ISOLATION OF LIVER PROTEINS WITH ISOELECTRIC POINT 6.75 AND A STUDY OF SOME OF THEIR PHYSICOCHEMICAL PROPERTIES

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Two proteins corresponding in some of their physicochemical characteristics and antigenic properties of serum γG-globulin and its heavy chain or fragment, but with low stability in solution, were isolated by electrodecantation from a rabbit liver homogenate in buffer containing 2.5 M urea at the isoelectric point 6.75.

Comparison of the properties of electrophoretic fractions of tissue proteins with the serum proteins is interesting not only as a part of the study of specific organ proteins and proteins common to all organs, but also in connection with the study of serum proteins at the sites of their synthesis or breakdown. Tissue proteins with electrophoretic mobility corresponding to γG-globulin of the blood serum have been investigated, although the value of the results obtained is reduced by the fact that the whole fraction of protein heterogeneous as regards some of its properties was studied.

A method of obtaining tissue proteins with different isoelectric points (pI), and a rabbit liver protein with pI 6.75, corresponding to the isoelectric point of one fraction of serum γG-globulin, was isolated and its physicochemical and antigenic properties studied.

EXPERIMENTAL METHOD

The liver of a healthy rabbit weighing 2.5-3 kg was washed in the cold to remove blood by perfusion through the blood vessels with physiological saline a temperature not exceeding 2°C, followed by perfusion with cold phosphate buffer, pH 6.75 and ionic strength 0.2, containing 2.5 M urea (the principal buffer solution). The liver tissue was then triturated in the cold with quartz sand with gradual addition of 20-30 ml of the principal buffer solution until a homogeneous mass was obtained. The homogenate was centrifuged for 1 h in a refrigerator centrifuge at 10,000 rpm and 0°C. Using a multimembrane electrodecanter of the author's own design [5], a protein fraction which was stationary or had minimum mobility at pH 6.75 was isolated from the resulting supernatant (70-90 ml).

After preliminary cooling of the electrodecanter system, the supernatant was introduced as the bottom layer into the separating chamber up to half its volume, and the liquid was propelled with a peristaltic pump having a throughput of 5 ml/h. A voltage of the order of 35 V, giving a current of 0.3 A, was applied to the electrodes. Between 4 and 5 h later, a solution containing practically stationary protein at this pH began to flow out through the uppermost outlet pipe of the separating chamber. From the beginning of treatment of the liver to obtaining the purified protein, its temperature did not exceed 5°C.

The molecular weight of the protein was determined by gel-filtration on Sephadex G-200, with elution by phosphate buffer, pH 6.75 and ionic strength 0.1, containing 2.5 M urea. The column measured 150 × 1.2 cm. Preparative separation of the two fractions which appeared was carried out on a column measuring 70 × 4 cm.

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Electrophoresis in agar gel on slides was carried out in the buffer used to isolate the protein by electrodecantation.

The optical rotatory dispersion (ORD) was studied on a precision spectropolarimeter [6] in the waveband 579-296 nm.

The amino-acid composition of the protein was determined by chromatographic separation on paper [1]. The hydrolysate was treated by ascending chromatography 4 times: twice in a butanol–acetic acid–water (4:1:5) mixture and twice in the same mixture but with a different ratio of its components (4:1.5:2.5).

The antigenic properties of the isolated protein were studied by the microdiffusion method in agar gel. The gel was made up in the principal buffer solution. Precipitating serum against rabbit serum γG-globulin was obtained by immunizing guinea pigs with the latter as antigen.

EXPERIMENTAL RESULTS AND DISCUSSION

By electrodecantation 10-15 ml of a solution containing 0.5-1.2% of a protein with pI 6.75 and from 0.3 to 0.6% of carbohydrates was obtained from the tissue of one liver. The opalescent carbohydrate component was eluted in the free volume during gel filtration on Sephadex G-200, and the protein was separated into two fractions with molecular weight of 150,000 and 50,000 (from the gel-filtration results) (Fig. 1).

