RELATIONSHIP BETWEEN IODINATION AND CONFORMATION OF THYROGLOBULIN

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Sheep thyroid slices synthesize an immediate non iodinated precursor of thyroglobulin * : prethyroglobulin. The sedimentation coefficient of Pre-Tg is 17 S (Nunez et al., 1965 a). The sedimentation coefficient of the in vitro iodinated Tg (^{125}I-Tg) is 18.4 S instead of 19 S for Tg. We assumed that these differences are connected with conformational modifications produced in the protein molecule by its iodination. This work describes successively : 1) the separation of Pre-Tg and ^{125}I-Tg by DEAE-Sephadex column chromatography ; 2) some experiments on the relationship between iodination and conformation of the halogenated molecules.

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* Abbreviations used : Pre-Tg : pre-thyroglobulin ; Tg : preformed thyroglobulin ; ^{125}I-Tg : in vitro labeled Tg ; SDS : sodium dodecyl sulfate ; MIT : monoiodotyrosine ; DIT : diiodotyrosine.

a) DEAE-Sephadex column chromatography on Pre-Tg

Sheep Pre-Tg (17.3 S) is prepared by incubation of thyroid slices with ^{3}H-Tyr (Nunez et al., 1965 a) and then analysed by DEAE-Sephadex column chromatography (Fig. 1 a) : Tg and Pre-Tg are partially separated. The non iodinated

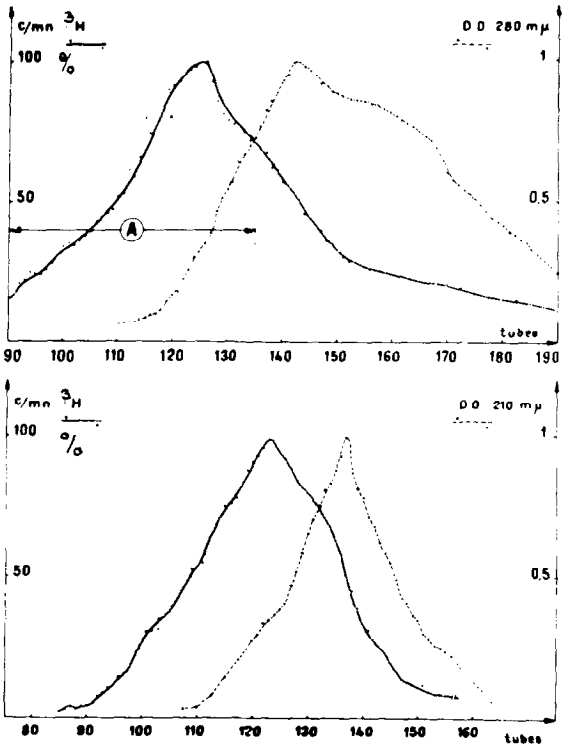


Fig. 1. DEAE-Sephadex A-50 column chromatography of Pre-Tg. Sheep thyroid slices are incubated in a medium containing ^{3}H-Tyr and propylthiouracil (10^{-3} M). The labeled pre-thyroglobulin, purified by filtration on Sephadex G 200, is analyzed with a DEAE-Sephadex column (350 x 15 mm) equilibrated with a phosphate buffer (pH 6 ; 0.05 M). The elution is conducted with the same buffer containing increasing ClNa concentration (0 to 0.5 M). Fig. 1 a : separation of Pre-Tg and Tg. Fig. 1 b : the fraction A, initially eluted from the first column, is analyzed on a second one.

Pre-Tg is less retained by the exchanger than the halogenated molecules of Tg. The rechromatography of the fraction A is shown by Fig. 1 b. The relative positions of the two maxima (^{3}H radioactivity and optical density) have not changed. Pre-Tg is enriched 700 fold. The peak of stable thyroglobulin (O.D.) is much less spread out : the second fractionation confirms the heterogeneity of Tg (Robbins, 1963 ; Roche et al., 1960 ; Ui et al., 1961).

b) DEAE-Sephadex column chromatography on ^{125}I-Tg

^{125}I-Tg and Tg can also be partially separated by DEAE-Sephadex column chromatography (Fig. 2 a) : ^{125}I-Tg is less retained than Tg but the two peaks overlap. After re-

