THYROID HORMONE SYNTHESIS AND REACTIVITY OF HORMONE-FORMING TYROSINE RESIDUES OF THYROGLOBULIN

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1. Introduction

The biosynthesis of thyroid hormones [thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3)] which occurs in thyroglobulin (Tgb) is critically dependent upon the native structure of the protein [1–3]. It is now well established that among the tyrosine residues of Tgb (~140 in porcine Tgb) only 40–45 are accessible to iodination into 3-iodotyrosyl and 3,5-diiodotyrosyl residues and that only few of the latter can couple to form thyroxyl and triiodothyronyl residues. In addition, it is also likely that iodination of the iodinable 40–45 tyrosyl residues occurs in a rigid sequential order, preferential hormonal residue synthesis occurring from early iodinated tyrosyl residues [4–6]. Thyroid peroxidase which catalyzes both iodination and coupling might play a role in the choice of tyrosyl residues to be iodinated and later coupled [7].

Thyroid hormone synthesis obtained by thyroid peroxidase-catalyzed iodination of very poorly iodinated human thyroglobulin was considerably decreased when the protein was affected by limited structural modifications [8]. Here, we show that such changes which do not modify the coupling capacity of the hormonogenic sites are sufficient to induce a change in the sequential order of iodination of thyroglobulin hormonogenic tyrosines resulting in decreased efficiency of the hormone-forming function.

2. Materials and methods

2.1. *Thyroglobulin preparations*

Iodine-poor thyroglobulin preparations (0.01–0.04%, g/100 g) were obtained from human colloid goitre. Tgb-1 or native Tgb was prepared in conditions maximally limiting proteolytic attack [9,10]. Tgb-2 was prepared according to [11]; this preparation disclosed a very limited proteolysis as shown by SDS–polyacrylamide gel electrophoresis (see fig.1 of [8]).

2.2. *Controlled hydrolysis of thyroglobulin by subtilisin*

Tgb-1 was dissolved at 0.2% in 5 mM Na-phosphate (pH 7.2) and digested with subtilisin in an enzyme/substrate ratio of 1:100 (w/w). After 0.5 h at 25°C, the reaction was stopped with fluorophenylmethylsulfone (2.5 mM) and the reaction mixture was lyophilized. The hydrolysate (Tgb-3) is mainly formed of 3 fragments of M_r 195 000, 160 000 and 57 000 as shown by SDS–polyacrylamide gel electrophoresis.

2.3. *Treatment of thyroglobulin by guanidine*

Tgb-1 was dissolved at 0.06% (w/v) in 6 M guanidine–HCl in 50 mM Na-phosphate (pH 7.2). After 0.5 h at 20°C, the solution was dialyzed against 100 vol. water and lyophilized. This preparation was designated Tgb-4.

2.4. *Thyroglobulin iodination*

Tgb preparations (Tgb-1–Tgb-4) were iodinated as follows:

(1) Iodination with ^{125}I: in 50 mM Na-phosphate (pH 7) in a final volume of 1 ml, were added 0.6 mg Tgb (1 nmol) 30 nmol $Na^{125}I$ (~0.5 μCi), 0.5 mg glucose and 1 unit thyroid peroxidase. After 2 min preincubation at 37°C, the reaction was started by the addition of 1 unit glucose oxidase (250 units/mg, grade I Boehringer) and stopped 1 min later by addition of 100 μl 50 mM $NaHSO_3$. Iodide in excess was

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eliminated by filtration of the reaction mixture on a column of Sephadex G-25 (0.9 × 5 cm) equilibrated in buffer. The labeled protein was dialyzed for 6 h at 4°C against 1 l water and freeze-dried. In these conditions, 20 ± 1.5 iodine atoms are bound to Tgb whatever the preparations used (Tgb-1–Tgb-4).

(2) Iodination with ^{131}I: The Tgb iodinated with ^{125}I (1 nmol) which still contains thyroid peroxidase and glucose oxidase was dissolved in 1 ml the same phosphate buffer and supplemented with 0.5 mg glucose and increasing amounts of $Na^{131}I$ (20–130 nmol). After 15 min incubation at 37°C, the reaction was stopped by addition of 100 μl 50 mM $NaHSO_3$, iodide was eliminated by Sephadex filtration and the doubly labeled protein was freeze-dried after dialysis against water.

2.5. *Other techniques*

Thyroid peroxidase was isolated from ox thyroids according to [12] and purified on Sephacryl S-200 and Biogel HTP [13]. Specific activity of the preparation was 205 units/mg protein, 1 unit corresponding to the amount of enzyme necessary to oxidize 10^{-6} mol iodide/min under the conditions in [13]. Protein concentration of Tgb solution was measured spectrophotometrically at 280 nm using $E^{1\%}_{1\,cm} = 10.0$.

