Evaluation of the Homogeneity of Several Thyroglobulin Preparations*

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A technique for the preparation of thyroglobulin by fractional precipitation with either ammonium sulfate or potassium phosphate buffer was described in 1948 by Derrien et al. (1). These preparations were found to be homogeneous by boundary electrophoresis, but heterogeneous in their salting-out curves (1) and in the ultracentrifuge (2, 3). In the ultracentrifuge, a main component with a sedimentation coefficient of 19 S was seen, as well as small amounts of both faster and slower sedimenting components.

The heterogeneous appearance of these thyroglobulin preparations could be due to the presence of contaminating proteins. However, since it has been demonstrated that the thyroglobulin molecule will undergo dissociation under certain conditions of pH and ionic strength (3, 4, 5), it is also possible that the heterogeneity is due to dissociation and aggregation phenomena.

The present study was undertaken to evaluate whether this heterogeneity is physical or chemical in nature, as well as to explore other procedures for the preparation of thyroglobulin. The homogeneity of the various preparations obtained was evaluated by starch gel electrophoresis and by ultracentrifugation, since it was found that heterogeneity was detected only by these techniques, and not by boundary electrophoresis, paper electrophoresis, or starch column electrophoresis. It was possible to demonstrate that the components seen by starch gel electrophoresis correspond to those seen in the ultracentrifuge. The preparative procedures studied included, in addition to phosphate fractionation, alcohol fractionation, DEAE-cellulose chromatography, ultracentrifugation techniques, and elution from starch gels after electrophoresis.

The information obtained from these studies indicated that the heterogeneity present in thyroglobulin preparations is physical in nature and based on differences in molecular size. It is likely that dissociation and aggregation of the main thyroglobulin component are responsible for this heterogeneity and that these processes are accelerated by dialysis against distilled water and lyophilization.

EXPERIMENTAL PROCEDURES

Phosphate Fractionation—Sheep thyroid glands were brought on ice to the laboratory, dissected from the surrounding tissues, frozen, and sliced thinly in the frozen state. The slices were extracted with 0.9% NaCl overnight with stirring at 5°. After centrifugation of the mixture at 78,000 X g for 1 hour in a Spinco preparative ultracentrifuge, fractionation of the supernatant fluid was carried out by the method of Derrien et al. (1) at 0°. Potassium phosphate, 3.5 m, pH 6.7, was added to the NaCl extract to achieve a final concentration of 1.68 m, and the precipitate was collected by centrifuging at 78,000 X g for 30 minutes. The precipitate was then dissolved in 1.5 m phosphate buffer, pH 6.7, and the centrifugation repeated. This procedure was carried out two additional times. The fourth 1.68 m precipitate was collected, dissolved in a small amount of distilled water, dialyzed extensively against distilled water, lyophilized, and stored in the dry state in the cold.

Thyroglobulin was also prepared from calf thyroids by this procedure and from human thyroids by fractionating between 1.64 m and 1.96 m phosphate. Most of the studies to be reported were carried out on sheep thyroglobulin.

Alcohol Fractionation—The alcohol fractionation procedure which was employed was based on Cohn method 10 (6) and was carried out at -5°. A 0.9% NaCl extract of thyroid slices was prepared as above and used as the starting material. Both biuret (7) and iodine (8) determinations were performed on the fractions obtained. After precipitation of a small amount of protein at an alcohol concentration of 20% in the presence of Na⁺ ion, 0.037 ionic strength, pH 5.8, the bulk of the thyroid proteins was precipitated at pH 5.4, 0.0185 M Zn²⁺, 0.034 M Na⁺, 20% alcohol. This second precipitate was reprecipitated in a solution containing 0.01 M Ba²⁺, 0.02 M Na⁺, and 16% alcohol at pH 5.6. After centrifugation, a small amount of protein with a low iodine content was found in the supernatant fluid. Treatment of the remaining precipitate with a solution containing 0.0942 M Zn²⁺, 0.012 M Na⁺, and 13% alcohol at pH 6.5 followed by centrifugation, brought into solution a fraction rich in iodine. This protein was reprecipitated by adjusting the solution to 25% alcohol, 0.008 M Zn²⁺, and 0.03 M Na⁺. This precipitate was taken up in sodium citrate, dialyzed extensively against distilled water, and then lyophilized.

