BIOSYNTHESIS OF THYROGLOBULIN BY ISOLATED THYROID CELLS AND CELL-FREE SYSTEM

Jacques Nunez, Jean Mauchamp, Vincenzo Macchia, Arlette Jerusalmi and Jean Roche

Laboratoire de Biochimie générale et comparée, Collège de France, Paris, France

Received May 19, 1965

The biosynthesis of thyroglobulin * proceeds from two lighter precursors (sedimentation coefficients between 3 and 8 S for the first and 12 S for the second) (Seed and Goldberg, 1963). The iodination of this protein seems to occur both at an intracellular (particulate) and an extracellular (colloid lumen) level (Nunez et al., 1965 b). It is independent of the synthesis of the polypeptide chains as shown by the experiments with puromycin and propylthiouracil (Seed and Goldberg, 1965 ; Maloof et al., 1964 ; Lissitzky et al., 1964 ; Nunez et al., 1965 a). Moreover, the polymerization of light precursors is independent of iodination in sheep thyroid slices (Seed and Goldberg, 1965 ; Nunez et al., 1965 a). Finally, the follicular structure is not necessary for iodination since isolated thyroid cells incorporate iodine into thyroglobulin (Raghupathy et al., 1965).

* The term thyroglobulin is reserved to the group of more or less iodinated proteins with an average sedimentation coefficient of 19 S.

In this paper we report experiments on the synthesis of thyroglobulin by isolated cells and cell-free systems.

I. Thyroglobulin biosynthesis by isolated thyroid cells

Raghupathy et al. (1965) have shown that isolated thyroid cells will incorporate iodine into intracellular thyroglobulin and that they are able to synthetize labeled proteins (TCA precipitating material) when incubated with labeled amino acids.

With a cell preparation obtained under the same conditions (Tong et al., 1962) we have found (fig. 1 a and 1 b) that ^{14}C-Tyr and ^{125}I are incorporated into light proteins (3-8 S) and thyroglobulin.

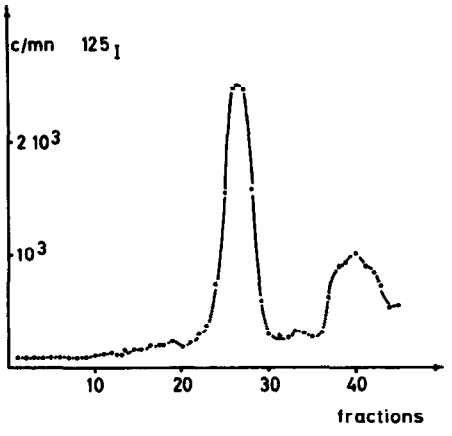

Fig 1a

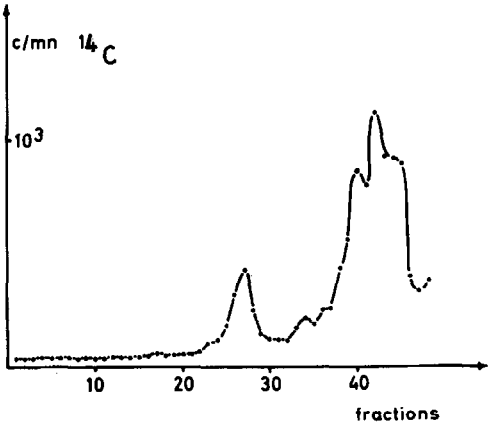

Fig 1b

Fig. 1. The thyroid cells are prepared according to Tong et al. (1962) from sheep glands ; they are incubated 12 h at 37°C in a medium containing a pool of amino-acids (Campagne and Gruber, 1962) and 20 % of beef serum. The cells are isolated by centrifugation then homogeneized in a buffer. After centrifugation at 105000 g for 1 h the supernatant is purified by filtration on a Sephadex G 25 column. The purified proteins are analyzed by sucrose gradient ultracentrifugation (10-25 %, 39000 r.p.mn).
Fig. 1 a : incubation with ^{125}I. Fig. 1 b : incubation with ^{14}C-Tyr.

II. Incorporation of amino-acids in proteins by a cell-free system

The subcellular fractionation of thyroid tissue does not permit a good separation of mitochondria from endoplasmic reticulum (Ekholm, 1961). We have used two particulate preparations. The first is defined as the fraction which sediments between 700 and 105000 g. The second is obtained between 15000 and 105000 g and contains variable amounts of microsomes. The most reproducible results have been obtained with the first preparation.