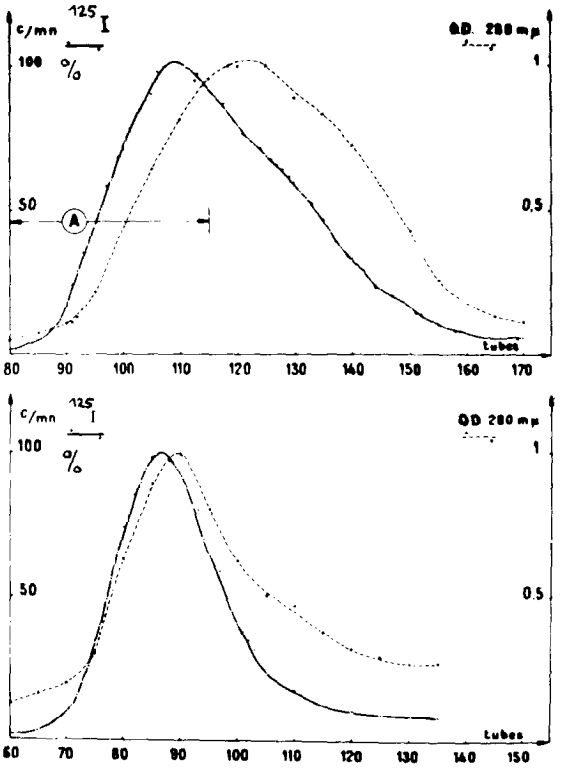


Fig. 2. DEAE-Sephadex A-50 column chromatography of ^{125}I-Tg. Sheep thyroid slices are incubated in a medium containing ^{125}I$^-$. The ^{125}I labeled thyroglobulin is purified by filtration on Sephadex G 200 and analyzed on DEAE-Sephadex column (conditions of Fig. 1). Fig. 2 a : separation of ^{125}I-Tg and Tg. Fig. 2 b : the fraction A initially eluted from the first column is analyzed on a second one.

chromatography of fraction A the positions of the radioactivity and the optical density peaks are almost the same (Fig. 2 b). ^{125}I-Tg is therefore also heterogeneous. The halogen content of Tg (30 iodine atoms) may be an average value.

The sedimentation coefficient of the fractions labeled with ^{125}I initially eluted from the column is equal to 17 S. That of the more retained fractions is 18.6 S (Fig. 3).

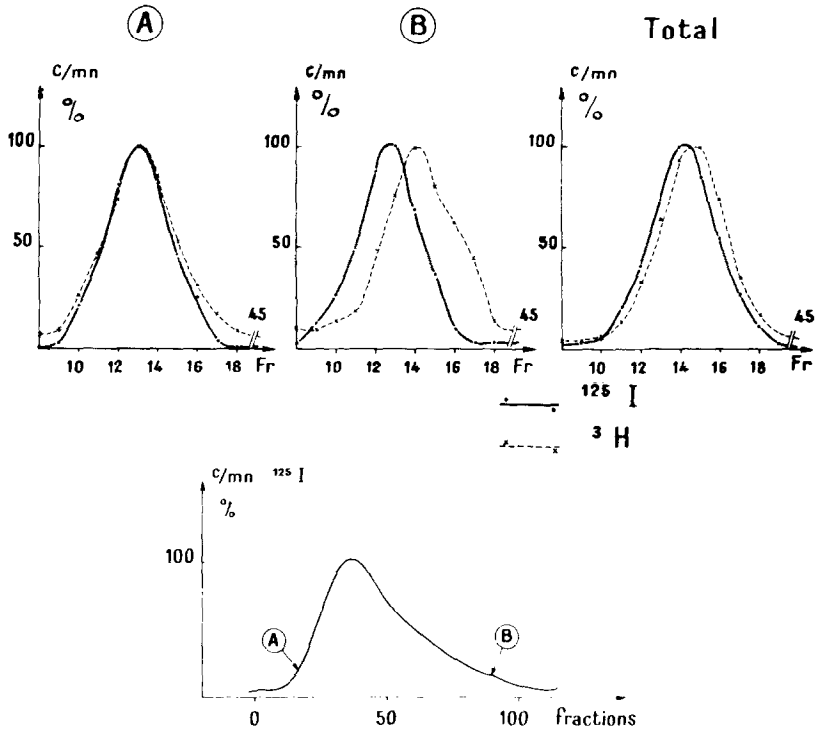


Fig. 3. Sucrose gradient ultracentrifugation of ^{125}I-Tg fractions separated by DEAE-Sephadex chromatography (conditions of Fig. 1). The total unfractionated preparation and the fractions A and B are compared to ^{3}H-Pre-Tg by sucrose gradient ultracentrifugation.