Centrifugal Fractionation—Phosphate-purified thyroglobulin, dissolved in 0.1 M KNO₃ at a concentration of 25 mg per ml, was centrifuged in the Spinco preparative ultracentrifuge exactly as described by Edelhoch (3). The middle fraction obtained from the second centrifugation was studied by starch gel electrophoresis in three ways: directly without further treatment; after dialysis against distilled water; and after dialysis against distilled water and lyophilization.

Solubility Curves—Solubility curves were determined for thyroglobulin prepared by both phosphate precipitation and alcohol
fractionation. A thyroglobulin solution containing 20 mg was pipetted into each of a series of Lusteroid centrifuge tubes. Aliquots of potassium phosphate solutions, pH 6.7, were added to achieve final salt concentrations of 1.2 to 1.8 M. All of the protein precipitated over this range. The tubes were equilibrated in an ice bath and were then centrifuged at 100,000 × g in the Spinco preparative ultracentrifuge at approximately 0° for 30 minutes. The supernatant fluid from each tube was diluted appropriately and the optical density at 280 μm was measured.

Starch Column Electrophoresis—Starch column electrophoresis was carried out by the technique of Fodin and Porath (9). Columns 35 × 2.5 cm were packed with potato starch in 0.02 M borate buffer, pH 8.0, and washed with the buffer before use. Fifty to 100 mg of the phosphate-purified thyroglobulin were applied in the buffer. During electrophoresis, the direction of migration of the protein was downward into the column. The column was immersed in an 8-liter cylinder filled with buffer which served both as an electrode chamber and as a means of cooling the column. The top of the column was connected by a standard taper, U-shaped tube to a second cylinder which served as the cathode chamber. Electrophoresis was run at 5°, 400 volts, 20 mA, for 22 or 48 hours. At the completion of the electrophoresis, a standard taper adapter with fritted glass disk was attached to the top of the column. The column was then inverted and attached to a fraction collector. Elution of the protein was carried out with the borate buffer at the rate of 20 ml per hour, and 1-ml fractions were collected and analyzed for their protein content.

Starch Gel Electrophoresis—Starch gel electrophoresis was run by both the horizontal and vertical methods of Smithies (10, 11). The conditions usually employed were 0.025 M borate buffer, pH 8.6, 150 volts for the horizontal technique, or 180 volts for the vertical, for 18 hours at 5°. The gels were then stained with amidoschwarz 10B in methanol-acetic acid. Starch gels from which protein was to be eluted were treated somewhat differently. They were run by the horizontal technique, since it was possible to electrophorese larger quantities of protein in this manner, and the electrophoresis was carried out either at room temperature for 16 hours or at 13° for 48 hours. Both of these procedures yielded a wider separation of the protein bands than was obtained by the usual conditions of 18 hours at 5°. Only a small strip of the gel was stained, and the unstained portion of the gel was cut in sections corresponding to the bands seen on the stained strip. Protein was eluted from these sections by electrodialysis in a manner similar to that employed by Moretti et al. (12).

Chromatography on DEAE-Cellulose Columns—DEAE-cellulose (Brown Company, Berlin, New Hampshire) was washed before use with 0.2 M K₃HPO₄ followed by 0.01 M potassium phosphate buffer of the pH at which the column was started. A 0.9-cm-diameter column packed with 2 g of DEAE-cellulose was used for 15 to 20 mg of the phosphate-purified thyroglobulin, and a column 2.5 cm in diameter containing 12 g of DEAE-cellulose was used for 150 to 200 mg of the protein. The protein was applied in 0.01 M potassium phosphate, pH 8 or pH 6.7, and washed in with the buffer. Linear gradients, employing the 0.01 M phosphate buffer in the mixing chamber, were used for eluting the protein. The specific conditions used for each column are described in the legend of Fig. 6. All columns were run at 5°.

