THE ULTRASTRUCTURE OF HUMAN THYROGLOBULIN

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The thyroglobulin has an important function in the metabolic processes by acting as a storeplace for the thyroid hormones thyroxin and triiodothyronine. The molecule, which comprises about 80% of the total protein concentration in saline extracts of the thyroid gland, is a glycoprotein with a sedimentation constant of about 19S and a molecular weight of 660,000–690,000. Many techniques used for the determination of the size and shape of other proteins have also been applied to the thyroglobulin molecule, such as viscosity measurements, determination of diffusion constant, depolarization of fluorescence, and electron microscopy. From viscosity data obtained for native thyroglobulin Edelhoch (1) concluded that the molecule possessed a rather high degree of spherical symmetry and compactness. Results from depolarization studies (2) using the fluorescence technique also suggested that the native thyroglobulin molecule behaved as a compact particle.

To our knowledge only two attempts have been made to elucidate the ultrastructure of thyroglobulin by the aid of electron microscopy. Jakoby and co-workers (3) examined crystals of the molecule in saturated ammonium sulphate by using the technique of shadowed carbon replicas but, due to residual salt, no fine structure was discernable. Similarly, the negative staining technique (4) employed by Valentine (5) gave no conclusive information with regard to the fine structure of the molecule.

Recent results (6, 7) obtained with the negative staining technique in studies of other macroglobulins prompted the present investigation of the ultrastructure of the thyroglobulin molecule with the same technique.

Materials and Methods

Preparation of Thyroid Extracts.—Thyroid glands from patients with colloid goiters were removed at operation and immediately frozen at −20°C. Several glands were collected—none of which was stored for more than 2 wk. The glands were sliced in the frozen state with a knife and soaked in phosphate-buffered saline (PBS) of pH 7.0 (0.02 M sodium phosphate, 0.17 M NaCl, and 2% butanol as preservative). The relative amount of buffer to thyroid tissue was 2:1. After continuous stirring at +4°C for 12 hr the particulated material was removed by centrifugation at 10,000 g for 45 mins. The supernatant was filtered and stored at +4°C until used.
Passive Hemagglutination-Inhibition Test.—The microtechnique described by Sever (8) was used. Serial twofold dilutions (0.025 ml) of the thyroglobulin preparation were prepared in phosphate-buffered saline of pH 7.2. The diluent contained 0.8% normal rabbit serum for stabilization purposes. 16 hemagglutinating units of human antithyroglobulin serum (0.025 ml) were added to each dilution of the thyroglobulin preparation and the tray was incubated for 1 hr at room temperature. Thyroglobulin-coated tanned sheep cells were then added in the form of a 1% suspension and the tray was incubated for another 2 hr at 28°C. The final cup exhibiting a complete inhibition of agglutination was considered to contain 1 unit of inhibitory activity. In order to detect possible contamination of IgM globulin in the tested thyroglobulin preparation, parallel tests were carried out using rabbit anti-human IgM serum and tanned cells coated with IgM instead of thyroglobulin.

Gel Filtration.—Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) was carefully washed in PBS and a column with a diameter of 5 cm and a length of 350 cm was packed with the gel. The 20-ml samples of thyroid extracts to be fractionated were applied to the top of the column. The flow rate was maintained at 1.2 ml/cm² per hour. The effluent from the column was passed through two Uvicords (LKB-Produkter AB, Stockholm, Sweden), coupled in series for extinction measurements at 280 and 254 nm, and then fractionated into samples of 10 ml. The transmission curves were converted to curves of absorbance.

Gel Precipitation.—Ouchterlony agar-diffusion studies (9) were carried out in 1% Noble-agar (Difco Laboratories, Detroit, Mich.) gel and PBS of pH 7.0. Rabbit antiserum was
prepared by 5 weekly intramuscular injections of equal parts of crude thyroid extract and Freund's complete adjuvants. The animals were bled from the marginal ear vein 8 wk after the last injection. The serum was collected and stored at −20°C without preservatives.

