# IODINATION AND BIOSYNTHESIS OF RAT THYROGLOBULIN

Serge Lissitzky, Marie Roques, Janine Torresani,
Claude Simon * et Simone Bouchilloux

Laboratoire de Biochimie Médicale, Faculté de Médecine, Marseille et * Département de Biologie, Centre d'Etudes Nucléaires, Saclay (S et O), France.

Received May 4, 1964

The heterogeneity of thyroglobulin with respect to iodine turnover has been demonstrated recently in several laboratories [see references in Bouchilloux et al (1964)]. Seed and Goldberg (1963) studying Tg formation by thyroid slices have given evidence for a 12S precursor protein in Tg synthesis. The presence of iodoaminoacids in the molecule has been considered to be the result of iodination of the tyrosyl residues of a non-iodinated precursor protein although no direct experimental evidence is at present available. This note concerns results giving direct experimental evidence 1/ of the heterogeneity of rat Tg 2/ that iodination occurs after peptide chain synthesis of Tg and 3/ that $^{14}C$-pulse labeled precursors of Tg are capable of being iodinated.

Rats (males, Wistar 280 g) were maintained in isotopic equilibrium with $^{125}I$ (Simon, 1963) or fed ad lib. without special dietary precautions with respect to iodine. In vitro incubations were carried out on thyroid glands taken immediately after death and bleeding of the animals. Each hemithyroid was cut into two parts with a razor blade. Incubation solution was Eagle's medium as used by Seed and Goldberg (1963) but in addition containing 0.17 µg KI per ml. Time conditions, added

---

Abbreviations : thyroglobulin = Tg ; MIT = 3-iodotyrosine ; DIT = 3,5-di-iodotyrosine

compounds and the order of their addition are listed in the legends of the figures or in the text. After incubation at 37°C with gentle shaking in an atmosphere of $O_2$, the glands were rapidly washed with Eagle's basic medium, frozen (-60°C) and homogeneized (0.15 M NaCl) with glass powder. The homogenate was centrifuged and the supernatant fractionated with $Am_2SO_4$ between 35 and 45 % of the saturation at room temperature followed by two reprecipitations at 50 % saturation to insure elimination of non protein material. The solution obtained (soluble fractionated proteins) was centrifuged on a 5-20 % sucrose gradient containing 0.1 M NaCl according to Martin and Ames (1961) in the SW 39 rotor of Spinco model L ultracentrifuge for 5 hr at 39,000 RPM. $^{131}I$ and $^{125}I$ radioactivities were counted in a well-type scintillation spectrometer with automatic sample changing and $^{14}C$ in a Packard Tri-Carb liquid scintillation spectrometer after dissolution of the samples in the counting fluid in the presence of hyamine. $^{14}C$-countings were corrected for the influence of $^{125}I$. Algal protein hydrolysate-$^{14}C$ (0.64 mc/mg) and $^{131}I$ (carrier free) were obtained from CEA (Saclay, France) and $^{125}I$ (carrier free) from the Atomic Energy of Canada.

Following a 20 min pre-incubation in Eagle's medium, thyroid slices were pulse labeled for periods of 20 sec to 5 min with $^{131}I$. Iodine in the soluble fractionated proteins was sequentially incorporated into components having respective sedimentation constants of $S_{3-8}$, $S_{12}$ and $S_{19}$.

Fig. 1 compares the incorporation of $^{14}C$-amino acids into the soluble fractionated proteins of $^{125}I$-equilibrated rat thyroid glands and their consecutive iodination by $^{131}I$ with and without pre-incubation with puromycin. At 20 min no $^{14}C$ is incorporated in the $S_{19}$ peak (thyroglobulin) (fig. 1A) whereas a consecutive pulse-labeling of 20 sec with $^{131}I$ shows this tracer in 3 peaks corresponding to $S_{19}$, $S_{12}$ and $S_{3-8}$. After 90 min incubation, $^{14}C$ is found (fig. 1B) in zones corresponding to $S_{3-8}$, $S_{12}$ and $S_{19}$ confirming the observations of Seed and Goldberg (1963) obtained with sheep thyroid slices. After 3 hr incubation, $^{14}C$ is almost entirely found in the $S_{19}$ peak. Although puromycin inhibits thyroglobulin synthesis completely, no modification of consecutive $^{131}I$-incorporation is observed (fig. 1C). This indicates that, in the absence of Tg synthesis,