The eluate was collected in fractions of 3 ml (2-g column) or 10 ml (12-g column). The protein content of the eluates was determined either by the method of Lowry et al. (13) or from the optical density at 280 μm. The absorbancy of a 1% solution of sheep thyroglobulin at 280 μm, 1-cm light path, was 10.4. When eluates from the DEAE-cellulose columns were subjected to electrophoresis, ultracentrifugation, or trypsin digestion for the preparation of peptide maps, they were first pooled, dialyzed extensively against distilled water, and then lyophilized.

It should be noted in reference to the chromatography of thyroglobulin on DEAE-cellulose that the capacity of this resin for thyroglobulin is much lower than the capacity reported for hemoglobin by Peterson and Sober (14). In the present study it was found that 1 g of the resin will retain approximately 50 mg of thyroglobulin. The amount of resin used in these experiments was therefore a 3-fold to 6-fold excess.

Ultracentrifugal analyses were run in a Spinco model E analytical ultracentrifuge. Sedimentation coefficients were corrected to water at 20°. The moving boundary electrophoretic analyses were carried out in a Perkin Elmer apparatus, with the cell cooled with ice, at 190 volts, 9 mA of current. The protein samples were dialyzed against 2 liters of the buffer used for 16 hours before each analysis.

Peptide Patterns—Peptide patterns were obtained on trypsin digests of the phosphate-purified thyroglobulin, as well as of DEAE-cellulose column eluates, by the two-dimensional chromatography-electrophoresis technique described by Katz et al. (15). Crystalline trypsin (Worthington Biochemical Corporation, Freehold, New Jersey) was added in an amount equal to 1% of the protein to be digested. During the digestion the pH was maintained at approximately 8.0 by the addition of small amounts of NaOH. The course of the reaction was followed by the ninhydrin method of Moore and Stein (16), and the reaction was terminated when a plateau was reached with one addition of enzyme. An aliquot equivalent to 4 to 8 mg of the protein was concentrated to dryness under nitrogen and applied in water to Whatman No. 3 paper. Chromatography was carried out in the first dimension in butanol-acetic acid-water, 4:1:5. This was followed by electrophoresis in the second dimension in pyridine-acetic acid-water, 1:10:289, pH 3.5, at 2000 volts for 75 minutes. The sheets were stained with 0.2% ninhydrin in acetone, with heating.

RESULTS

Electrophoretic Behavior of Thyroglobulin Preparations—When phosphate-purified sheep thyroglobulin at a concentration of 1.5 per cent was studied by boundary electrophoresis in barbital-citrate buffer, pH 8.6, 0.1 ionic strength, only one component was detected (Fig. 1). At this protein concentration it should have been possible to detect small amounts of proteins with different electrophoretic mobilities. Similarly, by paper electrophoresis in the same buffer, the preparation appeared homogeneous.

Thyroglobulin prepared by phosphate fractionation was further studied by starch column electrophoresis, since this method is considered to exhibit greater resolving power than boundary electrophoresis for components with very similar electrophoretic mobilities. Again, after electrophoresis for either 22 or 48 hours, only a single component was observed in the elution pattern (Fig. 2). Since the recovery of protein eluted from the column...
since the same picture was also obtained when the electrophoresis iodinated protein in each band.

