COVALENT ASSOCIATION OF THYROGLOBULIN SUBUNITS IN A THYROID POLYRIBOSOMAL CELL-FREE SYSTEM

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1. Introduction

Synthesis of 19 S thyroglobulin (Tg) may occur in a thyroid polyribosomal system [1–3]. Evidence was presented to support the concept of a mechanism based on an exchange of newly synthesized subunits of the native thyroglobulin [1]. No information however is available on the nature of the bond linking the exchanged material to the thyroglobulin.

In this paper we report experiments showing that covalent bonds are involved in this process.

2. Experimental

Beef thyroid polyribosomes were prepared according to the method of Kondo et al., as modified in [1]. The incubation medium contained in μmoles per ml: ATP 0.5; GTP 0.05; phosphoenolpyruvate 10; KCl 70; $MgCl_2$ 7.5; β-mercaptoethanol 15; tris-HCl (pH 7.8) 50, and 10 μg pyruvate kinase per ml. One μCi of a mixture of 16 ^{14}C-labeled amino acids was added per ml (Radiochemical Center Amersham, U.K., CRB 104, Specific activity 52 mCi/matom of carbon).

The ribosomal content was adjusted to a $O.D._{280m\mu}$ value of 4 per ml. Thyroid post-microsomal supernatant filtered through Sephadex G-25 was used as source of enzyme. The amount of added protein was 2 mg per ml. Incubations were conducted for 90 min at 37°. The reaction was stopped by chilling the tubes in an ice bucket. The ribosomes were pelleted by spinning the tubes at 105,000 *g* for 120 min. The supernatant was extensively dialysed against tris-HCl buffer 0.05 M, pH 7.2, and concentrated by low pressure dialysis. Sucrose density gradient ultracentrifugations were performed according to Salvatore et al. [4]. Details are given in the legends of the figures. Sucrose density gradients in 6 M guanidine (Guanidine hydrochloride, Eastman, Rochester, U.S.A.) were prepared according to de Crombrugghe [5].

Samples were treated with Hyamine 10 X hydroxide and counted in a Packard Tricarb liquid scintillation counter at 40% efficiency.

3. Results

Sucrose density gradient ultracentrifugation of the labeled products obtained after incubation shows a well defined ^{14}C-radioactivity peak corresponding to the 19 S Tg optical density peak (fig. 1). When this material is submitted to sucrose density gradient ultracentrifugation in 6 M guanidine an appreciable fraction of the radioactivity remains in the heavy part of the gradient (fig. 2). The radioactivity in the lighter fraction of the gradient could be due either to dissociation of heavier material or to the light material originally present among the labeled products (fig. 1).

A purified radioactive 19 S Tg fraction was prepared by sucrose density gradient centrifugation of the whole preparation. The tubes under the 19 S Tg optical density peak were pooled and concentrated, yielding a 19 S Tg preparation devoid of light material (fig. 3). A sucrose density gradient in 6 M guanidine of this isolated fraction shows two main optical density peaks, corresponding to undissociated 19 S Tg and to 12 S subunits (fig. 4). The radioactivity is distributed in three fraction: 54.8% in the 19 S peak, 19.9% in the 12 S peak and 22.2% in the light fraction.

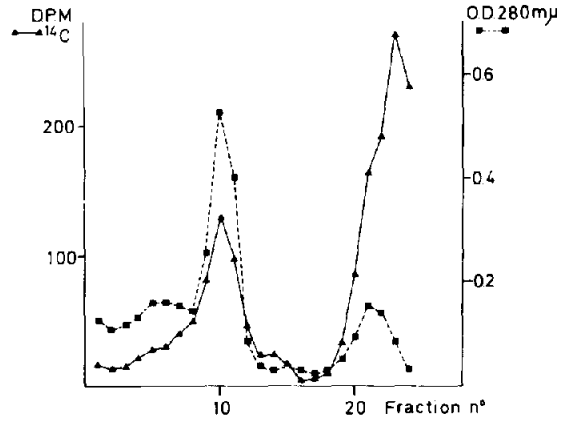

Fig. 1. Sucrose density gradient pattern of the soluble proteins, after incubation of thyroid polyribosomes and post-microsomal supernatant. Sucrose gradients; 5–25% in tris HCl 0.05 M (pH 7.2) Spinco SW 65 rotor; 180 min at 65,000 rpm. Centrifugation is from right to left (▲——▲ DPM, ■——■ O.D. 280 mμ).

