THYROGLOBULIN MATURATION IN RAT AND NOMENCLATURE OF THYROGLOBULIN-LIKE PROTEINS

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Experiments with surviving slices have shown that an amino acid-labeled S12 precursor protein was capable of associating into a TG* dimer (see below for nomenclature) in the absence of iodination (Seed and Goldberg, 1965 a ; Lissitzky et al. 1965 b ; Nunez et al. 1965). Maturation of the uniodinated TG dimer (Nunez et al. 1965 ; Goldberg and Seed, 1965) as shown by the increase of sedimentation constant from 16 or 17 to 19 was demonstrated in vitro and probably correlated with iodination of the molecule (Seed and Goldberg, 1965 b). That iodination is subsequent to peptide chain synthesis of TG either in vitro or in vivo has been well documented (see Lissitzky et al. 1965 b). It is still unproved that iodination in vivo is necessary for the maturation of the uniodinated TG dimer. The present experiments have been carried out to give information on this point.

Wistar rats (male, 300 g) receiving 5 µg iodine per day and in isotopic equilibrium with ^{125}I were injected for four days with a mixture of propylthiouracil 5 mg and T_3 10 µg and were sacrificed 1 to 24 hr after a single ^{131}I injection. Treatment of thyroid tissue and methods have been described previously (Lissitzky et al. 1965b).

In vitro and in vivo studies had shown that newly iodinated TG molecules were more sensitive to alkali- or SDS-dissociation into

* The abbreviations used are : TG, thyroglobulin ; PTU, propylthiouracil ; SDS, sodium dodecylsulfate ; MIT, 3-iodotyrosine ; DIT, 3,5-di-iodotyrosine ; T_4, thyroxine ; T_3, 3,5,3'-tri-iodothyronine ; SRA, specific radioactivity ; PBS, phosphate-NaCl buffer pH 6.8 µ = 0.15.

S12 subunit and presented a lower sedimentation coefficient than preformed S19 TG (Lissitzky et al. 1964, 1965 b ; Sellin and Goldberg, 1965). In PTU + T_3-treated rats in which thyroid iodine content is not modified as compared with T_3- treated (Table I) or saline-treated animals, sucrose gradient centrifugation of an unfrozen saline extract of fresh thyroids obtained 24 hr after ^{131}I injection, shows a single ^{131}I-protein peak with a lower sedimentation coefficient than ^{127}I-preformed TG dimer (fig. 1A) .

