THYROGLOBULIN SYNTHESIS IN A THYROID POLYRIBOSOMAL CELL-FREE SYSTEM.

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Thyroglobulin (Tg), the major protein of the thyroid gland, has a molecular weight of 660.000 and a sedimentation coefficient close to 19 S (Edelhoch 1965). It contains 8,5 % carbohydrate, under the form of 23 oligosaccharides of 2 types: a small unit containing only mannose and N-acetylglucosamine, and a larger unit, with a complex structure, containing in addition sialic acid, fucose and galactose (Cheftel et al. 1964, Spiro 1965).

Thyroid polyribosomal cell-free systems have been used by several authors in an attempt to dissociate the steps leading to the completion of the Tg molecule (Cartouzou et al., 1967; Nunez et al., 1967; Morais and Goldberg, 1967; Soffer 1967; Kondo et al., 1967, 1968a, 1968b). Conflicting results were reported. Although such systems can synthesize protein fragments immunochemically related to thyroglobulin, all but one group came to the conclusion that additional factors, related to the membrane components of the cell, are required for the completion of the molecule (Nunez et al.,1967)

This report presents evidence for the synthesis of 19 S Tg in a polyribosomal cell free system, and suggests as explanation a mechanism based on an exchange between newly synthesized subunits and subunits of the native Tg molecule.

EXPERIMENTAL

Beef thyroid polyribosomes were prepared according to the method described by Kondo et al. (1968a). A modification was adopted in the homogenizing procedure: a glass Teflon homogenizer was chosen instead of the "Ultra-Turrax"

apparatus. Polysomal profiles were recorded in the ISCO gradient fractionator. The whole polysomal preparations were incubated in a medium containing (in μmoles per ml): ATP, 0,5 GTP 0.05; phosphoenolpyruvate 10; KCl, 70; $MgCl_2$ 7.5; β-mercaptoethanol 7.5; Tris-HCl (pH 7.8) 50;and 10 μg pyruvate kinase per ml. One μCi of a mixture of 16 ^{14}C-labeled aminoacids was added per ml. (The Radiochemical Center Amersham, CRB 104, specific activity 52 μCi/m atom of carbon). Ribosomal content was adjusted to a $A_{260\ m\mu}$ value of 4 units. Post-microsomal supernatant of rat liver or beef thyroid was used as source of enzymes, after filtration through Sephadex G-25. In other experiments, this supernatant was replaced by the "pH 5 enzyme" fraction of beef thyroid gland. The amount of added protein was about 1 mg per ml.

Incubations were conducted for 90 min at 37°C. The reaction was stopped by quickly chilling the tubes. The ribosomes were pelleted by spining the tubes at 105.000 x g for 120 minutes. The supernatant was dialysed and concentrated by low pressure dialysis. Sucrose density gradient ultracentrifugation was performed according to Salvatore et al (1964); details are given in the legends of the figures. Samples were treated with Hyamine x 10 hydroxide (Packard) and counted at 50 % efficiency.

RESULTS

Polyribosomal profiles showed the presence of several successive peaks corresponding to the different classes of polyribosomes. A distinct peak corresponding to clusters containing 40 to 50 ribosomes occured in the heavy part of the gradient in 7 out of 10 preparations. As their lighter counter part these heavy polyribosomes were very sensitive to ribonuclease.

Sucrose density gradient ultracentrifugation of the labeled products obtained after the incubation of the whole polysome mixture with thyroid post-microsomal supernatant, revealed the presence of a small but reproductible amount of ^{14}C radioactivity in the 19 S region. This represented from 10 to 20 p. cent of the total radioactivity on the gradient;

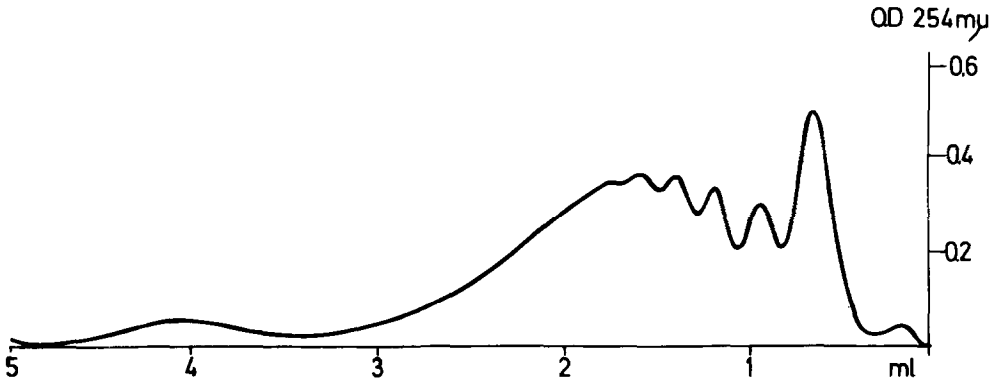

Fig. 1.- Beef thyroid polysome profile, as recorded in the ISCO gradient fractionator. A polysome suspension ($A_{260\ m\mu}$ = 3 units) was layered on a 15-40 % sucrose-gradient (in Tris-HCl 0.05 M (pH 7.2), KCl 10 mM, $MgCl_2$ 2 mM), and centrifuged at 45.000 rpm for 30 minutes in the Spinco Sw 65 rotor. Centrifugation is from right to left.

in some experiments this quantity was as high as 40 p. cent. The major part of the radioactivity remained in the lighter fraction of the gradient in a broad "3-8 S" region (fig. 2A, 3A).