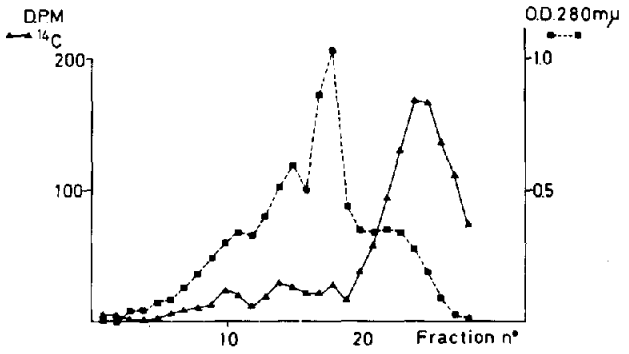

Fig. 2. Sucrose density gradient pattern of the soluble proteins, after incubation of thyroid polyribosomes and post-microsomal supernatant. Sucrose gradients; 0–15% in tris-HCl 0.05 M (pH 7.2), containing 6 M guanidine, Spinco SW 65 rotor, 17 hr at 50,000 rpm. Centrifugation is from right to left (▲——▲ DPM, ■——■ O.D. 280 mμ).

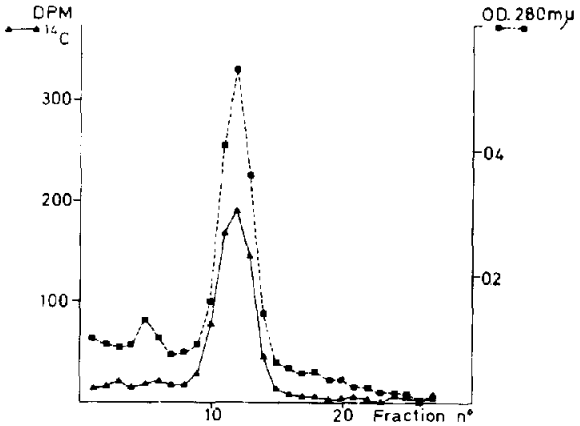

Fig. 3. Sucrose density gradient pattern of the isolated 19 S Tg fraction. Sucrose gradients; 5–25% in tris-HCl 0.05 M (pH 7.2) Spinco SW 65 rotor; 180 min at 65,000 rpm. Centrifugation is from right to left (▲——▲ DPM, ■——■ O.D. 280 mμ).

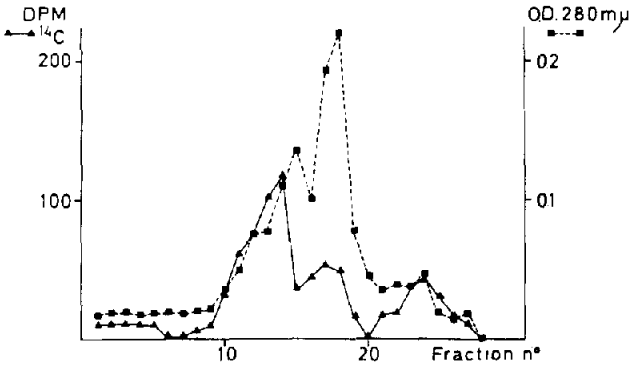

Fig. 4. Sucrose density gradient pattern of the isolated 19 S Tg fraction. Sucrose gradient 0–15% in tris-HCl 0.05 M (pH 7.2), containing 6 M guanidine; Spinco SW 65 rotor; 17 hr at 50,000 rpm. Centrifugation is from right to left (▲——▲ DPM, ■——■ O.D. 280 mμ).

