SPECIFIC SYNTHESIS OF THYROGLOBULIN ON MEMBRANE BOUND THYROID RIBOSOMES*

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1. Introduction

Since the original work of Palade [1], some exportable proteins have been demonstrated to be synthesized on polysomes bound to endoplasmic reticulum. Albumin [2–4] and β-lactoglobulin [5] are in this group. Thyroglobulin synthesis which represents up to 60% of thyroid protein synthesis has been extensively investigated in several laboratories [6–10] and is thought to occur on very large polysomes [6]. Using an immunoprecipitating method derived from that of Holme et al. [11] we present evidence here that thyroglobulin synthesis takes place on polysomes bound to the endoplasmic reticulum.

2. Materials and methods

Sheep thyroid slices were preincubated in Krebs–Ringer-bicarbonate buffer for 30 min as described previously [8]. Nascent peptides on polysomes were pulse labelled for 10 min with ^{3}H-leucine, 7 μCi/ml (4,5-^{3}H-leucine, 5 Ci/mmole, NEN Chemicals). The slices were then carefully chopped and homogenized with 10 strokes of a loose fitting Potter Elvehjem homogenizer in TKM buffer (250 mM sucrose, 50 mM Tris-HCl pH 7.4, 25 mM KCl, 5.25 mM $MgCl_2$, 1 mM dithiothreitol, 0.25 mM EDTA). Free and bound polysomes were prepared from a 800 *g* postnuclear supernatant according to Kimmel [12] with slight modifications. Briefly, a 27,000 *g* pellet obtained by centrifuging the postnuclear supernatant 10 min at 20,000 rpm in rotor 65, was resuspended in 4 ml TKM buffer made 25% rat liver supernatant and containing 100 U/ml heparin and 3 mg/ml yeast RNA. Sodium deoxycholate and Brij 58 were then added to a final concentration of 1% each. The bound polysomes released from the reticulum matrix were purified from this suspension by centrifugation at 270,000 *g* for 5 hr through a discontinuous sucrose gradient consisting of 4 ml 0.5 M sucrose layered on 3 ml 2 M sucrose in TKM buffer made 500 mM KCl in order to minimize unspecific adsorption of labelled material on polysomes. Free polysomes were prepared by directly layering the 27,000 *g* supernatant on identical sucrose gradient without any detergent treatment. Centrifugation conditions were the same as for bound polysomes. Control experiments, where either detergent treatment of the 27,000 *g* pellet was omitted or detergent treatment of the 27,000 *g* supernatant was performed, have shown that contamination of bound material with free ribosomes was negligible, and conversely. The RNA content of the 27,000 *g* pellet, as measured with the method of Fleck and Munro, was found to be about 60% of the total RNA of the 800 *g* supernatant.

Immunoprecipitation of the nascent peptides has been found to be far more specific than precipitation

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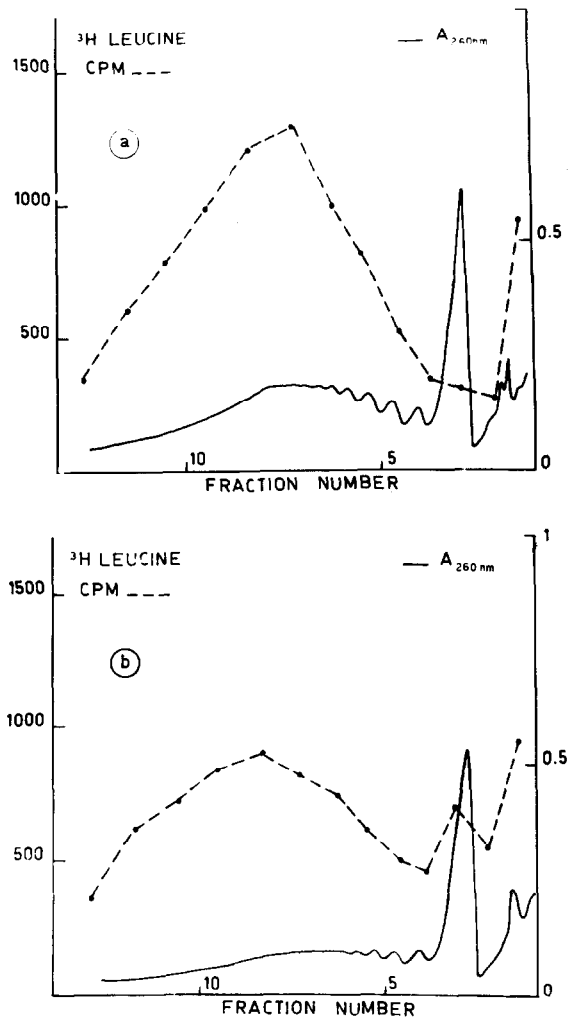

Fig. 1. Sucrose density gradient profiles of free (a) and bound (b) polysomes from thyroid gland labelled for 10 min with 7 μCi/ml ^{3}H-leucine. 200 λ of the polysome suspension were layered on 25–50% linear sucrose gradient in TKM buffer. Centrifugation was performed for 75 min at 40,000 rpm in a Spinco Rotor SW56. Fractions of 12 drops were collected and counted in Bray's solution.