Iodoamino acid separation and estimation were performed by ion-exchange chromatography in a Technicon Autoanalyzer after digestion with pronase and leucylaminopeptidase [14]. Radioiodine radioactivity was counted in a Packard Autogramma spectrometer.

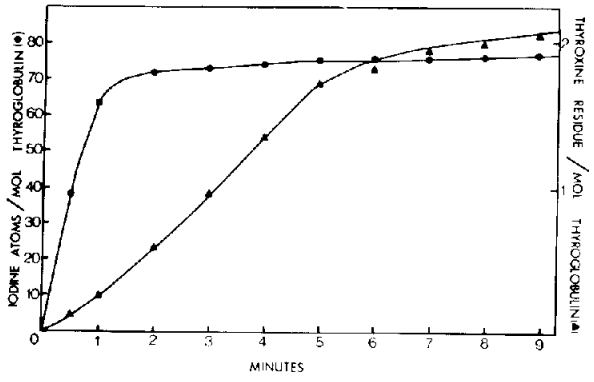

Fig.1. Kinetics of iodination and thyroxine formation in thyroglobulin. Tgb-1 (1 μM) was incubated for 9 min in 50 mM Na-phosphate (pH 7.2) in presence of 100 μM iodide labeled with ^{125}I, 1 unit/ml thyroid peroxidase, 5 μg/ml glucose oxidase and 0.5 mg/ml glucose at 37°C. Iodination level and thyroxine content were measured in samples of medium after different incubation times as in section 2.

3. Results and discussion

Fig.1 shows that the thyroid peroxidase-catalyzed iodination of thyroglobulin (Tgb-1) occurs much more rapidly than the formation of thyroxine residues. Indeed after 1 min incubation, iodination is almost complete while hormone synthesis is very low. A very similar kinetics was obtained with Tgb-2. In spite of

Table 1
$^{125}I/^{131}I$ ratio in thyroxine and 3,5,3′-triiodothyronine synthesized in different preparations of thyroglobulin as a function of iodination level (see fig.2)

	Atoms ^{131}I/mol thyroglobulin prelabeled with ^{125}I[a]	$^{125}I/^{131}I$ ratio in	
		T_3	T_4
Tgb-1	0		
	9.5	2	2.32
	19.4	1.83	1.84
	30.1	1.84	1.69
	41.5	1.78	1.55
	49.8	1.84	1.45
	62.4	1.91	1.40
	75.6	1.91	1.41
Tgb-2	10.5	1.33	1.09
	20.6	0.75	0.64
	31.5	0.62	0.57
	39.6	0.55	0.44
	54.2	0.54	0.41
	66.7	0.57	0.37
	75.9	0.55	0.36
Tgb-3	11.4		1.20
	19.8	0.50	0.77
	32.4	0.25	0.46
	55.5	0.16	0.40
	72.9	0.16	0.41
Tgb-4	11.4	0.66	0.64
	20.8	0.40	0.39
	34.2	0.37	0.32
	42.7	0.36	0.30
	54.5	0.26	0.21
	67.2	0.26	0.20
	76.7	0.25	0.19

[a] The Tgb preparations (Tgb-1, native; Tgb-2, weakly proteolyzed; Tgb-3, subtilisin treated; Tgb-4, guanidine treated) contained ~20 iodine atoms/mol