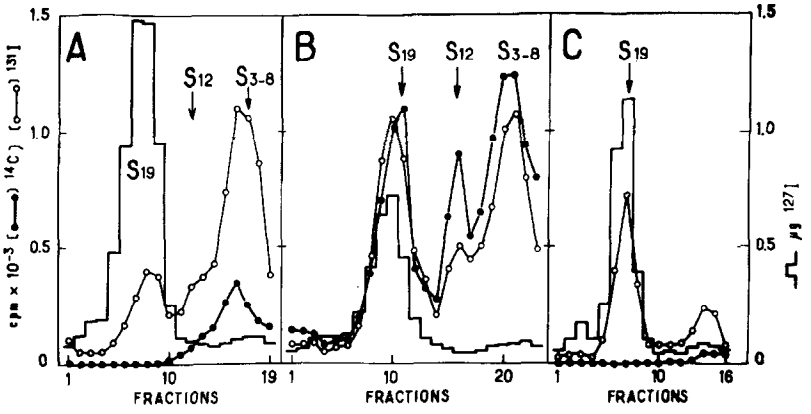


Figure 1 - Gradient centrifugation of soluble fractionated thyroid proteins pulse-labeled with $^{14}$C-algal protein hydrolyzate and $^{131}$I.
Half hemithyroid of 8 $^{125}$I-equilibrated rats (50 µg/day) were pre-incubated for 20 min in 2 ml of medium and then incubated in the same medium containing the $^{14}$C-amino acids (30 µc). After a 20 or 90 min incubation, a short pulse of $^{131}$I was given and the glands were treated as described in the text to obtain the soluble fractionated proteins.
A - incubation in the presence of $^{14}$C-protein hydrolyzate for 20 min followed by a 20 sec-pulse with $^{131}$I (200 µc).
B - incubation in the presence of $^{14}$C-protein hydrolyzate for 90 min followed by a 40 sec-pulse with $^{131}$I (200 µc).
C - pre-incubation and incubation in the presence of puromycin (500 µg). Incubation in the presence of $^{14}$C-protein hydrolyzate for 90 min followed by a 5 min-pulse with $^{131}$I (20 µc).

---

iodination of preformed protein molecules is not modified. In these experiments, $^{125}$I-values reflect the actual content in $^{127}$I of the fractions isolated. The specific radioactivity of iodine ($^{131}$I/$^{127}$I) is, for short time intervals of incubation, the highest successively in peaks $S_{3-8}$, $S_{12}$ and $S_{19}$ and decreases with the time of labeling. The precursor character of the $S_{3-8}$ and $S_{12}$ components in the biosynthesis of Tg ($S_{19}$) is very likely for the following reasons : 1/ with time $^{14}$C-radioactivity moves from the $S_{3-8}$ to the $S_{12}$ and the $S_{19}$ peaks 2/ actinomycin D, as shown previously (Seed and Goldberg, 1963), has little effect on the labeling of these protein components 3/ digestion with Pronase of a 20 sec-$^{131}$I-pulse labeled $S_{3-8}$ peak liberates 50 % of its radioactivity as MIT ; under similar conditions peak $S_{19}$ gives rise to MIT (60 %) and DIT (10 %) 4/ incubation with $^{14}$C-mannose reveals the same

kinetics of incorporation of radioactivity into the $S_{3-8}$, $S_{12}$ and $S_{19}$ areas as observed with the $^{14}C$-amino acids. 5/ $S_{3-8}$ material labeled in the presence of $^{14}C$-mannose or $^{14}C$-amino acids is TCA-insoluble 6/ all the fractions analyzed by sucrose-gradient centrifugation had been previously purified by a 35-45 % ammonium sulfate fractionation indicating that, if thyroglobulin precursor proteins are concerned, identical solubility properties are observed for Tg and its precursors.

Heterogeneity of $S_{19}$ Tg is indicated by the shifts of the $^{14}C$- and $^{131}I$- peaks towards the lighter fractions as compared with the $^{127}I$- peak (fig. 1A and 1B). Moreover dialysis at 0°C against 0,01M $NH_4OH$ of thyroid glands without preparation (fig. 2) or placed into conditions of iodine equilibrium with $^{125}I$ and injected with a single dose of $^{131}I$ for 1 min to 16 hr before death, dissociates the $S_{19}$ peak into a $S_{12}$ component [half-molecules, Edelhoch (1960)], the specific radioactivity of which is higher than that of the $S_{19}$ peak (table I).

