopeptides (seven and nine amino acid residues) of known sequence that contain two differently spaced tyrosine residues. The reaction efficiency in each synthetic step, determined according to Eastlake *et al.* (9), always exceeded 98% and in most instances 99%. Because the product of all efficiencies was 96–97%, the final peptides were used without further purification. Amino acid compositions agreed well with the theoretical values.

Enzymatic iodination of these peptides with thyroid perox-

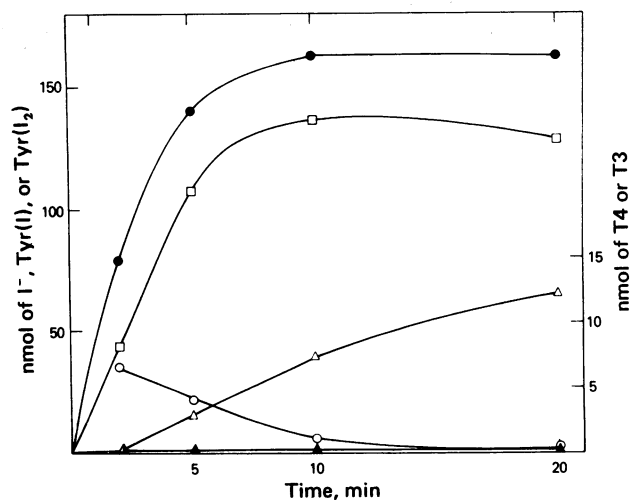


FIG. 1. Kinetics of formation of monoiodotyrosine [Tyr(I), O], diiodotyrosine [Tyr(I₂), □], thyroxine (T₄, Δ), and triiodothyronine (T₃, ▲) during the enzymatic iodination (incorporated I⁻, ●) of a linear random tyrosine-lysine copolymer.

idase showed that small peptides can serve as models for thyroglobulin, provided that they contain at least *two* tyrosine residues, so that intramolecular coupling can take place.

The two peptides, in which the tyrosine residues are separated by three (peptide I) or by five (peptide II) lysine residues, were almost completely iodinated, and both formed thyroxine as well as triiodothyronine (Table 2). However, peptide II produced more than twice the amount of hormone that peptide I did. Atom models of peptides I and II show that the polypeptide chains of both can easily be arranged in such a manner that they form a loop with all lysine side chains pointing towards the outside and the two aromatic rings of tyrosine being sandwiched together inside the loop (Fig. 2).

However, while it is very easy to align these rings in peptide II in such a manner that they assume an antiparallel position (Fig. 3A), this is not possible with peptide I where, at best, the alignment shown in Fig. 3B can be made without exerting strain. In that alignment there is steric hindrance due to the close proximity of two bulky iodine atoms (see also Fig. 2).

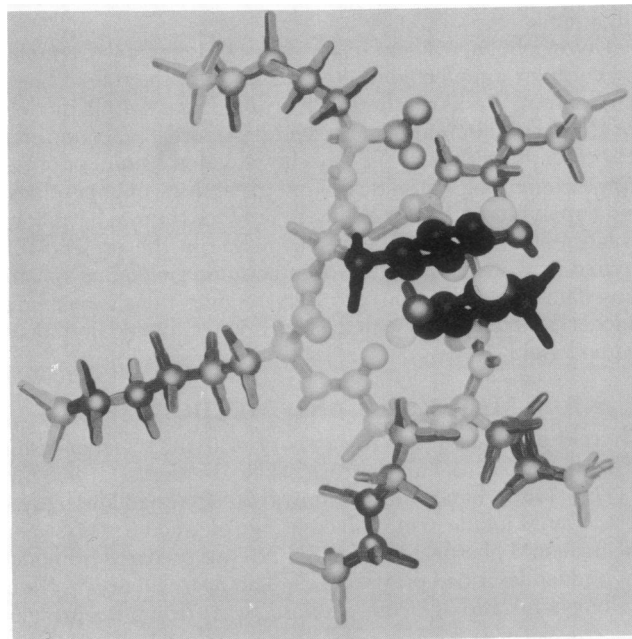


FIG. 2. Atom model of peptide I showing a conformation in which coupling can take place. The black balls represent the two aromatic rings and the big white balls, the iodine atoms. The lysine side chains point towards the outside.

Table 2. Enzymatic iodination (with thyroid peroxidase) of peptides Lys-Tyr-(Lys)_n-Tyr-Lys

Peptide	Incorporated iodine, atom/mol	% of incorporated iodine in	
		Triiodo-thyronine	Thyroxine
I (n = 3)	3.64	2.0	4.7
II (n = 5)	3.50	1.6	14

Consequently, one would expect that in peptide I, coupling of a diiodotyrosine residue with a monoiodotyrosine residue (with formation of triiodothyronine) is more favored than in peptide II, because in monoiodotyrosine the interfering iodine atom is missing. One would also expect that in peptide II, in which no steric hindrance inhibits the coupling of two diiodotyrosine residues, thyroxine should be formed more easily than in peptide I. The experimental data (Table 2) are in agreement with these assumptions.

Triiodothyronine is still present in both peptides after exhaustive iodination. This not only shows that coupling of monoiodotyrosine residues competes with their further iodination to diiodotyrosine residues, but also suggests that triiodothyronine residues are not further iodinated and that virtually all thyroxine residues are formed by coupling of two diiodotyrosine residues. A similar conclusion has been reached previously in experiments with thyroglobulin (2). Moreover, free triiodothyronine cannot be iodinated to thyroxine with thyroid peroxidase or lactoperoxidase (unpublished data).

The great advantage of synthetic oligopeptides over random copolymers is that the exact sequence of the amino acid residues and hence the spacing between the tyrosine residues is known.

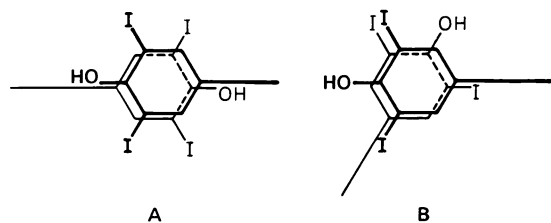


FIG. 3. Alignments of the two diiodotyrosine residues obtainable without exerting strain in peptide I (B) and in peptide II (A). The two diiodotyrosine residues are separated by three lysine residues in peptide I and by five lysine residues in peptide II.

These peptides can be synthesized with amino acids other than lysine for a study of the influence of the amino acid environment on iodination and coupling. Finally, the peptides can be synthesized with one or two ¹⁴C- or ³H-labeled tyrosine residues. This may be helpful in determining which of the two interacting tyrosine residues in a peptide is the acceptor and which is the donor of an aromatic ring in the course of the coupling reaction. It may also be helpful in elucidating the fate of the alanine side chain of diiodotyrosine, which remains when the aromatic ring of a diiodotyrosine residue is being transferred to another diiodotyrosine residue. The fate of this "lost side chain," which has been under discussion ever since Johnson and Tewkesbury proposed a mechanism for the coupling reaction (10), still remains obscure.

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