Difference in Isoelectric Point of 27S and 19S Thyroidal Iodoproteins Determined by Isoelectric Focusing (35490)

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The soluble proteins of the thyroid gland can be isolated by several methods which take advantage of their low solubility in approximately 50% saturation of ammonium sulfate (1), their ionic charge (2), and their molecular weight (3). Among the latter, sucrose density gradients separate soluble proteins of the human thyroid gland into fractions of values 3–8, 12 and 27S (4, 5). The 19S fraction, thyroglobulin, is a 669, 000-molecular weight glycoprotein and the 27S fraction is considered its dimer (6, 7).

The present work establishes that thyroglobulin and its dimer differ in isoelectric point. By means of isoelectric focusing (electrofocusing) on an acrylamide-gel support (8) the isoelectric point of the dimer is found to be at a lower pH than that of thyroglobulin. The relevance of this finding to the structure, iodination, and affinity to thyroid proteinases of the two iodoproteins is discussed.

Materials and Methods. Preparation of the 19 and 27S iodoproteins. Human thyroid tissue obtained from surgery, distant from the lesion, was homogenized in a pH 7.4 Tris–HCl buffer (0.05 *M* Tris–HCl in 0.1 *N* NaCl) then centrifuged at 4° for 1 hr at 4,000*g*. The supernatant was loaded on a 5–20% sucrose density gradient in a Spinco Model L2 centrifuge, in a rotor SW 65 and spun at 125,000*g* for 5.5 hr.

To prepare ^{125}I-labeled iodoproteins, the fragments obtained at surgery were organ-cultured as previously described (9). The culture was continued for 3 days in NCTC-109 medium plus 5% horse serum. Twenty-four hr before the end of the culture 15 μCi of ^{125}I/ml were added to the culture medium. The rest of the procedure was the same as for the unlabeled iodoproteins with the exception that after the first centrifugation an overnight dialysis of the supernatant ensued against the pH 7.4 buffer.

Gel electrofucusing was performed using 3–10 Ampholine (LKB) on a 5% polymerized acrylamide-gel support, in the apparatus described by Davis (10). The gel consisted of two sections; one containing the protein, which comprised the part of the gel that, after electrofucusing, ranged in pH from about 8.5 to 4.0, and the other part of the gel, which had pH values below 4.0 after electrofocusing. To prepare the gels for electrofocusing, the volume of the 0.6-cm i.d. × 12-cm length tubes which was occupied by the gel-Ampholine mixture with a pH less than 4.0 after electrofocusing was determined. This volume (1.7 ml) was filled with gel mixture, catalyzer, and Ampholine, and then was polymerized. The protein sample in 0.3 ml of the Tris–HCl buffer (pH 7.4) was mixed with the gel mixture, the catalyzer and the Ampholine, made up to 1.3 ml and polymerized on top of the first gel section. This two-step loading procedure was used since 19 and 27S at a pH below 4.0 would not migrate towards the anode. The gels were then electrofocused for 72 hr at 4°, with 0.4% ethanolamine in the upper reservoir in contact with the protein-carrying section of the gel and 0.2% sulfuric acid in the lower reservoir. Seventy-two hr was the minimal time required to obtain sharp bands in this preparation. The current was maintained at 0.5 mA/tube throughout the run. After the migration the tubes were fractured in a steel vise, the gels were liberated and fixed in 5% trichloroacetic acid. The fixation was omitted

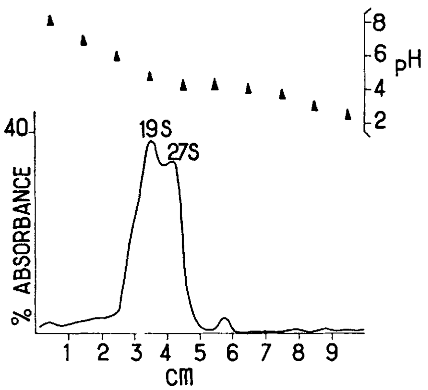

FIG. 1. Densitometric profile of a stained gel electrofocusing of 19 and 27S thyroid iodoproteins. 0.5 mg of each 19 and 27S were electrofocused for 72 hr at 4° at a current of 0.5 mA. The small peak to the right of the 27S peak corresponds to the boundary between the two sections of the gel. The cathodic end of the gel is at the left and the anodic to the right of the profile. (upper) pH curve obtained after electrofocusing control gels without protein.