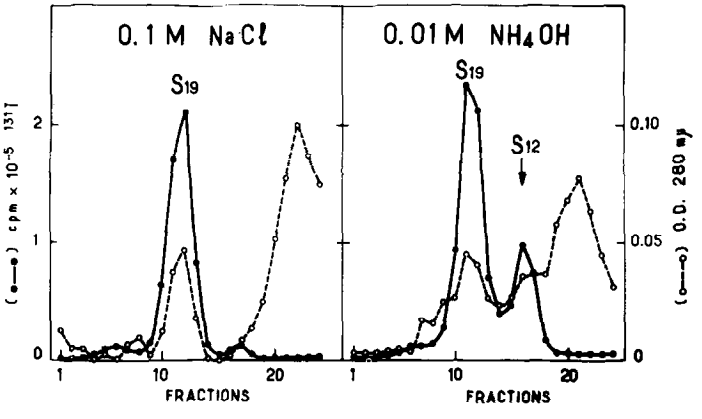


Figure 2 - Gradient centrifugation of soluble thyroid proteins pulse-labeled with $^{131}I$ in vivo for 4 hr. The thyroids of 3 rats were dissected 4 hr after an intraperitoneal injection of 100 μc per rat of $^{131}I$. After dialysis for 12 hr at + 2°C against 0.1 M NaCl (left) or 0.01 M $NH_4OH$ (right), the glands were frozen, homogeneized in the presence of 0.1 M TRIS-NaCl pH 8.6 (1 ml), centrifuged (4,000 g) and the supernatant (0.2 ml) ultracentrifuged on a sucrose gradient containing 0.1 M TRIS-NaCl buffer pH 8.6.

---

The shorter the time after $^{131}I$ injection, the greater the dissociation into the $^{131}I$- labeled $S_{12}$ component (∿ 60 % for an in vivo pulse of 1 min). This indicates that newly iodi-

Table I. Specific radioactivities of $S_{19}$-thyroglobulin and alkaline formed $S_{12}$ component

| $^{131}$I-injection Time after (hour) | 0.1 | 2 | 9 | 16 | 24 |
|---|---|---|---|---|---|
| $S_{12}$ | 3.6 * | 20.0 | 46.7 | 23.6 | 14.0 |
| $S_{19}$ | 1.5 | 11.8 | 34.5 | 27.1 | 15.2 |

* Specific radioactivities expressed as % $^{131}$I injected per µg x $10^3$ $^{127}$I.

Lots of rats (4 to 6) maintained in isotopic equilibrium with $^{125}$I (5 µg/day) were injected with a single dose of $^{131}$I (50 to 100 µc) and killed after the stated time interval. The glands were dialyzed for 12 hr at 0°C against 0.01 M $NH_4OH$, frozen homogeneized, centrifuged (4,000 g) and submitted to a sucrose gradient centrifugation as described in the text.

nated Tg molecules are more sensitive to alkaline dissociation. The iodoamino acid composition of both the $S_{19}$ and alkaline formed $S_{12}$ fractions are almost identical, the specific radioactivity of both iodotyrosines and iodothyronines being higher in peak $S_{12}$ than in peak $S_{19}$ for times of labeling in vivo, up to 9 hr.

These results indicate that in vivo iodination of thyroglobulin is a very rapid phenomenon independant of and posterior to protein synthesis. Direct in vivo evidence for the heterogeneity of thyroglobulin is presented and evidence is found for a 12S and a likely 3 to 8S precursor protein in thyroglobulin synthesis.

More detailed results and discussion will be presented elsewhere.

## REFERENCES

Bouchilloux, S., Rolland, M., Torresani, J., Roques, M. and Lissitzky, S., Biochim. Biophys. Acta, in the press.

Edelhoch, H., J. Biol. Chem., 235, 1326 (1960).

Martin, R.G. and Ames, B.N., J. Biol. Chem., 236, 1372 (1961).

Seed, R.W. and Goldberg, I.H., Proc. Nat. Acad. Sci. U.S., 50, 275 (1963).

Simon, C., Biochim. Biophys. Acta, 74, 565 (1963).