exactly to the protein bands seen, indicating the presence of thyroglobulin was prepared from tissue labeled in vivo with I^131, as calf and sheep thyroglobulin. In addition, when human also indicated three different sized components, it seemed reason-

able that there was a correspondence between the components as seen by the two techniques. This was verified by a combination of density gradient centrifugation and starch gel electrophoresis. A 0.5-ml sample of phosphate-purified thyroglobulin at a concentration of 40 mg per ml in 0.1 M NaCl was layered on top of a discontinuous density gradient. The density gradient was formed by layering 2 ml each of 6, 8, 10, 12, and 14% sucrose in an 11-ml Lusteroid tube. All of the sucrose solutions also contained 0.1 M NaCl. After centrifugation at 97,000 X g in the Spinco preparative ultracentrifuge for 90 minutes, 0.5-ml layers were successively removed from the top of the tube by pipette, and the optical density at 280 m\(\mu\) was determined. All of the samples containing protein were studied directly without further treatment by starch gel electrophoresis. In 90 minutes the proteins had sedimented through the gradient to the extent that the optical density at 280 m\(\mu\) was maximal at a level 5 ml below the top of the tube and decreased on each side of this peak.

By starch gel electrophoresis all of the layers were shown to contain the middle band or main component of the phosphate-purified thyroglobulin. In addition, the upper layers also contained the faster migrating component of the starch gels and none of the slower component. The lower layers showed increasing amounts of the slower migrating component of the starch gels, and none of the fast. The samples from the middle layers contained protein corresponding to the middle band on starch gel and only traces of the other two components. This indicates that the fast band seen in starch gel electrophoresis corresponds
observed in their salting-out curves. Neither preparation gave a smooth salting-out curve, and when the change in optical density of the supernatant fluid per change in molarity of phosphate buffer was plotted, several regions of precipitation were observed in both curves.

**Elution of Protein from Starch Gels**—Attempts were made to isolate a more homogeneous preparation of thyroglobulin by elution of the middle band seen in starch gels from unstained gels. All such attempts resulted in preparations still having the three-banded pattern when examined again by starch gel electrophoresis. It was furthermore shown that when unstained starch gels containing sheep thyroglobulin purified by phosphate fractionation were cut in sections corresponding to the three bands seen in the stained portion of the gel, and the protein eluted from each of these sections and rerun separately in a second starch gel, the pattern seen in Fig 4 was obtained. Regardless of which section of the gel was eluted, the protein obtained gave a similar pattern. The middle band was always the heaviest, but small amounts of the protein of the other bands were also apparent. This indicated an interconversion of the proteins present in the three bands during the elution procedure.

**Centrifugally Fractionated Thyroglobulin**—Since starch gel elution experiments had indicated an interconversion of the three components of phosphate-purified thyroglobulin, it was thought desirable to obtain a preparation of thyroglobulin essentially free of the minor components so that the reasons for this interconversion could be elucidated. At that stage of the work a technique for obtaining an essentially homogeneous thyroglobulin preparation by repeated centrifugation was described by Edelhoch (3). Use was made of this procedure to obtain a thyroglobulin preparation containing only traces of the fast and slow components when studied by starch gel electrophoresis. Similarly, the density gradient centrifugation technique described earlier in the present report was also used to obtain a similar more homogeneous preparation (Fig. 5-1). It was observed with both of these centrifugally fractionated thyroglobulin preparations that dialysis against distilled water at 5°C for 18 hours markedly increased the amount of the fast migrating component seen on starch gels (Fig. 5-2). When the dialyzed, centrifugally fractionated thyroglobulin was lyophilized, in addition, starch gel electrophoresis showed an additional increase in both the fast and slow components (Fig. 5-3). It can be seen, therefore, that when centrifugally fractionated thyroglobulin was examined directly by starch gel electrophoresis without further treatment, it was considerably more homogeneous than the phosphate-purified thyroglobulin. However, if it was first dialyzed against water and then lyophilized, it then demonstrated the same three-banded pattern as phosphate- or alcohol-fractionated thyroglobulin.

Since all of the other preparations reported in this paper were subjected to dialysis and lyophilization, it is likely that these processes were responsible for the appearance of the three-banded pattern on starch gel electrophoresis.