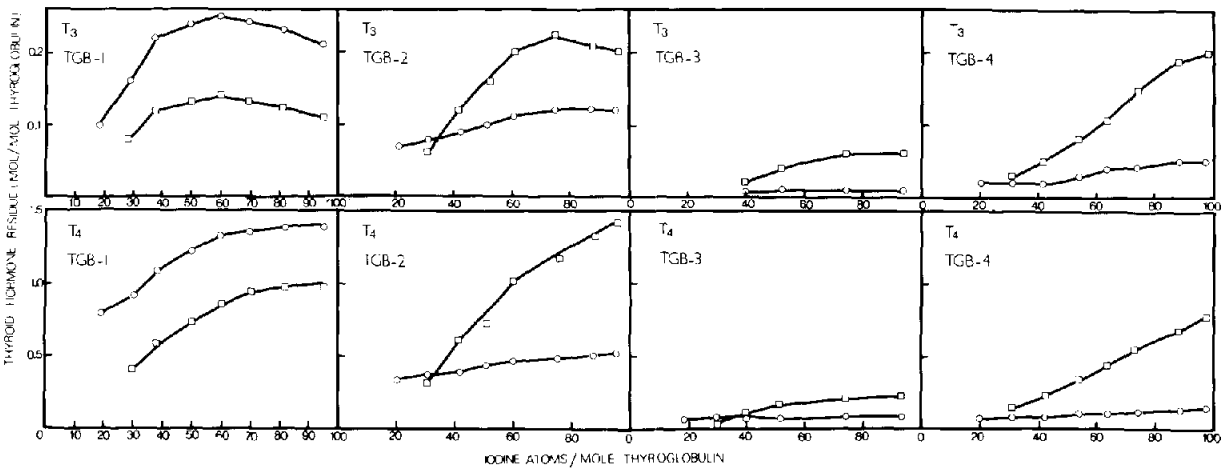

Fig.2. Effect of increased iodine (^{131}I) levels on thyroid hormone synthesis in hormone-free poorly iodinated [^{125}I] thyroglobulin preparations. The experiment was performed in two steps: (1) a short ^{125}I-labeling (1 min) was carried out in the presence of 1 μM thyroglobulin, 30 μM iodide labeled with ^{125}I, 1 unit/ml thyroid peroxidase, 1 units/ml glucose oxidase and 0.5 mg/ml glucose. ^{125}I-Labeled thyroglobulin (~20 atoms I/mol) was isolated by gel filtration on Sephadex G-25; (2) 1 μM ^{125}I-labeled thyroglobulin was iodinated with increasing concentrations of iodide labeled with ^{131}I (30–130 μM) as in section 2. T_4 and T_3 contents were measured from ^{125}I (○) and ^{131}I (□) labeling.

the absence of lag-time in the formation of hormone observed in [5], the reactions of iodination and coupling could be dissociated and this allowed to prepare iodinated Tgb almost deprived of hormone residues. Using such material, it is possible to investigate the coupling of iodotyrosine residues as a function of increasing iodine content of the protein thus allowing a study of the reactivity of hormonogenic tyrosine residues of thyroglobulin. Indeed, if a first iodination step using ^{125}I is performed to obtain in 1 min the incorporation of ~20 iodine atoms in the protein, the tyrosine residues iodinated with ^{125}I should represent those with the highest reactivity towards iodine. When iodination of the protein is pursued for 15 min using an other label (^{131}I), iodine will bind to other tyrosine residues of lower reactivity and coupling of iodotyrosine residues will occur to form iodothyronine residues containing both radioiodine isotopes. The value of the ^{125}I/^{131}I ratio in the formed hormone residues should be a measure of the reactivity of the hormonogenic tyrosine residues towards iodine. Such experiments have been performed using native Tgb (Tgb-1), two preparations of the protein at 2 degrees of limited proteolytic attack (Tgb-2 and Tgb-3) and a preparation of Tgb denatured by treatment with 6 M guanidine prior to iodination (Tgb-4).

The amounts of ^{125}I- and ^{131}I-labeled T_4 and T_3 formed in the 4 Tgb preparations as a function of increasing iodine level of the protein are illustrated in fig.2. To the exception of the native Tgb-1, the 3 other Tgb preparations contained more hormone residues labeled with ^{131}I than with ^{125}I. From the data of fig.2, the ^{125}I/^{131}I ratio for T_4 and T_3 were calculated at all iodination levels (table 1). For native Tgb-1, this ratio varies from 2.32–1.41 for T_4 and

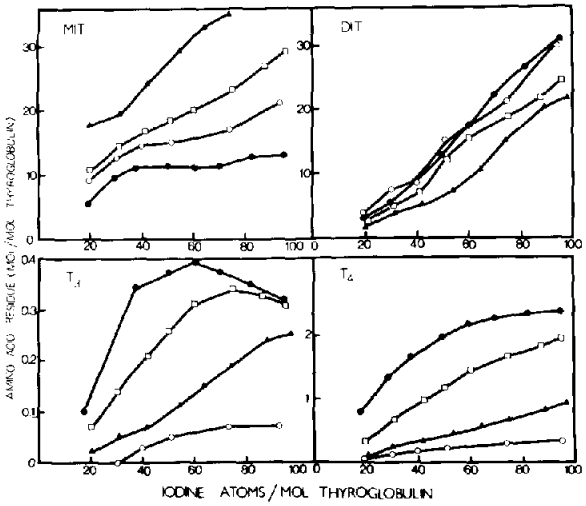

Fig.3. Iodoamino acid distribution in different preparations of thyroglobulin as a function of no. iodine atoms incorp./mol protein. Same experiments as in fig.2. (●) Tgb-1; (□) Tgb-2; (○) Tgb-3; (▲) Tgb-4.

from 2.0–1.9 for T_3 for iodination levels comprised between 29.5 and 95.5 iodine atoms/mol Tgb while for the other Tgb preparations the ratio is considerably lowered for equivalent iodination levels.