When the same samples were analyzed on Sephadex G-200, the major part of the radioactivity was eluted with the void volume. It should be mentionned that in the operating conditions a globular protein of about 150.000 M.W. (bovine gammaglobulin) would be retarded.

When the incubation was performed with liver post-microsomal supernatant (fig. 2B) or thyroid "pH 5 enzyme" (fig. 3B) no radioactivity appeared in the 19 S region. Interestingly enough, a striking difference was noted when in addition to these enzyme fractions, the incubation medium was supplemented with stable bovine Tg: in these conditions labeled 19 S Tg was detected (fig. 2C, 3C).

DISCUSSION.

A cell-free polysomal system prepared from beef thyroid gland is able to incorporate radioactivity in 19 S Tg. These data are in agreement with the results of Nunez

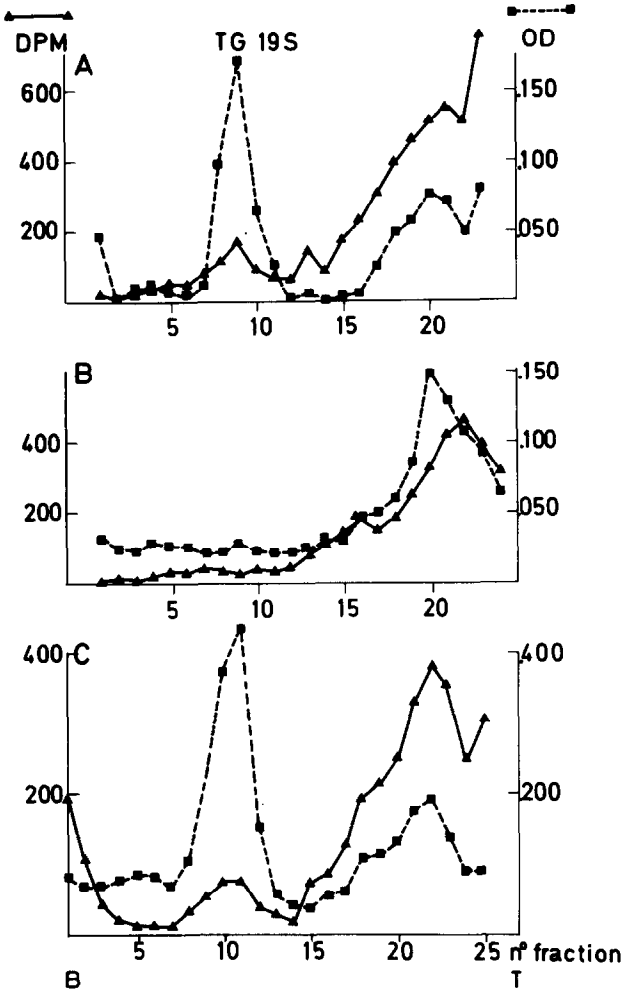

Fig. 2.- Sucrose density gradient ultracentrifugation pattern of the soluble proteins, after incubation (see experimental) of beef thyroid polyribosomes and A : beef thyroid post-microsomal supernatant, B : rat liver post-microsomal supernatant, C : rat liver post-microsomal supernatant, plus beef Tg (0.4 mg/ml).
Sucrose gradients; 5-25 % in Tris HCl 0.05 M (pH 7.2) Spinco Sw 65 rotor; 210 minutes at 60,000 rpm.
Centrifugation is from right to left. (▲——▲ DPM, ■-----■ OD 280 mμ).

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et al. (1967). Morais and Goldberg (1967) and Kondo et al. (1968b) reported also that products synthesized in thyroid polyribosomal cell free systems contain material immunochemically related to Tg. Cartouzou et al. (1967) and Soffer