**Immunoelectrophoresis.**—This was carried out in a 1 mm layer of 1% Nobel-agar gel in barbital buffer μ = 0.05, pH 8.6. Electrophoresis was run for 90 min at 25 v/cm and 30 ma. The reagents in the troughs were allowed to diffuse for 48 hr.

**Analytical ultracentrifugation** samples were concentrated and dialyzed against 0.01 m phosphate buffer of pH 7.4 with 0.14 m NaCl prior to centrifugation. The runs were performed in a Spinco Model E ultracentrifuge at 42,040 rpm at 20°C using a 12 mm 4° sector cell with a standard window. Pictures were taken at 4 min intervals and the sedimentation constant s20, w was calculated by conventional methods using η = 0.725 for thyroglobulin (10).

![Ultracentrifugal analysis of material from the second peak in Fig. 1. The photograph was taken 16 min after full speed (42,040 rpm) was reached. Sedimentation is from left to right. The peak has an s20, w value of 18.2.](image)

**Preparation of Material for Electron Microscopy.**—The purified material was dialyzed against a solution of 1% ammonium acetate and diluted in the negative contrast material. After a few seconds the excess fluid was withdrawn with a filter paper. The grids were left in the air to dry and then immediately examined in a JEM-5Y electron microscope at a magnification of 50,000 using double condensed illumination. The condenser aperture was 250 μ and the objective aperture 50 or 70 μ at an operation voltage of 80 kv. Two different stains were used: 2% solution of sodium tungstosilicate (STS) and 2% solution of phosphotungstic acid (KPT), both of pH 6.0.

**RESULTS**

A typical diagram of the elution of thyroglobulin material from a column of Sepharose 4B is shown in Fig. 1. As indicated by the absorbance values at 280 and 254 mμ the protein is distributed within the three central peaks. After pooling and concentrating fractions 155–170 and 206–214 respectively to an average protein concentration of about 3 mg/ml the two samples were subjected to analytical ultracentrifugation. Fraction 210, which had the highest protein concentration, was saved for electron microscopy. The protein peak represented by fractions 155–170 was shown to have a s20, w value of 26.7 and the peak represented by fractions 206–214 had a value of 18.2.
This paper will deal exclusively with the homogeneous 18-19S peak of which a Schlieren picture is shown in Fig. 2. This picture revealed no contaminating proteins with other $s_{20,w}$ values. However, since ultracentrifugation is an insensitive method for the tracing of contaminants, a double diffusion test in agar was performed. Fig. 3 shows the results obtained when a rabbit hyperimmune serum prepared against crude thyroid extract was tested against the same extract as well as against fraction 210 from the gel filtration run. The rabbit antiserum gave a single line of precipitation with the gel-filtered thyroglobulin preparation while additional weak lines of precipitation were obtained with the crude thyroid extract. When tested in immunoelectrophoresis against the same antiserum fraction 210 behaved as a single homogeneous component.

When fraction 210 was subjected to electron microscopy characteristic particles, apparently representing thyroglobulin molecules, were found in high concentration (Fig. 4). These particles had an elongated symmetrical structure with a length of approximately 220 A and an approximate maximal width of 110 A. Some of the particles seemed to be slightly distorted, probably due to forces acting during the drying process on the grid. When differently-oriented, apparently intact particles were examined at higher magnifications it became possible to deduce a model for the thyroglobulin molecule. Figs. 5 a–5 c show that the molecule has the shape of a small helix with two turns. It was not possible to determine the type of helix in all cases with certainty, but right-handed helices seemed to be in majority. The pitch varied from 40 A in the most compressed form of the molecule (Fig. 5 a) to 50 A in the more stretched out molecules (Fig. 5 b). The latter type of structures predominated on the grid. The difference in the pitch of this helical structure may be caused by forces acting during the drying process tending to stretch the molecule. Many particles appeared to have an even more elongated and loose structure as shown in Fig. 5 c. A configuration, seen on a few occasions, was a circular structure with an inner diameter in the range of 50 A and an outer diameter of 100 A (Fig. 6 a). This structure, which did not form a closed circle, most probably, represented the whole or major part of one of the helices, as seen end on. This assumption was further supported by the fact that the ring sometimes was
connected to a short threadlike structure of the same thickness (Fig. 6 b). In a recent study (Chesebro et al., reference 7) the IgM antibody molecule was found to retain most of its antibody activity when redissolved following drying in STS as for electron microscopy. The thyroglobulin similarly retained its hemagglutination-inhibiting activity after being exposed to the same drying process and its sedimentation constant remained unchanged indicating that denaturation and gross configurational changes of the particles did not occur.