Incubation of these particulate fractions in the presence of an ATP generating system, 105000 g super-

Table I

Preparation	Puromycin	c/mn/mg	Inhibition
I (Mitochondria + Microsomes)	-	1477	-
	$2.7.10^{-4}$ M	190	80 %
II (Microsomes)	-	4553*	-
	5.10^{-4} M	1583*	65 %

Preparation I. Final volume 1.8 ml. ATP 5 µmoles ; α-keto-glutarate 50 µmoles ; saccharose 150 µmoles; potassium phosphate buffer 0.02 M pH 7.4 ; particles of 290 mg of thyroid ; soluble fraction from a 1/5 homogenate in saccharose solution 0.25 M 0.2 ml ; ^{14}C-amino acids (Chlorella protein hydrolyzate) 20 µl.

Preparation II. Same conditions than I except α-ketoglutarate replaced by phosphoenolpyruvate 10 µmoles ; pyruvate kinase 50 µg. Microsomes from 1.3 g of thyroid.

Incubation is carried at 37°C during 60 mn for both preparations.

*These values have not been corrected by substraction of a not incubated blank which contains a variable amount of radioactivity.

natant (with or without intermediary precipitation of the supernatant at pH 5) and a labeled amino acid, was followed by TCA precipitation of the proteins according to the procedure of Allen and Schweet (1962).

Table I reports the results obtained and shows that puromycin inhibits incorporation of ^{14}C into TCA precipitable material, indicating that protein synthesis occurs in this preparation.

III. Cell-free thyroglobulin biosynthesis

After incubation with ^{3}H-Tyr (3,5-^{3}H-Tyr 14.4 C / mM ; 0.6 µmole per flask) the particles were eliminated by ultracentrifugation (105000 g, 1 h) and free tyrosine was eliminated from the soluble fraction by gel filtration (Sephadex G 25). The labeled proteins were analyzed

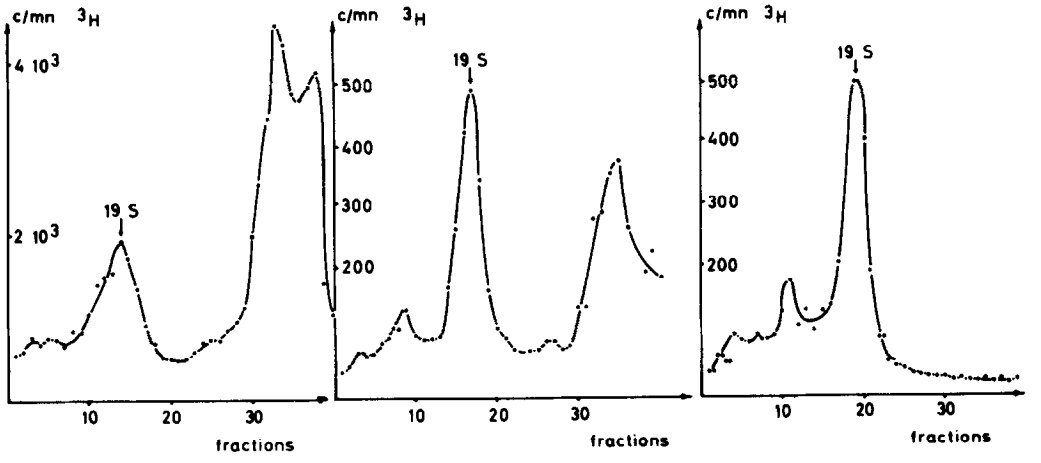

Fig 2a Fig 2b Fig 2c

Fig. 2. Sucrose gradient ultracentrifugation (10-25 % ; phosphate buffer 0.05 M pH 7.4 ; 39000 r.p.mn) of the supernatant fraction proteins purified by filtration on Sephadex G 25.
Fig. 2 a : preparation I (mitochondria + microsomes). Fig. 2 b : preparation II (microsomes). Fig. 2 c : 19 S protein purified on Sephadex G 200.

by sucrose gradient ultracentrifugation (10-25 % ; 39000 r.p.mn). Two main labeled fractions are observed (fig. 2 a, 2 b) : the first is light (3-8 S) ; the second has nearly the same migration as ^{125}I-thyroglobulin (19 S) prepared from sheep thyroid slices incubated with $^{125}I^-$; the positions of the ^{3}H radioactivity peak and that of the ^{125}I peak are slightly different. This result is not surprising since we have shown (Nunez et al., 1965 a) that thyroglobulin newly synthetized by thyroid sheep slices has a sedimentation coefficient smaller (17 S) than that of the "older" molecules (19 S). Identification of the heavier component with thyroglobulin was confirmed by sucrose gradient ultracentrifugation of a soluble fraction freed of light compounds by Sephadex G 200 filtration (fig. 2 c) : 90 % of the radioactivity of this purified fraction was found to be precipitable by TCA (Allen and Schweet, 1962). The same fraction was further treated with FDNB, the DNP-protein was hydrolysed (HCl 6 N) and the hydrolysate extracted with ether : only 12 % of the radioactivity was soluble in this solvent, suggesting that the ^{3}H-amino acid was not incorporated only or even mainly as the end groups, but presumably within the polypeptide chain.