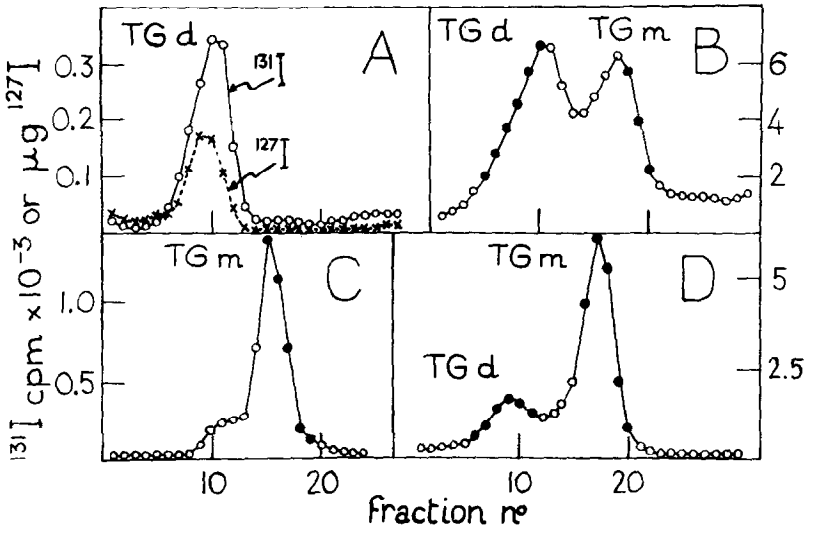

Fig. 1 - Effect of cold and alkali treatment on the dissociation of newly iodinated thyroglobulin dimer in PTU + T_3-treated rats.
17 rats in isotopic equilibrium with ^{125}I having received 5 µg $K^{127}I$ for 4 months, were injected with 5 mg PTU and 10 µg T_3 daily for 4 days and sacrificed 24 hr after the injection of 200 µc ^{131}I. Thyroid glands were pooled and a saline (0.15 M NaCl) extract was obtained : centrifugation on a sucrose gradient in PBS (5-20 %) in the SW25 rotor of Spinco L (24 hr, 24,000 rpm) of the extract before (A) and after (B) freezing for 24 hr at - 20°. The 3 fractions (full circles) of peak TGm (B) were concentrated under reduced pressure against PBS and recentrifuged (SW39, 6 hr, 39,000 rpm) (curve C). The 6 fractions (full circles of peak TGd (curve B) were pooled and concentrated by dialysis under reduced pressure against 0.01M NH_4OH for 4 hr and centrifuged in the rotor SW25. The fractions indicated by full circles in C and D were used for pronase digestion
An extract of the thyroids of rats treated with T_3 only and submitted to centrifugation after freezing in the same conditions (see B) showed no TGm peak. TGd, thyroglobulin dimer ; TGm, thyroglobulin monomer (S12).

TABLE I

Effect of PTU + T_3 treatment on time course of ^{131}I uptake, ^{127}I thyroid content and specific radioactivity of iodine in thyroglobulin and its subunits in rats in isotopic equilibrium with ^{125}I.

rats	time after ^{131}I injection (hr)	^{131}I uptake (% of injected dose)	^{127}I (µg per gland	SRA° of TG fractions S19	SRA° of TG fractions S12●	SRA S12 / SRA S19 †	^{131}I-cold-labile TG dimer × (%)
untreated controls (4)	1	1.00	7.7	0.03	0.10	3.4	13.7
	6	4.44	7.2	0.16	0.30	1.8	3.7
	24	5.07	9.1	0.11	0.18	1.5	3.7
PTU + T_3 treated (6)	1	0.62	6.7	0.004	0.013	3.2	10.0
	6	0.99	6.3	0.018	0.139	7.9	28.1
	24	0.43	9.1	0.05	0.051	10.2	23.9

Rats in isotopic equilibrium with ^{125}I were injected with 5 mg PTU and 10 µg T_3 daily for 4 days. A single dose of ^{131}I was given subcuteneously (50, 25 and 25 µc for T_3-treated controls 1, 6 and 24 hr before sacrifice ; 500, 250 and 100 µc for PTU + T_3-treated animals 1, 6 and 24 hr before sacrifice).
Into brackets in column 1 is the number of rats used for each time interval.
A saline (0.15M NaCl) extract of the freshly obtained thyroid glands was frozen for 16 hr before sucrose gradient centrifugation (5-20 %) in the SW 39 rotor of Spinco L for 5.5 hr at 39,000 rpm. Sucrose solutions made with PBS.

° Specific radioactivity : % of ^{131}I injected dose per µg ^{127}I.
● corresponds to ^{131}I-cold-labile TG dimer
† SRA of cold-labile TG dimer over SRA of remaining cold-stable TG dimer.
× % of ^{131}I-TG dimer which dissociates into S12 TG monomer under the influence of freezing and thawing the extract.

TABLE II

Distribution of ^{131}I in iodoaminoacids and their specific radioactivity in thyroglobulin fractions from PTU + T_3-treated rats.

nature of the protein digested	mean SRA* of the protein fraction	Distribution of ^{131}I (%) in and SRA* (figures into brackets) of				$\frac{^{131}I\text{-MIT}}{^{131}I\text{-DIT}}$
		MIT	DIT	T_4	T_3	
cold-labile TG dimer	3.9	59.3 (9.22)	17.8 (1.56)	0.5 (0.13)	0.6 (0.11)	3.8
alkali-labile TG dimer	1.6	64.0 (4.77)	18.8 (0.92)	0.7 (0.06)	0.2 (0.09)	3.4
cold- and alkali-stable TG dimer	0.3	42.8 (0.60)	33.2 (0.25)	2.3 (0.03)	0.4 (0.05)	1.3

Cold-labile TG dimer represents the TGm peak of experiment described in fig. 1C ; alkali-labile and cold- and alkali-stable TG dimers are respectively the TGm and the TGd peaks of experiment described in fig. 1D. Digestion with pronase and paper chromatographic analysis described in Lissitzky et al. (1965 b).