**DISCUSSION**

In the present investigation a simple one step purification procedure similar to the gel filtration technique described by Salvatore et al. (11) was employed. A longer column was used, however, in order to obtain a better separation of the 19S thyroglobulin molecules from the 27S component.

Little is known about the tertiary structure of thyroglobulin. From viscosity data Edelhoch (1) suggested that the thyroglobulin molecule possessed a rather high degree of spherical symmetry and compactness. Edelhoch obtained an axial ratio of 9 for the molecule assuming the thyroglobulin to be a rigid ellipsoid of revolution. The polarization data by Steiner and Edelhoch (2) suggested that the 19S thyroglobulin molecule behaved like a compact rigid particle. In the present study the axial ratio of the thyroglobulin molecule was found to be about 2 as compared with 9 for the theoretical ellipsoid of revolution. Furthermore, the molecule did not have the appearance of a compact particle but revealed the intricate structure of a helix, possessing some flexibility. The discrepancy between our electron microscopy data and the data of previous investigators may at least partly be explained by the fact that the latter data were based on the assumption that the thyroglobulin molecule had the shape of an ellipsoid rather than that of a helix.

It is interesting to note the presence of circular structures in certain preparations. Such structures are sometimes abundantly found in stored myxovirus preparations as a resultant of the break-down of the helical nucleocapsid, probably involving the breakage of bonds linking successive turns in the intact nucleocapsid (Caspar and Klug, reference 12).

The thyroglobulin molecule has been the subject of study in two previous electron microscopical investigations. Valentine (5), using the negative staining method, reported that the molecule was almost undetectable. He could picture some vague round objects of about 140 A in diameter.

It has also been our experience that occasionally it can be very difficult to obtain good contrast of the thyroglobulin molecule. The question of how to explain the different size and shape of the thyroglobulin molecule in Valentine’s study as compared with our data must however at the present time be left open. Thyroglobulin crystals have been examined by Jakoby et al. (3) as shadowed carbon replicas. Such preparations disclosed units of 228 ± 9 A in diameter, the fine structure of which could not be seen because of residual salt. It was
proposed that this unit represented a polymer of thyroglobulin. Since the type of preparation and the method used in their investigation differed considerably from ours no further comparison between their results and those presented here can be made.

SUMMARY

Thyroglobulin molecules purified in a single step procedure by gel filtration were studied in the electron microscope using the negative staining technique. The molecule had the shape of a flexible helix with two turns. Its length was about 220 Å and the maximal diameter of the coiled part of the molecule was estimated to be 110 Å. The pitch varied between 40 and 50 Å. Thyroglobulin molecules dried in sodium tungstosilicate on a carbon film as for electron microscopy retained their hemagglutination-inhibiting activity and 19S sedimentation constant when redissolved in physiological buffer.

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BIBLIOGRAPHY

FIG. 4. Typical electron micrograph of purified human thyroglobulin molecules. Arrows indicate some of the screwlike particles. × 150,000. Scale line = 100 Å.
Figs. 5 a–5 c. Electron micrographs of selected thyroglobulin particles at higher magnifications. It can be seen that the molecule has the shape of a flexible helix with two turns. Compare pictures of models below each micrograph. × 400,000; the scale lines represents 100 Å.

Fig. 6 a. Circular structure seen on a few occasions in the purified preparations. This configuration probably represents the major part of one of the helices as seen end on. Note the opening of the circle (arrow). × 400,000. The scale line represents 100 Å.

Fig. 6 b. The ring structure connected to a short threadlike appendage (arrow) of the same thickness as the uncoiled part of the helix. × 400,000. The scale line represents 100 Å.