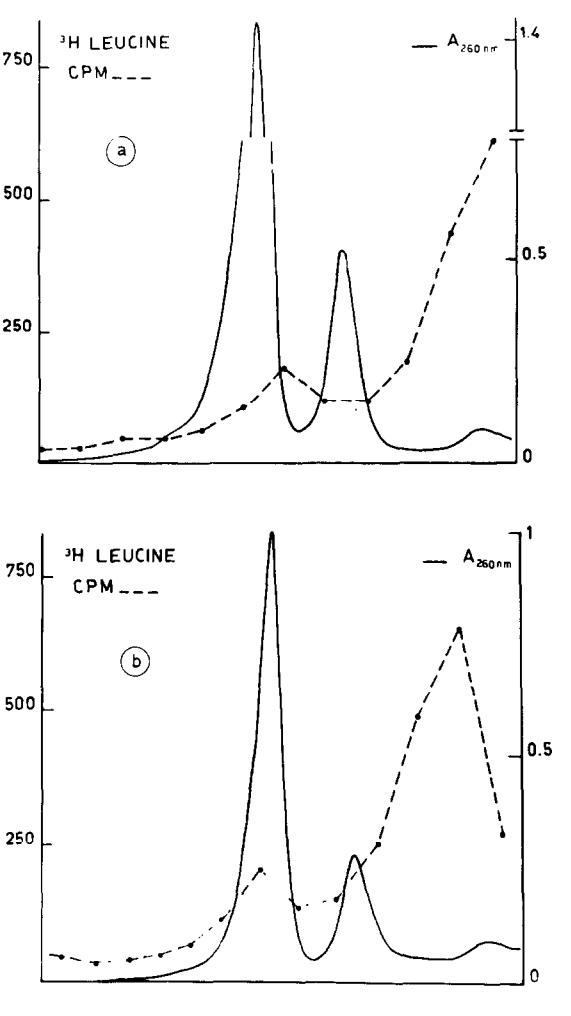

Fig. 2. Sucrose density gradients profiles of free (a) and bound (b) polysomes dissociated by EDTA treatment to release labelled nascent peptides. Polysome pellets were suspended in 200 λ 140 mM NaCl, 15 mM EDTA, 10 mM phosphate buffer pH 7.4, and layered on 10–30% linear sucrose gradients in the same buffer. Centrifugation was for 175 min at 40,000 rpm in a SW 39L Spinco Rotor. 50 λ aliquots from 12 drop fractions recovered from the gradients were digested in soluene and counted.

of whole polysomes [11]. Therefore, the labelled nascent peptides were released from polysomes by resuspending the pellets of free and bound polysomes in 140 mM NaCl, 15 mM EDTA, 10 mM phosphate buffer pH 7.4. The suspensions were placed on 10–30% sucrose gradients made in the same buffer and the released peptides were separated from the ribosomal subunits by centrifugation at 40,000 rpm during 175 min in SW 39 L rotor. The fractions of the gradients lighter than the 40 S subunits (fract. 1, 2, 3) were pooled and diluted to 2.1 ml with phosphate buffer without EDTA. A 100 λ aliquot of the resulting solution was digested in soluene 100 (Packard) and counted.

Table 1
Immunoprecipitation of nascent peptides from thyroid polysomes.

Nascent peptides released from:	Serum	Total radioactivity in samples (cpm)	Radioactivity in immunoprecipitates (cpm)	Percent of total radioactivity
Free polysomes	Control	3, 120	263	8.4
Free polysomes	Antithyroglobulin	3, 120	283	9.0
Bound polysomes	Control	3, 190	225	7.0
Bound polysomes	Antithyroglobulin	3, 190	1,813	58.5

Fractions no, 1, 2, 3 of fig. 2a and 2b were pooled. These fractions contain labelled nascent peptides released from free and bound polysomes without any contamination with ribosomal material. Immunoprecipitation of the peptides was performed using either a control or an antithyroglobulin serum (see text).

Immunoprecipitation of the nascent peptides was performed in 1 ml samples of the same solution using the double antibody technique. 10 λ of either guinea pig control serum or antithyroglobulin serum were added. After 5 min incubation at 37° and cooling at 0°, 200 λ sheep serum anti guinea pig immunoglobulin were added to all samples and new incubations, first for 5 min at 37° and then overnight at 4° were performed. The samples were centrifuged for 30 min at 3,000 rpm and the immunoprecipitates were washed twice before soluene digestion and counting.

3. Results and discussion

The data presented are those of one typical experiment out of five giving similar results. Fig. 1 shows free and bound polysome profiles. Slight degradation of bound polysomes by ribonuclease has probably occurred during the detergent treatment of the 27,000 *g* pellet as suggested by the shape of the profile [13]. Mere inspection of the profiles reveals that specific activity of bound polysomes is greater than that of free polysomes. Assuming that translation rates, leucine distribution in the protein synthesized and specific activity of the leucine pool are the same for both groups of polysomes, this would be compatible with the hypothesis that bound polysomes are synthesizing larger proteins.

Nascent peptides released from bound polysomes must indeed be heavier than those obtained from free polysomes as indicated by the fact that they enter the sucrose gradient (fig. 2) while peptides from free polysomes do not. The centrifugation patterns of fig. 2a, b were highly reproducible.

Table 1 demonstrates that only peptides from bound polysomes were specifically precipitable by antithyroglobulin serum. We would thus conclude that thyroglobulin is synthesized only on bound polysomes. Assuming that only half completed thyroglobulin peptides are immunoprecipitable, bound polysomes would synthesize almost only thyroglobulin. Bound polysomes could thus possibly be a source of passably pure thyroglobulin coding mRNA.

Experiments are in progress to determine the maximal sedimentation coefficient value of thyroglobulin nascent peptides. This could provide information about the sedimentation coefficient of thyroglobulin monomers.

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