* % of ^{131}I injected dose x 10^3 per µg ^{127}I.

If the extract was frozen (-20°) and thawed before centrifugation, a fraction of the ^{131}I-peak (cold-labile TG dimer) dissociated into an ^{131}I-S12 subunit (TGm) with higher specific radioactivity than the mean specific radioactivity of the whole remaining dimer (fig. 1B, 1C and table I). Additional dissociation of the latter was obtained by dialysis against 0.01M NH_4OH (fig. 1D). In intact rats, cold- or alkali-labile TG may be demonstrated only at short time intervals after ^{131}I injection (Lissitzky et al. 1964). Table I illustrates the large amount of cold-labile TG dimer in PTU + T_3-treated rats as compared with T_3-treated controls.

Iodoamino-acid composition of cold-labile and alkali-labile newly iodinated TG dimer (table II) shows a higher MIT/DIT ^{131}I-ratio in the most labile TG dimers and a decreasing specific radioactivity of iodotyrosines in TG species which are more stable towards cold or ammonia. Moreover, the hormone contents of iodinated TG dimers increases with increasing stability of the molecules (table II). If care is taken to avoid freezing the glands or the thyroid extracts, no or very small amounts of ^{131}I-S12 peak is observed in PTU-treated or in untreated rats after time intervals longer than 1 to 2 hr after ^{131}I injection. This important observation has been extended to other species (man, sheep) (unpublished) and appears to be a general phenomenon.

From these observations the following conclusions may be formulated 1/ appearance of iodinated S12 TG monomer is the consequence of dissociation of labile iodinated TG dimer and appears more readily if the dimer is poorly iodinated 2/ uniodinated TG dimer is the most probable substrate for iodination in vivo 3/ maturation of TG dimer (as shown by increasing sedimentation constant from 16-17 to 19) is accompanied by increasing amounts of iodine in the molecule 4/ as maturation proceeds, the DIT and hormone content of TG increases 5/ it is not possible to say at present if iodination and iodothyronine formation is the cause or the consequence of TG maturation 6/ PTU would inhibit TG maturation by decreasing iodine organification.

There is much evidence that TG iodination occurs at the cell-colloid interface (Wollman and Wodinsky, 1955 ; Stein and Gross, 1964 ; Nadler, 1965). It is most likely that maturation of iodinated TG dimer takes place during its migration through the highly viscous contents of the lumen. Physical (Edelhoch, 1965) and chemical (Lissitzky et al. 1965 a, 1965 b) investigations have shown that

S19 TG is formed from two identical S12 subunits. A S27 iodoprotein has been purified and probably represents a dimer of S19 (Salvatore et al. 1965 ; Vecchio, 1965). All these proteins have an identical aminoacid composition (Bouchilloux et al. 1964 ; Vecchio, 1965), TG heterogeneity being related to variable iodoaminoacid content. It should also be mentioned that the S12 subunit is capable of associating in the absence of iodination and that maturation of uniodinated S16-17 TG is likely to be linked to iodination. The above observations permit us to propose a tentative unified nomenclature for thyroglobulin-like proteins.

Thyroglobulin monomer (TG1) will represent uniodinated S12 subunit (MW 320,000) and thyroglobulin dimer (TG2) the association of two of these monomers. The maturation of the dimer under the influence of iodination increases its sedimentation constant from 16 or 17 to 19. The iodinated S19 TG dimer could associate to give a thyroglobulin tetramer (TG4) (S27 iodoprotein) or other polymers such as TG hexamer (TG6) (S35 iodoprotein). Thyroglobulin dimer is the basic expression to designate the main specific thyroid protein component irrespective of its degree of maturation under the influence of iodination. The degree of structural evolution of the molecule and the procedure to demonstrate it may then be precisely designated, for example : S18 cold-labile ^{131}I-labeled thyroglobulin dimer, S19 cold-stable iodinated thyroglobulin dimer, S17 uniodinated thyroglobulin dimer.

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