BIOSYNTHESIS OF THYROGLOBULIN IN VIVO : FORMATION AND POLYMERIZATION OF SUBUNITS IN THE RAT AND GUINEA PIG (°)

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The biosynthesis of thyroglobulin (°°) has been studied so far in vivo by autoradiography (Nadler et al., 1964) and in vitro by following the incorporation of ^{3}H- or ^{14}C- labeled aminoacids in thyroid slices (Seed and Goldberg, 1963; Lissitzky et al., 1964; Nunez et al., 1965). In the present work, the time course of formation of the thyroglobulin subunits and their polymerization has been studied after administration of a pulse label of ^{3}H- leucine to rats and guinea pigs.

The smallest subunit which appears during the biosynthesis of rat thyroglobulin in vivo has been characterized: it has the same solubility properties of thyroglobulin, a sedimentation coefficient of about 5S and a molecular size, as determined by gel filtration corresponding to one quarter of 19S.

The dimer 12S - which seems to be a precursor of 19S thyroglobulin from studies in vitro - has been found in vivo only in the guinea pig, whereas it does not accumulate in rat thyroid. The rate of polymerization of thyroglobulin subunits has been found to be much higher in rats than in guinea pigs.

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(°°) Thyroglobulin is by definition the 19S ultracentrifugal component of the thyroid soluble extract (Robbins and Rall, 1960). Other thyroid iodoproteins and their subunits will be referred to by their sedimentation constant.

EXPERIMENTAL

Male rats (Sprague-Dawley, 100-150 g) and guinea pigs (Wistar, 200-250 g) were injected intravenously with a pulse label (10 μC per g body weight) of 4,5-^{3}H-L-leucine (New England Nuclear Corporation, specific activity = 5 Curies/mMole) and were killed - in groups of 4-5 animals - 10, 30, 60, 120 and 360 minutes later. The soluble thyroid extracts in standard buffer (0.1 M KCl, 0.02 M sodium phosphate, pH = 7.4) were prepared as previously described (Salvatore et al., 1964) and subjected to repeated salting out (°) (three times between 1.4 and 1.8 M $(NH_4)_2SO_4$ at 0°C). The purified extracts contained only thyroglobulin-like proteins (no immunochemical cross-reaction with plasma proteins). The radioactivity was determined by liquid scintillation counting (with an error<2%) and the protein concentration by absorbancy at 280 and 210 mμ.

The incorporation of radioactivity into the thyroid purified proteins of rats and guinea pigs at various times of pulse labeling is reported in Table I.

Table I

Time-course of incorporation of ^{3}H-leucine in rats and guinea pigs* soluble thyroid proteins.

Pulse label (minutes)	dpm per mg of purified thyroid proteins** Rat	Guinea pig
10	4,000	450
30	6,100	750
60	8,500	1,500
120	10,500	2,000
360	12,500	3,100

* The animals were injected i.v. with 10 μC (2×10^{-9} Moles) per g body weight of ^{3}H-L-leucine.

** Mean values of 4 animals.

(°) Preliminary experiments have shown that by this method all the thyroglobulin-like proteins (6S, 12S, 19S and 27S) are separated from contaminant proteins present in the crude extract.

The purified extracts were analyzed by sucrose gradient centrifugation (see Salvatore et al., 1964). The distribution of the radioactivity among the various protein fractions, 10 minutes and 6 hours after the pulse label, is shown in fig. 1. In the rat at early times most of the radioactivity is associated with a slow sedimenting component; nevertheless, an appreciable amount of a tritiated component sedimenting together with the rat unlabeled thyroglobulin is already evident even 10 minutes after the injection of the ^{3}H-L-leucine. After 6 hours, more than 80 per cent of the total radioactivity is associated with 19S. No sedimenting peak between the slow component and the 19S was clearly detectable at all times of labeling, although a broad intermediate tail was occasionally evident. In order to check for the presence of minute amounts of a 12S protein, which is usually found by labeling thyroid slices, the gradient fractions 16 through 24 were pooled, concentrated by vacuum dialysis and resubmitted to sucrose gradient analysis. In this case also, no 12S was detected. On the contrary, in the guinea pig a 12S peak is clearly evident 10 minutes after the pulse label, and becomes the main radioactive