**Chromatography on DEAE-Cellulose Columns**—The elution pattern obtained when thyroglobulin purified by phosphate fractionation was applied to DEAE-cellulose columns was reproducible with a given elution gradient, but markedly different patterns were obtained with the different buffers employed. Approximately 94% of the protein applied was eluted from the column. The pattern obtained when both salt concentration and pH were changed simultaneously is shown in Fig. 6A. Because of the incomplete separation of peaks obtained with this
gradient, use was made of gradients employing constant pH and increasing salt concentrations. The pattern obtained at pH 8 with increasing phosphate concentration is shown in Fig. 6B and with increasing benzoate concentration at pH 8 in Fig. 6C. Because of the possibility of protein denaturation on exposure to the resin at the somewhat alkaline pH of 8 (18), elution was also performed at pH 6.7 with increasing phosphate concentration (Fig. 6D).

The chromatography of thyroglobulin on DEAE-cellulose was carried out before the above mentioned experiments which indicated interconversion of the three components seen in starch gels and in the ultracentrifuge. The eluates were therefore examined by starch gel electrophoresis and in the ultracentrifuge to determine whether any separation of the three components had occurred. Figure 7A shows the ultracentrifugal pattern for phosphate-purified thyroglobulin, and the patterns obtained from several DEAE-cellulose peaks are shown in Fig. 7B through 7F. The sedimentation coefficients calculated from these are presented in Table I. All of the peaks yielded patterns similar to that of the phosphate-purified thyroglobulin, except Peak 1, Column C, in Fig. 6, which had small amounts of two additional components. None of the DEAE-cellulose peaks showed only a single component in the ultracentrifuge. Peaks 1, 2, 3, 4, 5, 6, and 10 from Column D of Fig. 6 were studied by starch gel electrophoresis. Each showed the same three bands obtained with the phosphate-purified thyroglobulin. None of the peaks exhibited only one band in the starch gel. Since the gradient used was a very gradual one, it is likely that if the two minor components present in phosphate-purified thyroglobulin were chemically distinct proteins, they would have been eluted specifically so that they would have been present in some peaks and absent in others. Instead, their behavior was identical with that of the main component, and they were present to the same extent in all of the column eluates. It is of interest that the peak studied from the column run at pH 8 had two additional components in its ultracentrifugal pattern, with sedimentation coefficients of 12 S and 8 S. These are similar to components reported by Edelhoch (3) at alkaline pH values, and may have been produced by exposure of the protein to the resin at the slightly alkaline pH of 8.0.

The primary reason for investigating DEAE-cellulose chromatography in the present study was to determine whether the three components of purified thyroglobulin preparations could be separated by this technique. The fact that they are not is consistent with the other data presented which indicates that all three represent different physical forms of the thyroglobulin molecule. However, since the elution patterns obtained showed such a marked degree of heterogeneity, it was considered of interest to attempt to gain some information concerning the cause of this heterogeneous appearance. Extensive denaturation was considered unlikely, since the protein in the eluted fractions had the same behavior in the ultracentrifuge and in starch gels as the phosphate-purified thyroglobulin applied to the column. Peptide patterns were next obtained on the tryptic digests of phosphate-fractionated thyroglobulin and of Peaks 1, 5, 7, 8, and 9 of Column D, Fig. 6. Fig. 8A shows the peptide pattern obtained from phosphate-purified thyroglobulin, and Fig. 8B shows the pattern obtained from Peak 9. All of the patterns obtained from the peaks were similar to the pattern from the phosphate-purified thyroglobulin. This indicates that the peptide structure of the protein in each of these peaks is essentially the same.

A possible explanation for the heterogeneity of thyroglobulin on DEAE-cellulose columns could be its elution as several different sized units. The low ionic strength medium in which proteins are applied to the column would favor the dissociation of the thyroglobulin molecule (3), whereas the increasing salt concentration of the gradient would favor its reassociation. The smaller units would be eluted at lower ionic strengths than the large units, since their total charge would be smaller (19). Such dissociation-association equilibria have been proposed to explain the chromatographic heterogeneity of hemoglobin (19).
Homogeneity of Thyroglobulin Preparations

**TABLE I**

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<th>Sedimentation coefficients of phosphate-purified sheep thyroglobulin and DEAE-cellulose column peaks</th>
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* An additional component with a sedimentation coefficient of 6 S, which comprised a very small percentage of the total protein, was observed in more concentrated solutions. At the concentration used in all of the experiments reported in this table, this component was not present in sufficient quantity to permit measurement of its sedimentation coefficient.

