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Iodine-induced changes in thyroglobulin half-sized subunits[1]

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Summary. The two half-sized subunits of 19 S thyroglobulin have been separated and analyzed. They share the same peptide composition and carbohydrate content. The only difference was the iodine level, which was about three times higher in the faster electrophoretic subunit.

Thyroglobulin is the major iodoprotein synthesized in the thyroid gland as a framework for thyroid hormones. It is a large glycoprotein of 660,000 and has a sedimentation coefficient of 19S[2]. Van der Walt et al.[3] have shown that, upon reduction, this molecule is easily dissociated into 2 non-identical half-sized subunits having different electrophoretic mobilities in sodium dodecyl sulfate gel electrophoresis. The same authors inferred that the different electrophoretic behavior observed was due to some minor differences resulting from the amino acid analysis of the 2 separate polypeptides. In contrast with this finding we now show that these 2 half-sized molecules have similar peptide and carbohydrate compositions. Since the only remarkable difference found is in the iodination level, it is suggested that iodination is a specific post-translational event which is responsible for the observed changes in the electrodynamic properties of thyroglobulin apparent subunits.

Material and methods. Purification of 19 S thyroglobulin. Hog thyroids were obtained at the local abattoir immediately after the death of the animals. Thyroids were minced with scissors and soaked for 15 min with 0.1 M sodium phosphate buffer, pH 7.2. After a fractionated (1.4–1.8 M) ammonium sulfate precipitation, the extracted proteins were filtered on a Sepharose 6B (1.3×80 cm) column equilibrated with the extraction buffer. The peak fractions were pooled and appropriately concentrated. The protein concentration was estimated spectrophotometrically using an E1% (1 cm) of 10 at 280 nm. Its homogeneity was tested by analytical ultracentrifugation in a Spinco model E ultracentrifuge.

Isolation of porcine thyroglobulin subunits. To separate the 2 major components of hog thyroglobulin observed under denaturating and reducing conditions (i.e. 0.1% sodium dodecyl sulfate, 0.01% 2-mercaptoethanol), preparative slab gel electrophoresis was employed. The slab gel (5% acrylamide) had the following dimensions: 0.3×14×12 cm. About 2 mg of sodium dodecyl sulfate-treated thyroglobulin were extensively reduced with 2-mercaptoethanol and layered on the top of the gel and electrophoresed for 4 h at 50 mA. At the end of this period the gel was removed and a side slice cut and briefly stained, to localize the 2 major peptides. On this basis the 2 portions of the gel, containing the faster and the slower moving bands, were cut into very small pieces, immersed in 2 ml of electrode buffer (without sodium dodecyl sulfate) and shaken vigorously for 12 h at room temperature. The extracted proteins were then concentrated by lyophilization.

Carbohydrate and iodine determinations. The carbohydrate content of both unfractionated hog 19 S and isolated polypeptides was estimated according to the method of Roe[4]. Organic iodine was determined by the procedure of Palumbo et al.[5].

Limited enzymatic proteolysis in sodium dodecyl sulfate by *Staphylococcus aureus* SV8 protease. Limited enzymatic digestion of the 2 peptides and the native protein was accomplished using the *Staphylococcus aureus* SV8 protease (EC 3.4.21., Miles Lab.). Each sample, consisting of a mixture of the enzyme and protein (0.1 mg/mg of protein), was incubated in 0.5% sodium dodecyl sulfate at 37 °C for 30 min according to the method of Cleveland et al.[6]. The digestion was stopped by adding an excess of (0.75 M) 2-mercaptoetheanol and boiling for about 1 min.

Analytical sodium dodecylsulfate polyacrylamide gel electrophoresis. Before electrophoresis samples, whose concentration in sodium dodecyl sulfate was adjusted to 1%, were incubated at room temperature for about 30 min, and then

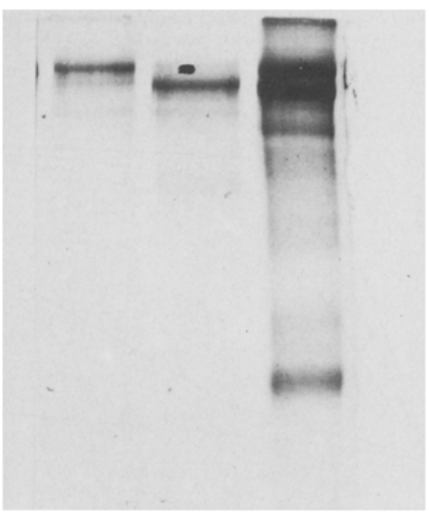

Figure 1. Purification of hog 19 S thyroglobulin reduced subunits by preparative gel electrophoresis. From the left: slots 1 and 2 show the isolated components (faster and slower migrating bands of the doublet of near 330,000). Slot 3 depicts the electrophoretic pattern of reduced hog 19 S thyroglobulin. Lighter materials, other than the 330,000 doublet may be noticed in this preparation. The gel contained 7.5% acrylamide.

boiled (2 min) in a bath at 100 °C. Polyacrylamide slab gels (15% acrylamide) were prepared as described by Laemmli[7].