These results suggest that the hormonogenic tyrosine residues of native Tgb (Tgb-1) have a higher reactivity towards iodine than those of Tgb-2, Tgb-3 and Tgb-4 and that the first residues to be iodinated are the first to couple into hormonal residues, in agreement with [4–6]. In contrast, this conclusion is not valid for the other Tgb preparations. The structural modifications undergone by the protein allowed some tyrosine residues to be more reactive towards iodine than the hormone forming residues present in native Tgb-1.

Alterations of the native structure of the protein in Tgb-2, Tgb-3 and Tgb-4 are well illustrated by the increase in MIT residues formed as compared to Tgb-1 (fig.3), a consequence of the accessibility to iodine of tyrosine residues masked in native Tgb. As compared to Tgb-1, hormone synthesis is deeply modified in Tgb-2–Tgb-4. For the highest iodination level (95.5 iodine atoms/mol Tgb), Tgb-4 formed only 41% of the T_4 and 80% of the T_3 synthesized in Tgb-1; for Tgb-3, hormonal synthesis yield is yet more decreased since only 13% of the T_4 and 20% of the T_3 present in Tgb-1 has been estimated. In contrast, at high iodination levels, the number of hormone residues synthesized in Tgb-2 is close to that found in Tgb-1. However, hormone synthesis efficiency in Tgb-2 is largely diminished. Indeed, for low levels of Tgb iodination (20–30 iodine atoms/mol), Tgb-2 synthesized 1–3-times less hormones than Tgb-1.

Thus, it appears that the structural alterations generated by the very limited proteolytic attack undergone by the protein in Tgb-2 have modified only the sequential order of iodination of tyrosine residues by allowing some non-hormone forming tyrosine residues to be more reactive to iodine than the hormone forming residues involved in Tgb-1. At low iodination levels iodine binds preferentially to these residues at the expense of hormone forming sites which can be iodinated only at higher levels of iodination. However, once iodinated hormonogenic tyrosines preserve their aptitude to couple into iodothyronine residues since Tgb-2 at high iodination levels can synthesize almost the same number of hormone residues as native Tgb. Disruption of hormone synthesis sites occurred only after large structural alterations of the protein either by breakage of numerous covalent (Tgb-3) or non-covalent (Tgb-4) bonds in the peptide chains, whereas slight structural alterations (Tgb-2) are sufficient to modify the reactivity of hormone forming tyrosine residues.

It is thus clear, that the efficiency of Tgb as an hormone forming protein is related to its native state. The structure of the protein is especially well adapted to form T_4 and T_3 residues at low iodination levels since limited alteration of the native structure modifies the order of iodination of hormone forming tyrosine residues and results in a decreased synthesis efficiency.

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References

[1] Lamas, L., Covelli, T., Edelhoch, H., Cortese, F. and Salvatore, G. (1971) in: Further Advances in Thyroid Research (Fellinger, K. and Höfer, R. eds) p. 201, Springer Verlag, Berlin, New York.
[2] Rolland, M., Montfort, M. F., Valenta, L. and Lissitzky, S. (1972) Clin. Chim. Acta 39, 95–108.
[3] Rolland, M., Montfort, M. F. and Lissitzky, S. (1973) Biochim. Biophys. Acta 303, 338–347.
[4] Lamas, L., Taurog, A., Salvatore, G. and Edelhoch, H. (1974) J. Biol. Chem. 249, 2732–2737.
[5] Gavaret, J. M., Deme, D., Nunez, J. and Salvatore, G. (1977) J. Biol. Chem. 252, 3281–3285.
[6] Deme, D., Pommier, J. and Nunez, J. (1976) Eur. J. Biochem. 70, 435–440.
[7] Deme, D., Pommier, J. and Nunez, J. (1978) Biochim. Biophys. Acta 540, 73–82.
[8] Maurizis, J. C., Marriq, C., Michelot, J., Rolland, M. and Lissitzky, S. (1979) FEBS Lett. 102, 82–86.
[9] Rolland, M. and Lissitzky, S. (1976) Biochim. Biophys. Acta 427, 696–704.
[10] Marriq, C., Rolland, M. and Lissitzky, S. (1977) Eur. J. Biochem. 79, 143–149.
[11] Bouchilloux, S., Rolland, M., Torresani, J., Roques, M. and Lissitzky, S. (1964) Biochim. Biophys. Acta 93, 15–30.
[12] Pommier, J., Deme, D. and Nunez, J. (1975) Eur. J. Biochem. 51, 329–336.
[13] Alexander, N. M. (1977) Endocrinology 100, 1610–1620.
[14] Rolland, M., Aquaron, R. and Lissitzky, S. (1970) Anal. Biochem. 242, 307–317.