† The sedimentation coefficient of this component at infinite dilution was $S_{20, w} = 19.7$ S. This peak accounted for approximately 90% of the protein of the preparation.

**Fig. 8.** Tracings of peptide patterns of trypsin digests (A) of phosphate-purified thyroglobulin and (B) of Peak 9 from DEAE-cellulose Column D, Fig. 6. Chromatography in butanol-acetic acid-water, 4:1:5, to the right, followed by electrophoresis at pH 3.5. Origin in lower left corner, negative pole at the top.

Another factor influencing the chromatographic behavior of thyroglobulin could be its iodine content. It is likely that thyroglobulin isolated from pooled thyroid glands represents a spectrum of molecules of different iodine content. Since the pH of the phenolic hydroxyl of the iodinated tyrosines is lower than that of tyrosine, molecules with a greater iodine content would have a greater number of negative charges in the pH range in which the chromatography was carried out and would tend to be eluted at higher ionic strengths. When iodine determinations were performed on the eluate from several columns, it was found that the fractions emerging last had a higher iodine content. For example, when iodine determinations (8) were performed on the seven main peaks of Column B, Fig. 6 (Tubes 187 through 223), the amount of iodine present was found to be 0.01, 0.73, 0.74, 0.97, 1.0, 0.98, and 1.0%, respectively. However, evidence that iodine content alone was not responsible for the complex elution pattern of this column was obtained by rechromatography of pooled Tubes 202 through 206 (on a 2-g DEAE-cellulose column with a gradient from 0.01 to 0.2 M potassium phosphate, pH 8.0). Instead of emerging as a single peak on rechromatography, the protein was eluted over a wide range of salt concentration as many peaks.

If the behavior of thyroglobulin on these columns is considered a result both of dissociation-association phenomena, as well as of differences in the extent of iodination, elution patterns as complex as those actually obtained can be imagined.

**DISCUSSION**

In their original publication on the preparation of thyroglobulin by salt fractionation, Derrien et al. (1) noticed heterogeneity in the salting-out curves of this protein. Since all of the fractions which they obtained had similar iodine to nitrogen ratios, they concluded that the protein was chemically homogeneous and that its behavior could be explained by an aggregation of the molecules in strong salt solutions. It is particularly tempting to accept such an explanation for the heterogeneous behavior of thyroglobulin in the ultracentrifuge and in starch gel electrophoresis as well, since the dissociation of thyroglobulin has been reported by several investigators. Heidelberger and Pedersen (4) reported in 1956 that at pH 3 and pH 12, the thyroglobulin molecule appears to undergo dissociation. Lundgren and Williams (6), and more recently O'Donnell et al. (2) reported the transformation of the native thyroglobulin molecule to another form, termed alpha, in solutions of low ionic strength. Ekehloch (3) has further studied this transformation and has reported that it involves a dissociation of the thyroglobulin molecule. He was able to achieve this dissociation at alkaline pH values or by the addition of dodecylsulfate. Although he reported that, under the conditions which led to dissociation of the 19-S component, the 28-S component also disappeared, his paper does not establish whether the minor components seen in thyroglobulin preparations represent aggregated and dissociated forms of thyroglobulin or chemically distinct proteins.

The present study has indicated that the main component of phosphate-purified thyroglobulin and the two minor components differ primarily in their molecular size. Although they have similar electrophoretic mobilities, as indicated by boundary, paper, and starch column electrophoresis, they are separated by starch gel electrophoresis, which is known to act as a molecular sieve (17). In addition, a correlation has been presented between the ultracentrifugal behavior and the starch gel electrophoretic behavior of these components; the component sedimenting the fastest corresponds to the slow band on starch gel, whereas that sedimenting the slowest corresponds to the fast band in starch gels. The middle band in the starch gel corresponds to the S-19 component seen in the ultracentrifuge, and therefore to a molecular size of approximately 670,000 (3), whereas the other components would be larger and smaller than this.