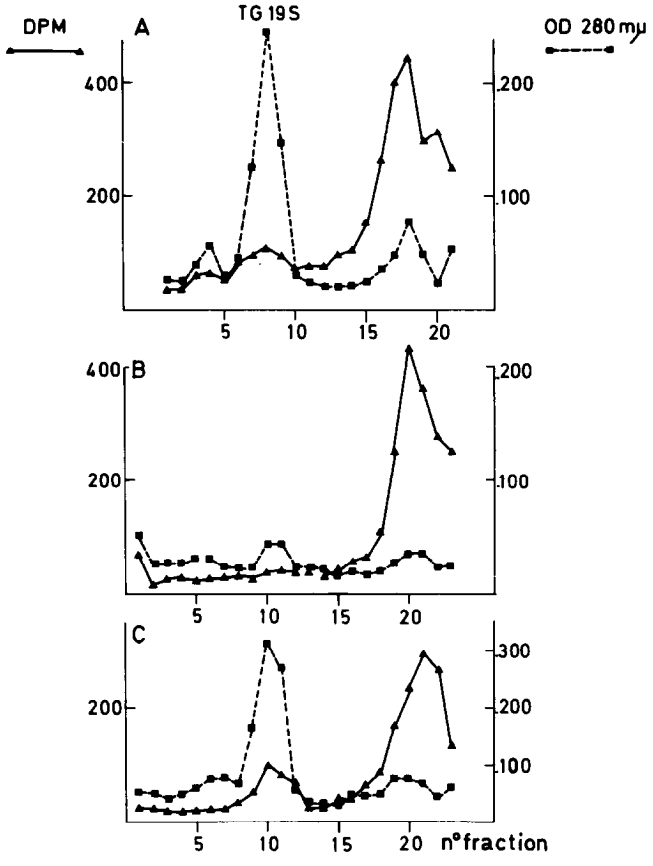

Fig. 3.- Sucrose density gradient ultracentrifugation pattern of the soluble proteins after incubation (see experimental) of beef thyroid polyribosomes and A : beef thyroid post-microsomal supernatant, B : beef thyroid "pH 5 enzyme", C : beef thyroid "pH 5 enzyme" plus beef Tg (0.4 mg/ml). Sucrose gradients 5-25 % in Tris-HCl 0.05 M (pH 7.2); Spinco Sw 65 rotor; 210 minutes at 60,000 rpm. Centrifugation is from right to left (▲——▲ DPM, ■-----■ OD 280 mμ)

(1967) however claimed that in their hands such systems were unable to produce any detectable Tg related material. The discrepancies between these reports are very likely caused by differences in the polyribosomal preparations due either to the mechanical destruction of the polyribosomes or to the elimination of large aggregates during the preparation of the polyribosomes.

In Kondo et al. (1968 b) experiments the incubation medium did not contain stable Tg, which could explain the absence of labeled 19 S Tg. This point however was not investigated. Our data demonstrate that in order to obtain labeled Tg, stable Tg must be present in the incubation medium ; when absent the radioactivity remained in the "3-8 S" area. Although there is suggestive evidence that this material is similar to the "3-8 S" detected after incubation of thyroid slices, no direct proof is as yet available. In view of the results on sucrose density gradient centrifugation and on Sephadex G-200 filtration it is likely that the synthesized material has not yet adopted a globular conformation. The addition of the carbohydrate moiety of the molecule, which is thought to be a late event in the synthesis of Tg, may be required for this step (Cheftel and Bouchilloux, 1968 a, 1968b; Spiro and Spiro, 1966). Recent work however has shown that the synthesis of a part of the carbohydrate moiety, "the inner core", containing mainly mannose may occur at an early stage, and is related to the peptide assembly,(Herscovics, 1969). The size and the number of Tg subunits is open for the discussion. Lissitzky et al. (1968) presented evidence for the existence of eight subunits of 80.000 M.W. ; Pierce et al. (1965) found subunits of about 120.000 M.W. From the studies of de Crombrugghe et al. (1966) and Nissley et al. (1969) it appears that the size of the Tg subunit is about 150.000 M.W., which corresponds to a quarter subunit of Tg. Polyribosomes of 50 ribosomes would contain the information for a protein of this size, as inferred from the studies of Heywood et al. (1967) on the synthesis of myosin.

In order to explain the presence of labeled 19 S Tg, in our cell-free system, we have considered three alternatives: 1° Passive adherence of labeled peptides to stable Tg. 2° Polymerisation of subunits into Tg, 3° Exchange between newly synthesized subunits and stable subunits in the Tg molecule.

Passive adherence has been disregarded for the following reasons: addition of increasing amounts of stable Tg after the incubation does not increase the proportion of radioactivity associated with Tg. Moreover in a system containing liver polyribosomes and thyroid post-microsomal supernatant no ra-

dioactivity is detected in the Tg peak. Polymerisation of subunits is also unlikely to occur (see fig. 1B, 2B): labeled Tg is only present if there is stable Tg in the medium. Exchange of subunits is a possible explanation of our results. The formation of "hybrids" would also explain the fact that the labeled Tg has a sedimentation coefficient close to 19 S rather than a lower value (Nunez et al., 1967). At the present time we have no information concerning this mechanism. Lissitzky et al. (1965) showed that the "3-8 S" fractions isolated from slice experiments is converted in 19 S Tg when incubated in the presence of a post-microsomal supernatant. The possibility that this exchange may be enzyme-dependent is currently under investigation.

In conclusion, the appearance of labeled Tg in a thyroid polyribosomal cell-free system may be the result of two sequential steps:

1° the synthesis of a subunit of Tg. Polyribosomes of 50 ribosomes would contain the information for a protein of 150.000 M.W., which is the size of the peptide moiety of a quarter subunit of Tg;

2° the exchange of these newly synthesized subunits with subunits of native Tg.

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