18.4 S for ^{125}I-Tg and 19 S for Tg may be average values. The following experiments seem to agree with this assumption.

c) MIT/DIT ratio of ^{125}I-Tg fractions

Pre-Tg is totally dissociated by SDS ; ^{125}I-Tg is only partially dissociated but more than Tg (Sellin and Goldberg, 1965). It appears therefore that more the molecules are iodinated more they resist this detergent. To demonstrate this conclusion ^{125}I-Tg is treated with SDS ; the dissociated fraction is separated from the resistant one by sucrose gradient ultracentrifugation ; each fraction is hydrolysed and

the MIT/DIT ratio established : this ratio is 1.5 times higher for the dissociated fraction ; thus the dissociable molecules are the less iodinated.

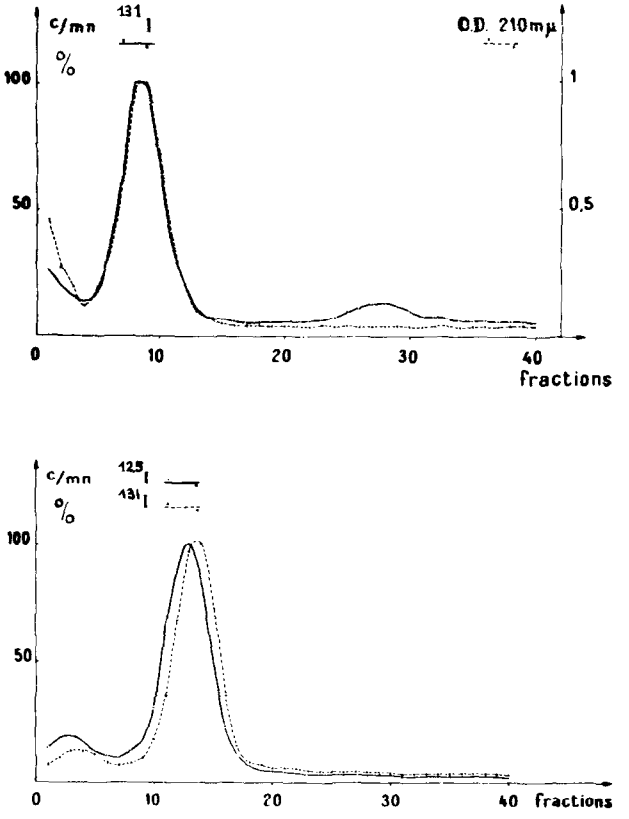


Fig. 4. Sucrose gradient ultracentrifugation (10-25 %, 39000 r.p.m., 7 h) of Tg iodinated enzymatically. Tg (5 mg) is incubated 1 h with 50 μg horseradish peroxydase, 10 μg glucose-oxydase, 18 μg glucose at pH 7 and purified by filtration on a column of Sephadex G 200. Fig. 4 a : Tg halogenated with 8 atoms. Fig. 4 b : mixture of ^{131}I-Tg (8 atoms) and ^{125}I-Tg (39 atoms).

In another experiment ^{125}I-Tg is fractionated by sucrose gradient ultracentrifugation ; each fraction of the radioactivity peak is hydrolysed and the MIT/DIT ratios established. The molecules contained in the fractions with a lower

sedimentation velocity have a MIT/DIT ratio 1.5 times higher than those taken near the bottom of the tube. The ^{125}I-Tg molecules are therefore not only distributed along the peak according to a diffusion pattern but also as a function of their sedimentation coefficient which itself depends on the level of iodination.