It was of interest that strikingly similar starch gel patterns were obtained from phosphate-fractionated thyroglobulin from the three species studied, sheep, calf, and human, from alcohol-fractionated sheep thyroglobulin as well as from all of the DEAE-cellulose column fractions. This repeated occurrence of the three-banded pattern suggested a relationship between the three components seen, particularly since in the case of human thyroglobulin it was demonstrated that all three bands contained iodine. Information has been obtained by two separate techniques that interconversion of these components occurs. When each of the three bands was eluted separately from starch gels, dialyzed against distilled water, and lyophilized, and then
reexamined by starch gel electrophoresis, each again showed a three-banded pattern. Similarly, when more homogeneous samples of thyroglobulin, prepared by centrifugation techniques, were subjected to dialysis against water and then to lyophilization, the three-banded pattern was again obtained on starch gel.

The fact that it was possible to isolate a more homogeneous preparation by centrifugation would appear to be due to the fact that thyroglobulin and its dissociation products do not equilibrate rapidly, resulting in a relatively stable system (3). The centrifugation procedures, in contrast to elution from starch gels, or to chromatography on DEAE-cellulose, yielded thyroglobulin preparations in sufficient concentration to allow examination by starch gel electrophoresis without any intervening treatment which would subject the preparation to changes in ionic strength, as in dialysis, or to the energy changes which may occur during lyophilization. After elution from starch gel or from DEAE-cellulose, both dialysis against distilled water and lyophilization were carried out before the preparations were examined by starch gel electrophoresis. It has been demonstrated that these procedures produce a heterogeneous appearance in even the centrifugally fractionated material, and it is likely that they were also responsible for the heterogeneous appearance of the other preparations.

Preparation of thyroglobulin by centrifugal techniques is therefore of use only when it is desirable to study one physical form of the molecule under the very limited conditions which do not cause its transformation. Under other conditions it becomes as physically heterogeneous as thyroglobulin prepared by alternate techniques. For chemical studies, centrifugal fractionation has no advantages, since physical heterogeneity is of no importance in these studies. In addition, only very small amounts of protein are available by the centrifugal techniques.

Since the heterogeneity of phosphate-fractionated thyroglobulin has been shown to be physical and not chemical in nature, this preparation is a satisfactory starting material for procedures requiring large amounts of chemically homogeneous protein. This is particularly true in the case of structural studies, in which the large molecular size of thyroglobulin necessitates the use of substantial amounts of material. Such structural studies are presently being carried out in this laboratory on phosphate-fractionated thyroglobulin.

SUMMARY

The homogeneity of thyroglobulin prepared by phosphate fractionation, alcohol fractionation, chromatography on diethylaminoethyl (DEAE)-cellulose columns, and elution from starch gels after electrophoresis has been evaluated by starch gel electrophoresis. All of these preparations presented a similar three-banded starch gel pattern, with a strong middle band and two other faint bands. The heterogeneity seen in starch gels was correlated with that seen in the ultracentrifuge, where about 90% of the protein was found to have a sedimentation coefficient of 19 S, with small amounts of 6-S and 28-S components also present.

The three components observed by starch gel electrophoresis were shown to be interconvertible. Similarly, thyroglobulin obtained in a relatively homogeneous form by centrifugation techniques was demonstrated to undergo transformation during dialysis against distilled water and lyophilization, with the result that the three-banded pattern was again obtained.

Chromatography of thyroglobulin on DEAE-cellulose columns produced complex elution patterns. The elution peaks obtained were shown to have three bands on starch gel electrophoresis and were also shown, by the peptide mapping technique, to contain the same protein.

From the information obtained it was concluded that the heterogeneity seen in thyroglobulin preparations is not chemical but rather physical in nature, resulting from dissociation and aggregation of the molecule, and that these processes are accelerated by dialysis against distilled water and by lyophilization.

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