Electrophoresis in agar gel in the principal buffer, pH 6.75, corresponding to minimal mobility of the protein, revealed two distinct fractions, presumably because of endosmosis, in the zone of mobility of serum γG-globulin. The fraction with lower molecular weight is less affected by endosmosis than the protein with a molecular weight of 150,000. This is easily explained by the difference in molecular weight in the presence of a small difference in the value of pI. An increase in the vigorousness of the conditions used to isolate the protein from the liver homogenate, i.e., a decrease in the rate of passage of the fractionation mixture through the chamber with an increase in the strength of the current, did not result in only one component, although an almost pure fraction with the corresponding isoelectric point was obtained from serum γG-globulin even when milder conditions were used for the fractionation [4]. It was impossible to determine the isoelectric spectra of the resulting protein fractions because of their instability in ampholytes.

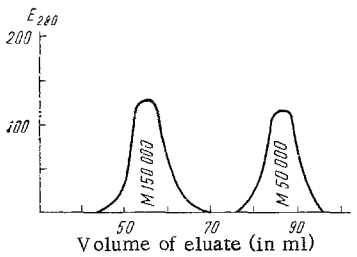

Fig. 1. Elution curve of two rabbit liver proteins isolated by electrodecantation at pH 6.75. Sephadex G-200 column, 150 × 1.2 cm.

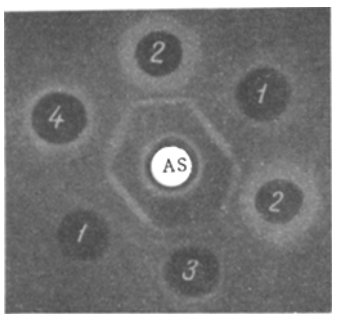

Fig. 2. Results of gel diffusion reaction between antiserum (AS) against rabbit serum γG-globulin and rabbit serum γG-globulin (1), total protein with isoelectric point 6.75 (2), protein left in solution after incubation of total protein at 38°C (3), and rabbit blood serum (4).

After preliminary separation on a Sephadex G-200 column, the ORD of the two fractions was studied. The fraction with molecular weight 150,000 had the characteristic ORD constants of serum γG-globulin: $\lambda_c = 203$, $a_0 = -300$, and $b_0 = 0$. The protein with molecular weight 50,000 had the values $\lambda_c = 213$, $a_0 = -346$, and $b_0 = +23$, most probably corresponding to the heavy chain of the γG-globulin molecule or to a fragment of it. These results are in good agreement with the hypothesis [7] of the possible existence of cellular antibodies, which can be represented as whole molecules of 7S-antibodies and as heavy chains joined instead of light chains to certain components of the cell membranes.

The isolated protein has comparatively low stability. Heating in an incubator at 38° causes clouding of the solution in 30-40 min and the formation of numerous floccules after 5-7 h, which usually do not fall as a precipitate. A solution with an apparent structure, which was lost on shaking, was thus formed. After brief centrifugation a precipitate was formed, containing up to two-thirds of the previously dissolved protein. The precipitate did not dissolve in high concentrations of urea, on increasing the ionic strength of the solvent, on addition of Ca, Mg, or other ions, and so on. Trypsin caused hydrolysis of the protein and solution of the precipitate. A preliminary study of the amino-acid composition of

the precipitated protein showed some increase in the hydrophobic and a decrease in the hydrophilic amino acids compared with serum γG-globulin. This probably is responsible for the low resistance of the protein in solution.

A preliminary increase in the urea concentration to 4 M prevented the formation of a precipitate on heating. A similar effect was produced by raising the pH to 9.6. A decrease in the urea concentration in the principal buffer solution led to a sharp decrease in the yield of protein, and the fraction with molecular weight 50,000 disappeared and there was a substantial decrease in the fraction with molecular weight 150,000. Investigation of the supernatant by gel-filtration and electrophoresis revealed that a protein belonging to the fraction with molecular weight 50,000 and some of the fraction with molecular weight 150,000 was thrown down into the precipitate.

The antigenic properties of the protein isolated from the liver homogenate corresponded to those of the serum γG-globulin (Fig. 2). After heating, with a decrease in the protein concentration in the supernatant, there was a decrease in the intensity of the microdiffusion reaction. This indicated precipitation of a protein corresponding in its antigenic structure to serum γG-globulin.

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