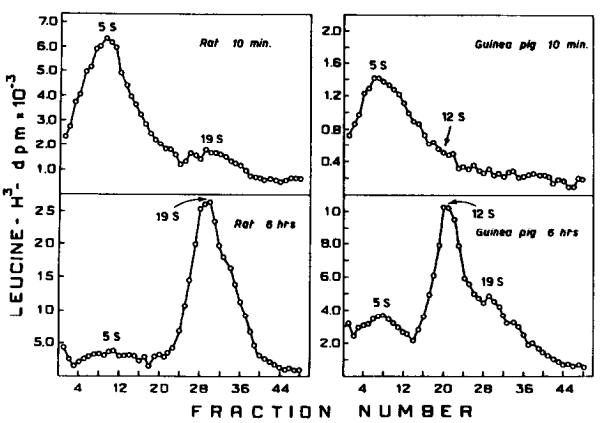

Fig. 1. Sucrose gradient ultracentrifugation patterns of purified (see text) thyroid extracts from rats (left) and guinea pigs (right). Pulse labeling in vivo, with 10 μCuries (2×10^{-9} Moles) of 4-5 ^{3}H-L-leucine per g body weight, 10 minutes (top) and 6 hours (bottom) before sacrifice. Sucrose gradient, 5-20%; SW39 rotor, Spinco Mod. L2-HV ultracentrifuge; equivalent time of centrifugation at 38,000 RPM = 6 hours, +4°C. Total protein: 1 mg. Sedimentation is from left to right. The number above the peaks are approximate sedimentation coefficients calculated on the basis of a reference rat thyroglobulin.

protein after 6 hours. The slow sedimenting component decreases while the 19S increases very slowly throughout the experimental period. The relative proportion of the various labeled ultracentrifugal components as a function of the pulse-labeling time, is shown in figs. 2 and 3.

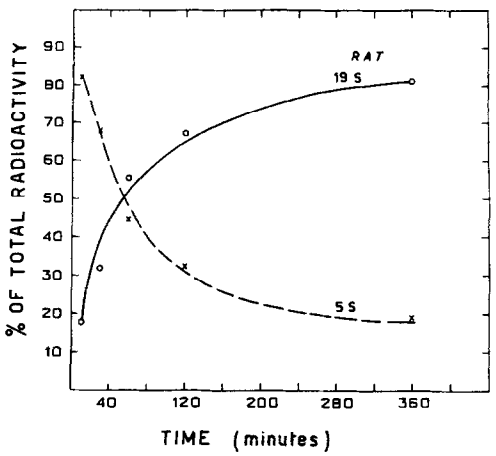

Fig. 2. Time-course of the formation and polymerization of thyroglobulin subunits in rat thyroid glands. Pulse labeling in vivo as in figure 1. Ordinates: relative proportions of the areas under the sucrose gradients peaks of each ultracentrifugal component; abscissae: time in minutes.

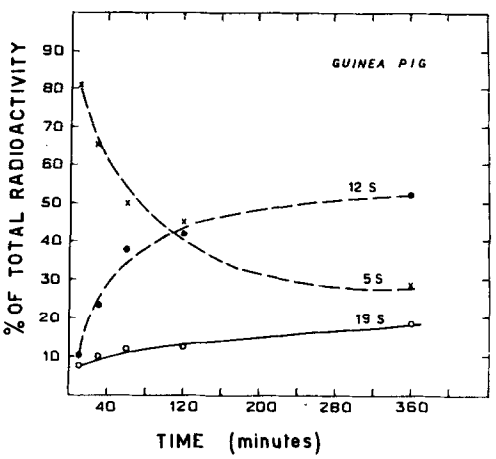

Fig. 3. Time-course of the formation and polymerization of thyroglobulin subunits in guinea pig thyroid glands. Details as in figure 2. Note the presence of the 12S component, absent in rat thyroid extracts.

The slow sedimenting component has been further characterized. Twenty-two rats were injected with 4 millicuries each of ^{3}H-L-leucine and killed 30 minutes later. The thyroid soluble extract was purified, as previously indicated, and fractionated by sucrose gradient preparative centrifugation in the SW 25.2 rotor of the Spinco mod. L2-HV centrifuge. The six fractions near the top of the peak of the slow sedimenting component were pooled and concentrated as described above. Sucrose gradient analysis of this preparation showed that its sedimentation rate was higher than that of bovine serum albumin (4.2S) and lower than that of rabbit γ-globulin (6.5S). Even after 10 hours of centrifugation, only one peak having a sedimentation coefficient of about 5S was observed. The same preparation was analyzed by filtration through a Sephadex G-100 column (previously calibrated with proteins of known molecular weight) to determine its molecular size (see Andrews, 1964; Auricchio and Bruni, 1964). The elution volume of the major peak obtained was smaller than that of rabbit γ-globulin, corresponding, for a globular protein, to a molecular weight of about 170,000 (one quarter of thyroglobulin).