Results and discussion. The data presented below provide some evidence from gel electrophoresis indicating possible similarity in the polypeptide composition of the 2 major components of reduced 19 S porcine thyroglobulin.

Figure 1 shows the 2 components of hog thyroglobulin after purification, and the electrophoretic pattern of the whole reduced 19 S. The latter profile consists of a major doublet ($M_r \cong 330{,}000$), of 2 significant bands ($M_r \cong 140{,}000$ and 70,000 respectively) and of some minor components. The origin of such lighter materials has recently been discussed[8]. It has been found that the amount of the species, other than the 330,000 doublet, is linearly related to the iodine content of the native 19 S (0.79% w/w in the present case). The 330,000 dalton doublet has been already reported[9] in reduced 19 S hog thyroglobulin.

The analysis of carbohydrates revealed that both subunits contain the same amount of sugar which, moreover, does not differ from the carbohydrate content of the original unfractionated 19 S, being near 10% w/w (within the experimental error).

From figure 2 it is evident that the electrophoretic patterns produced by the limited enzymatic digestion of the 2 species are essentially indistinguishable. The same figure shows that the enzymatic digestion of the native unfractionated 19 S (which contain lighter materials) produced an electrophoretic pattern very similar to those produced by each of the 2 isolated polypeptides. The only remarkable difference observed between the 2 subunits relates to the iodine content, which is significantly higher in the faster-moving species. In fact the iodine analysis of these subunits reveals that the faster-moving band (see fig. 1) has an iodine content about $2\frac{1}{2}$ times higher than the slower one (0.69% and 0.27 w/w respectively).

Van der Walt et al.[3] have described the time-course of the reduction of bovine 19 S thyroglobulin. From these data it appears that, upon reduction, there is a decrease in the non-dissociable (i.e. iodine rich) 19 S and a concomitant increase in the faster component of 300,000 dalton. From the same data, it is also evident that the amount of the slower component does not change with the reduction time. This fact is in agreement with the possibility that the faster-moving species, which originates from the undissociable 19 S, contains a higher iodine level than the slower band, but it does not say very much about the respective peptide compositions.

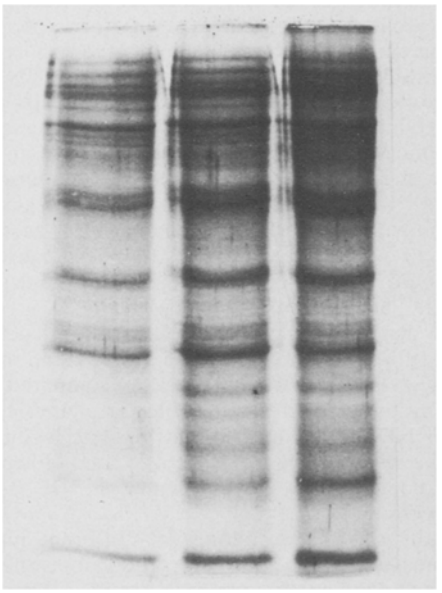

Figure 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (15%) of peptides obtained by limited proteolysis in 0.5% sodium dodecyl sulfate at 37 °C for 30 min (*Staphylococcus aureus* SV 8 protease) of hog thyroglobulin reduced major electrophoretic components. From the left: digestion products of the slower migrating component, of the faster migrating component and of the reduced unfractionated 19 S hog thyroglobulin. The size of these bands ranges from approximately 40,000 dalton to about 5,000 dalton. Very likely 2 of these bands are produced by the SV 8 protease. The gel contained 15% acrylamide.

Since the peptide maps of the 2 subunits as well as the carbohydrate content have been found to be essentially identical it is possible that the 2 half-sized molecules have similar or even identical primary structures and that the change in the electrophoretic mobilities is related to the significant difference in the relative iodine contents. However, a larger iodine content per se does not seem likely to result in changes in the electrophoretic mobility of such large molecular species as the half-sized thyroglobulin subunits. More probably, the observed change in electrophoretic mobility is due to iodine-induced changes in the hydrodynamic volumes of the 2 molecular species. Perhaps it is possible that, during the peroxidatic iodination of thyroglobulin to form thyroid hormones, new types of bonds (besides the disulfide bridges) are formed. For example, peroxidases are known to cross-link phenolic groups[10].

Alternatively, since iodination modulates the protein conformation[11], sodium dodecyl sulfate may be bound to a different extent according to the iodination level of thyroglobulin. Consequently, although the 2 peptides have the same molecular weight, primary structure and carbohydrate content, they carry different amounts of sodium dodecyl sulfate molecules and, for this reason, have different electrophoretic behavior.

Another observation of some interest is the finding that the significant presence (in the unfractionated 19 S molecules) of material lighter than 330,000 (see fig. 1), does not result in the appearance of new electrophoretic species upon proteolysis as compared to the proteolytic pattern produced by each of the isolated polypeptides. This fact may suggest that some kind of internal homology exists in the thyroglobulin chain. This finding is reinforced by recent data[12] which show that the elementary polypeptide chain of guinea-pig thyroglobulin is really composed of homologous repeating segments.

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