This experiment has been repeated in the presence of puromycin : under such conditions we have observed only a very small incorporation of labeled amino acid into thyroglobulin (10 % of the control).

IV. Nature of the radioactivity linked to the particles incubated in a cell-free system

The particles separated by ultracentrifugation

(105000 g) after incubation in a cell-free system contain about 20 % of the total radioactivity incorporated. The particles were extracted by digitonin and the solution obtained purified by Sephadex G 25 filtration. This extract was analyzed by sucrose gradient ultracentrifugation. As shown in fig. 3 light compounds only were liberated from the particles.

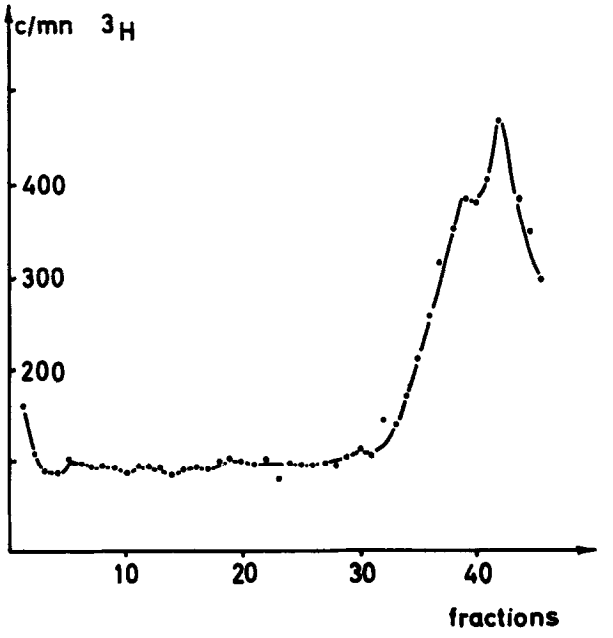

Fig. 3. The particles are extracted with digitonine (0.5 % at 4°C, 30 mn) after centrifugation and washing. The proteins are purified by filtration on Sephadex G 25 and analyzed by sucrose gradient ultracentrifugation.

The results described above show that both the incorporation of amino acids into light proteins and polymerization into thyroglobulin can be achieved by isolated cells and obtained in a cell-free fraction both independently from iodination. It remains to be established whether polymerization occurs only after release

of the light precursors from the ribosomes or perhaps immediately before.

REFERENCES

Allen, E. and Schweet, R., J. Biol. Chem., 237, 760 (1962).
Campagne, R.N. and Gruber, M., Biochim. Biophys. Acta, 55, 353 (1962).
Ekholm, J., J. Ultrastruct. Res., 5, 573 (1961).
Lissitzky, S., Roques, M., Torresani, J., Simon, C. and Bouchilloux, S., Biochem. Biophys. Res. Commun., 16, 249 (1964).
Maloof, F., Sato, G. and Soodak, M., Medicine, 43, 375 (1964).
Nunez, J., Jacquemin, C., Brun, D. and Roche, J., Biochim. Biophys. Acta (1965 b and c), in press.
Nunez, J. Mauchamp, J., Macchia, V. and Roche, J., Biochim. Biophys. Acta (1965 a) in press.
Raghupathy, E., Kerkof, P.R. and Chaikoff, I.L., Biochim. Biophys. Acta, 97, 118 (1965).
Seed, R.W. and Goldberg, I.H., Proc. Natl. Acad. Sci. U.S., 50, 275 (1963).
Seed, R.W. and Goldberg, I.H., J. Biol. Chem., 240, 764 (1965).
Tong, W., Kerkof, P. and Chaikoff, I.L., Biochim. Biophys. Acta, 60, 1 (1962).

This work was supported by a grant from Délégation Générale à la Recherche Scientifique (Commission : Biologie Moléculaire).

We thank Miss J. Osty for assistance with these experiments.