when the bands of precipitation in the gels were cut to be rerun at pH 7.4 in acrylamide-gel electrophoresis (10) to check for the identity and integrity of the separated proteins. In every run, a pH curve was obtained from gels electrofocused simultaneously without protein, they were sectioned at 1 cm, and each section was immersed in 5 ml of distilled water overnight, and the pH of the water was measured in a pH meter. To count the ^{125}I activity present in the gels, they were fixed overnight in the acid, sectioned, and then counted in a well-type scintillation counter. To stain the gels, after the overnight fixation they were placed in 20% formaldehyde made 5% with trichloroacetic acid for 48 hr, then stained overnight with 0.5% acid black I in 7.5% acetic acid and finally destained in distilled water. The densitometric readings were performed in a densitometer Apelab (Apelab 92-Bagneux, France).

Results and Discussion. Fig. 1 shows the densitometric profile of the 19 and 27S iodoproteins subjected to electrofocusing and staining. Above it, the pH curve corresponding to this run shows the 19S peak coinciding approximately with a pH 4.5. The two components are definitively separable. The iodoproteins were obtained after repeated gradient centrifugation of the 19 and 27S fractions from fresh human thyroid tissue. (The smaller distinct peak shown to the right of the iodoproteins corresponds to the boundary between the two sections of the gels as described in Materials and Methods). To explore further this separation of the bands of isoelectric precipitation of the iodoproteins, the *in vitro* ^{125}I-labeled 19 and 27S were electrofocused. When the 19S fraction was run with a marker of 27S, two bands of precipitation were formed as indicated by the two peaks of Fig. 2B. Then, in Fig. 2C, the 27S fraction can be observed clearly separated from the contaminating 19S, which is the one close to the cathode. Finally, in Fig. 2D, both the 19 and 27S fractions were added together in the same amount that each one was present in the two preceeding gels, again two distinct peaks are shown where the amount of radioactivity present was additive. A pH curve is depicted in Fig. 2A, obtained simultaneously with this run. The unevenly distributed counts seen between the cathode and the peak of 19S were always at a low level and their pattern was never reproducible. The identity and integrity of the proteins precipitated in each band was demonstrated by rerunning the homogenized gel containing the band of isoelectric precipitation in acrylamide-gel electrophoresis, where repeatedly 19 and 27S were, respectively, over 90 and 80% pure.

These observations show that 19 and 27S differ in another characteristic: their isoelectric point. Ui *et al.* (2) separated these iodoproteins in DEAE columns by increasing ionic strength eluants, 27S appearing well after 19S. This would indicate a difference in total net charge between these proteins at a given pH. Moreover, the same authors, as well as others (7), found that the iodine content of 27S was higher.

It is not probable that the only specific cause for the discrepancy in isoelectric point of 19 and 27S is due to the further iodination of the tyrosyl residues, with the drop in p*K* of the phenolic hydroxyl groups which this entails (though always remaining well above the pH where the isoelectric precipitation of 19 and 27S takes place) (11). If increasing degrees of iodination would modify the isoelectric point to any extent and as 19S is usually heterogeneous in its iodination (12, 13), the sharp peaks of radioactivity found