These experiments permit therefore the classification of Tg into two groups : the first one dissociable by SDS, with a higher MIT/DIT ratio and with a sedimentation coefficient lower than the average value of 19 S ; the second one, which resists SDS treatment, is more iodinated and may contain molecules with a sedimentation coefficient greater than 19 S. The properties of highly iodinated Tg preparations agree with such an assumption.

d) Properties of highly iodinated Tg

Tg is iodinated by an enzymatic method (Nunez et al., 1965 b). Various samples containing 1 to 100 atoms of I / molecule have been prepared **. The sedimentation coefficient of a sample containing 10 additional atoms I / mol remains at 19 S and that of preparations with 100 atoms I / mol increases to 20 S (Fig. 4). The transition occurs between 30 and 40 atoms. The MIT/DIT ratio decreases linearly between 1 and 30-40 atoms added ** and remains constant for higher halogen contents (Fig. 5). This can mean that the change of sedimentation coefficient corresponds to a change of reactivity of tyrosine residues. Moreover the molecules containing 10 to 30 atoms are highly dissociated by SDS ; those halogenated with 40 atoms are almost totally resistant.

** The halogen to be fixed is added to a molecule of Tg which already contains about 20-30 iodine atoms.

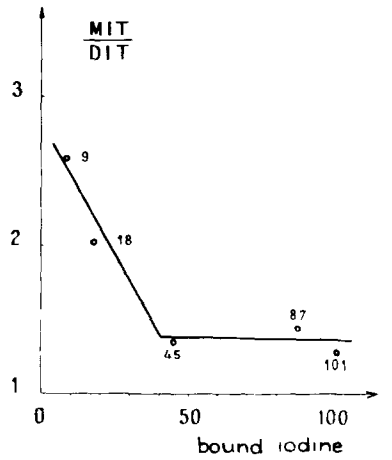
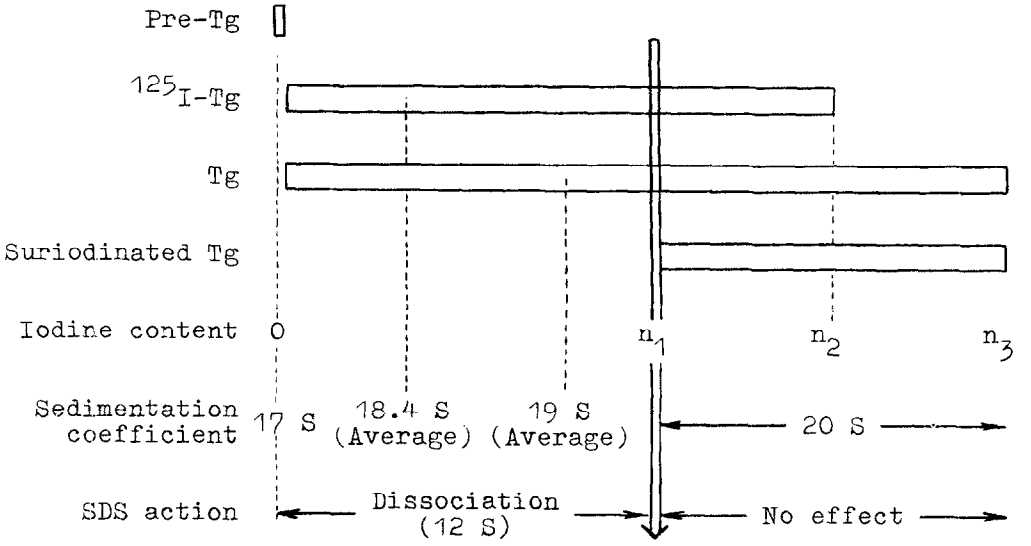


Fig. 5. MIT/DIT ratio of five enzymatically halogenated thyroglobulins containing 9, 18, 45, 87 and 101 iodine atoms (in addition of the normal iodine content (30 atoms).

e) Discussion

The scheme below summarizes these conclusions.



Our results confirm that Tg is heterogeneous. More the molecules are iodinated more their sedimentation coefficient is

great. This increase from 17 S to 20 S may be due to a change of shape or density. At least one transition occurs, when the iodine content reaches a value of 60-70 atoms. The molecules are no more dissociable by SDS.

We know that more Tg is iodinated more it resists autolysis (Pitt-Rivers, 1964). We can suppose that the conformational changes of Tg and its greater resistance to dissociation can explain its lower degree of autolysis and indirectly the diminished iodide release.

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