DISCUSSION

Previous studies on the biosynthesis of thyroglobulin have been carried out on thyroid slices of sheep (Seed and Goldberg, 1963, 1965a; Nunez *et al.*, 1965) and rats (Seed and Goldberg, 1965b; Lissitzky *et al.*, 1965). The present study is the first dealing with the formation and the polymerization of the subunits of thyroglobulin *in vivo*. A remarkable difference has been found between the results *in vitro* and *in vivo*: the 12S component, which accumulates in the rat *in vitro*, has not been found *in vivo*. This difference is not related to matters of technique or to biological variability. Parallel experiments (unpublished) carried out on rat thyroid hemilobes incubated in Eagle's medium between 10 minutes and 4 hours with ^{3}H-L-leucine, demonstrated the presence of significant amounts of the 12S component. This observation, together with the well-known dissociation pattern of thyroglobulin into 12S subunits (Edelhoch, 1960), makes it very likely that 12S is an intermediate in thyroglobulin synthesis. *In vivo*, however, the rate of its dimerization is faster than that of its formation from the smaller 5S component; therefore, it does not accumulate in the gland. The appearance of a labeled 12S component in experiments *in vitro*, is probably

related to an impairment of polymerization of the 12S subunits in thyroid slices.

The presence of high levels of tritiated 12S has now been observed during the biosynthesis of ^{3}H-thyroglobulin in guinea pigs. This species, however, is almost unique among mammals since its soluble thyroid extract contains 10-15 % of a stable 12S protein (Salvatore et al., 1965) which has been recently purified and characterized (Salvatore,G., Aloj, S., Salvatore, M. and Edelhoch, H., in preparation). Furthermore, a labeled 12S is clearly observed after administration of radioiodine to guinea pig, whereas in rat and in most mammals all the organic iodine is incorporated directly in the 19S and the 27S iodoproteins.

The reason for the different levels of the 12S component in rats and guinea pigs (stable 12S or ^{125}I-12S or ^{3}H-12S) is not clear at present. There is some evidence that the pattern of the soluble thyroid proteins is related to the physiological activity of the gland (Salvatore et al., 1965). The guinea pig is considered to have a low thyroid activity. In fact, the incorporation of ^{3}H-L-leucine in the purified soluble thyroid proteins is significantly smaller in the guinea pig than in the rat (see table I). Moreover, 6 hours after the pulse label of ^{3}H-L-leucine, 19S represents more than 80% in the rat and less than 20% in the guinea pig (see figs. 2 and 3). Since the formation of thyroglobulin from its precursors is much faster in rats than in guinea pigs, the 12S intermediate does not accumulate in the former, but does so in the latter species.

The immediate precursor of the 12S component has so far not been defined. Sucrose gradient analysis of crude thyroid extracts labeled with ^{3}H- or ^{14}C-aminoacids demonstrates the presence of a broad peak whose sedimentation coefficient was referred to as 3-8S (Seed and Goldberg, 1963; Lissitzky et al., 1964). The precursor-product relationships of the very heterogeneous 3-8S material with the larg units were not clear since the 3-8S fraction did not disappear even after a long incubation time. The present studies demonstrate the formation in vivo of a 5S component both in rats and guinea pigs and its precursor-product relationship with the larger units. If the 5S component has a molecular weight of 170,000, this molecule should have a high degree of asymmetry. In this case the elution volume obtained by gel filtration does not correspond to the true molecular weight. On the other hand, a small subunit whose molecular weight approximates 165,000 has been found among the dissociation products of the 27S iodoprotein by Vecchio et al.,(1966) and after the reduction of the disulfide bonds of

thyroglobulin by de Crombrugghe et al., (1966). The ^{3}H-labeled component observed in the present experiments is most likely equivalent to this subunit, which corresponds to one quarter of the thyroglobulin molecule.

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REFERENCES

Andrews, P., Biochem. J., 91, 222 (1964)

Auricchio, F. and Bruni, C.B., Biochem. Z., 340, 321 (1964).

de Crombrugghe, B., Pitt-Rivers, R. and Edelhoch, H., J. Biol. Chem., 241, 2766 (1966).

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

Lissitzky, S., Roques, M., Torresani, J., Simon, C. and Bouchilloux, S., Biochem. Biophys. Res. Communs, 16, 249 (1964).

Lissitzky, S., Roques, M., Torresani, J. and Simon, C., Bull. Soc. Chim. Biol., 47, 1999 (1965).

Nadler, N.J., Young, B.A., Leblond, C.P. and Mitmaker, B., Endocrinology, 74, 333 (1964).

Nunez, J., Mauchamp, J., Macchia, V. and Roche, J., Biochem. Biophys. Acta, 107, 247 (1965).

Robbins, J. and Rall, J.E., Physiol. Revs., 40, 415 (1960).

Salvatore, G., Salvatore, M., Cahnmann, H.J. and Robbins, J., J. Biol. Chem., 239, 3267 (1964).

Salvatore, G., Sena, L., Viscidi, E. and Salvatore, M. in Cassano, C. and Andreoli, M., Current Topics in Thyroid Research, Academic Press Inc., New York, p. 193, 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 (1965a).

Seed, R.W. and Goldberg, I.H., Science, 149, 1380 (1965b).

Vecchio, G., Edelhoch, H., Robbins, J. and Weathers, B., Biochemistry, 5, 2617 (1966).