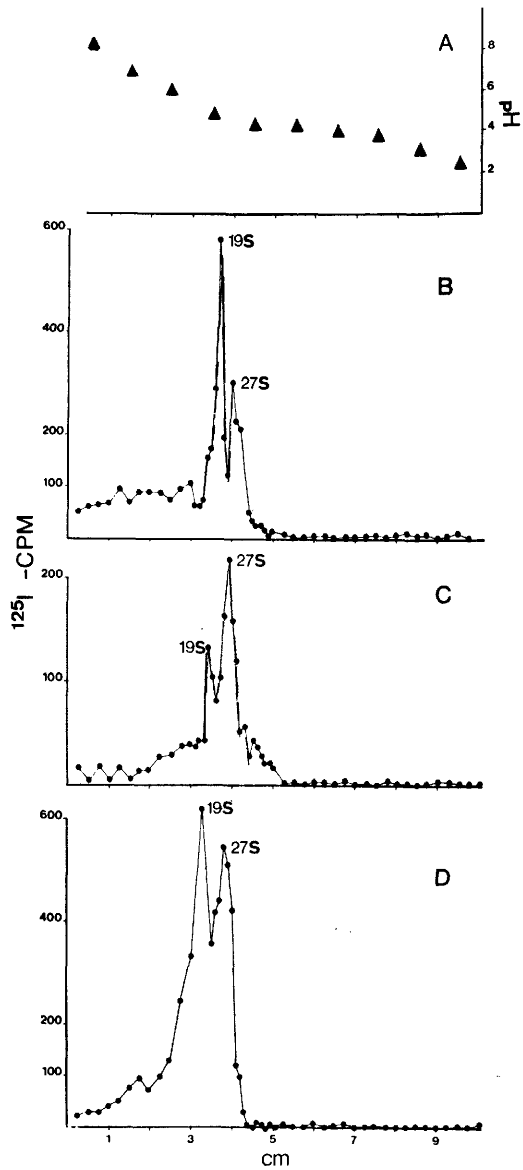

FIG. 2. Radioactivity profile of the electrofucusing of ^{125}I-labeled 19 and 27S thyroid iodoproteins: (A) pH curve obtained after electrofocusing control gels without protein; (B) radioactivity profile of 19S plus a 27S marker; (C) radioactivity profile of 27S; (D) radioactivity profile of 19 plus 27S, each one in the same amounts as in (B) and (C), plus same amount of 27S marker as in (B). The cathodic end of the gel is at the left and the anodic to the right of the profile.

at the point of isoelectric precipitation of 19S in Figs. 2B, C, and D could hardly be obtainable. Moreover the change in iodination from the 27S fraction to the most iodinated fraction of 19S is smaller than the change between the latter and the least iodinated of the 19S molecules (2, 13). Also it has been described that a high level of iodination of 19S brings an abrupt change in conformation (14), however this degree of iodination has not been reported for native 27S. It is more likely that the increased content of sialic acid in 27 over 19S (12) could play a role in their difference in isoelectric point, [the pK_a of the 3 carboxylic groups of sialic acid are at pH 2.6, 2.6, and 2.75 (15)].

Alternatively, the process of dimerization might expose different ionizable groups either by altering the tertiary structure of the two 19S molecules [as substrate-enzyme attachment would alter the conformation of the latter (16)] or by masking an uneven number of opposite charged groups in the apposed surfaces, leaving in either case, a different net charge on the surface of the dimer as compared to 19S.

This difference of net charge on the surface of 19 and 27S could also promote a different affinity of the iodinated proteins as substrates towards the thyroidal proteinases responsible for liberating the thyroid hormones. This, in view that 27S has a higher lability at 35 and 40° and to different denaturants (17) as compared to 19S, and according to some authors a higher content of thyroxine than the monomer (7), could suggest that the dimer is a better substrate and hormone source.

Summary. The 19S thyroid soluble iodoprotein (thyroglobulin) differs from the 27S thyroid soluble iodoprotein, its dimer, in isoelectric point. Purified 19 and 27S obtained from extracts of human thyroids after surgery or after *in vitro* culture of the same material, when submitted to isoelectric focusing showed distinct bands of precipitation. These results are interpreted to mean that the total net charge available on the surface of the dimer is different from that of the monomer. The implication of this finding to the structure, iodination, and possibility of

recognition by specific proteinases of the dimer is discussed.

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