4. Discussion

Native bovine 19 S Tg (M.W. 670,000 daltons) contains 101 disulfide bonds. Complete reduction of these bonds results in the formation of 4 polypeptide chains of M.W. about 165,000 daltons [6]. Air re-oxidation of the sulfhydryl groups restores products which are similar to the native protein [7].

Differences in the association of the 12 S subunits in the quaternary structure of human 19 S Tg have been demonstrated by using sucrose density gradients in 6 M guanidine [5]. These differences have been related to the degree of iodination of Tg. [5, 8, 9]. Investigations conducted on bovine 19 S Tg have led to the conclusion that there are 2 species of 19 S Tg molecules, according to their aptitude to dissociate

in 12 S subunits under the action of either guanidine or urea, agents which affect the non-covalent bonds.

Bovine 19 S Tg, contains thus both the dissociable and the non-dissociable species. The latter one is composed of 12 S subunits held together by one or a few S-S bonds, whereas in the former the 2 subunits are held together by non-covalent bonds and readily dissociate in 6 M guanidine. The 12 S contains 2 chains of 165,000 daltons which are linked by disulfide bonds to form dimers.

As far as 19 Tg synthesis concerns, it is likely that in our experiments the β-mercaptoethanol present in the incubation medium reduces native 19 S Tg into subunits. The reoxidation of the disulfide bonds occurs after dialysis, which removes the reducing agent. During this process completed newly labeled subunits are included in the restored 19 S Tg molecule. This property of Tg of recovering its initial characteristics after reduction and reoxidation is shared with other proteins [see ref. 7]. Preliminary experiments indicate that Tg synthesis also occurs in the absence of β-mercaptoethanol. The amount of non-dissociable Tg however, is less important. This shows that reduction and unfolding of the Tg molecule would favour the covalent association of subunit to Tg (unpublished results).

The experiments in 6 M guanidine indicate that the incorporation of these novel subunits involves the formation of disulfide bonds. Indeed in gradients performed in those conditions a large part of the radioactivity is located in the undissociated 19 S Tg area and in the 12 S region (which contains 6 S subunits held together by disulfide bonds). Although the S-S bond rearrangement could imply a "disulfide exchange enzyme", which does exist in the thyroid [10], no proof exists as yet that it actually plays a role in this cell-free system.

Acknowledgements

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References

[1] Ph.de Nayer and M.de Visscher, Biochem. Biophys. Res. Commun. 36 (1969) 639.
[2] J.Nunez, J.Mauchamp, A.Jerusalmi and J.Roche, Biochim. Biophys. Acta 145 (1967) 127.
[3] R.Morais and I.H.Goldberg, Biochemistry 6 (1967) 2539.
[4] G.Salvatore, M.Salvatore, H.J.Cahnman and J.Robbins, J. Biol. Chem. 239 (1964) 3267.
[5] B.de Crombrugghe, Thesis: Propriétés moléculaires de la thyroglobuline normale et pathologique (Librairie Universitaire, Louvain, 1968).
[6] B.de Crombrugghe, R.Pitt-Rivers and H.Edelhoch, J. Biol. Chem. 241 (1966) 2766.
[7] B.de Crombrugghe and H.Edelhoch, Biochemistry 5 (1966) 2238.
[8] B.de Crombrugghe and M.de Visscher, Arch. Intern. Physiol. Biochem. 77 (1969) 150.
[9] M.Andreoli, L.Sena, H.Edenhoch and G.Salvatore, Arch. Biochem. Biophys. 134 (1969) 242.
[10] F.de Lorenzo and G.Molea, Biochim. Biophys. Acta 146 (1967) 593.