Short Communications

Separation of calf-lens proteins by means of vertical-column zone electrophoresis*

The classical concept of the protein composition of the crystalline lens is that of four different fractions, one insoluble, albuminoid, and three soluble, α-, β-, and γ-crystallin. However, recent investigations employing immunological and agar microelectrophoretical methods have shown that the protein composition of the lens is more complicated than was previously believed[1-3]. Consequently new methods must be applied to the purification of the different proteins, for which purpose chromatography on cellulose ion exchangers seems to be very promising. A preliminary report on the separation of lens proteins on DEAE-cellulose has already been published[4]. However, the complexity of the chromatographic pattern makes a suitable prefractionation method highly desirable. For this purpose the preparative zone-electrophoretic procedure of PORATH has been applied to the lens proteins. This method was found to have higher capacity and to give better resolution than the starch-block electrophoresis technique used by BLOEMENDAHL AND TEN CATE[5].

Materials and methods

Calf eyes were removed 30–60 min after the death of the animal and immediately put on ice. The lenses were removed, freed from the capsule and homogenized in a Potter-Elvehjem homogenizer in an 0.05 *M* Tris buffer, pH 8.0 (5 ml per 3 lenses). The homogenate was centrifuged for 45–60 min at 15,000 $\times$ *g*, and the supernatant was dialyzed for 24 h against three changes of 750 ml 0.05 *M* Tris buffer (pH 8.0). All preparative procedures were conducted at 4°.

Vertical-column zone electrophoresis with cellulose as supporting medium was used for the separation of the proteins[6,7]. The column, 3.2 $\times$ 100 cm, was packed with cellulose powder (Munktell AB, Sweden), suspended in Tris buffer. The packing was carried out with a positive pressure of 25–30 lb./in.^{2}. The void volume of the column was 610 ml as tested with a color band. 12 ml of protein solution with a concentration of approx. 8 % was introduced 15 cm into the column at a rate of 3–4 cm/h to compensate for the electroendosmotic flow. The electrophoresis was performed in 0.05 *M* Tris buffer (pH 8.0) with a potential gradient of 5 V/cm for 46 h at 0.5°. The current was approx. 45 mA. To obtain optimum separation it was found necessary to treat the column with 0.2 *M* NaOH between runs.

The column was eluted at 4° with a flow rate of 20 ml/h. The eluate was collected in 5-ml fractions, the absorbancies of which were measured at 280 mμ in a Beckman DU spectrophotometer.

The pooled protein fractions were concentrated by ultrafiltration through

Abbreviations: DEAE-, diethylaminoethyl-; Tris, tris(hydroxymethyl)aminomethane.

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collodion shells (Schleicher and Schüll, Keene, New Hampshire), and then dialyzed for 24 h against three changes of the buffer used in the following experiments.

Free electrophoresis experiments were performed in veronal–acetate buffer, I = 0.15, at 1° in an Aminco Portable Electrophoresis Apparatus using a 3-ml Tiselius cell. Ultracentrifugation was done at room temperature in a Spinco Model E analytical ultracentrifuge at 52,640 rev./min in a standard 4°-sector cell. The buffer used was 0.1 *M* phosphate (pH 6.9).

Protein concentrations were measured by the method of LOWRY *et al.*[8] with human serum as standard.

All buffers contained 10 % (v/v) of a saturated solution of 5.7-dichloro-8-quinolinol as a chelating agent.

Results and discussion

The zone electrophoretic separation is shown in Fig. 1. Three distinct fractions, denoted as Fractions I, II and III, were obtained. The pattern is reproducible, if the column is treated with NaOH between runs. An untreated column causes the disappearance of the third peak, probably because of an increased electroendosmotic flow. The recovery was approx. 85 % in all experiments. Evidently some adsorption of the protein to the cellulose occurred, which explains the large elution volume of Fraction III.

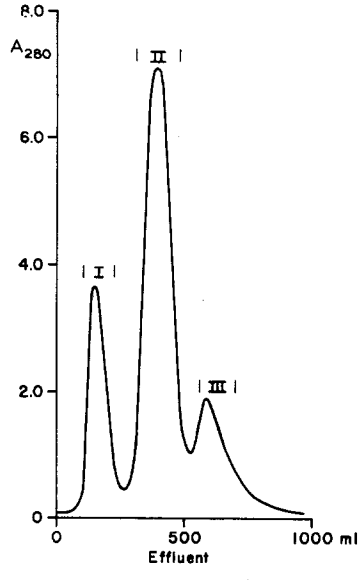

Fig. 1. Zone electrophoresis of calf-lens proteins in 0.05 *M* Tris buffer (pH 8.0); 46 h at 5 V/cm. Proteins with the highest mobility towards the anode are eluted first. The vertical lines indicate those parts of the fractions used for further analyses.

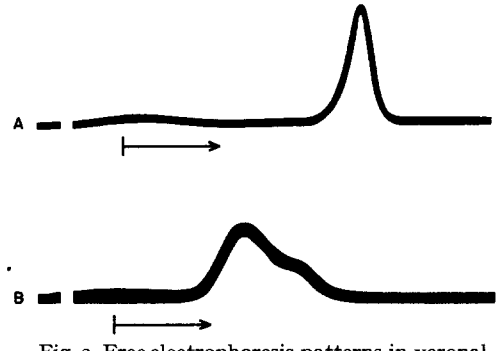

Fig. 2. Free electrophoresis patterns in veronal–acetate buffer, I = 0.15, pH 8.2. Current 8 mA, ascending boundaries; migration towards the anode. A: Fraction I, 21 mg/ml, 270 min, bar angle 20°. B: Fraction II, 15 mg/ml, 420 min, bar angle 45°. The vertical lines indicate the initial position of the boundaries and the arrows the direction of migration.

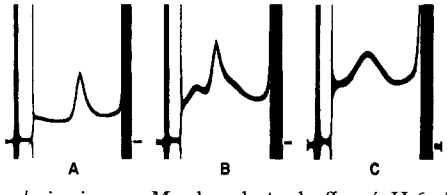

Fig. 3. Ultracentrifugation patterns at 52,640 rev./min in 0.1 *M* phosphate buffer (pH 6.9). A: Fraction I, 12 mg/ml, 24 min, phase-plate angle 60°. B: Fraction II, 24 mg/ml, 39 min, phase-plate angle 50°. C: Fraction III, 13 mg/ml, 167 min, phase-plate angle 35°. The times were measured after reaching full speed.

The fractions were pooled as indicated in Fig. 1, concentrated, dialyzed and subjected to electrophoretical and ultracentrifugational analysis.

The free-electrophoresis results are shown in Fig. 2. As can be seen, both Fractions I and II are negatively charged at pH 8.2. Fraction I is electrophoretically homogeneous at this pH, whereas Fraction II shows two peaks. Fraction III exhibited very low mobility at both pH 8.2 and 6.6. However, the protein partly precipitated in the electrophoresis cell during the run at the concentration and temperature used. The precipitate redissolved to a large extent at 6°. Because of this fact no definite conclusion can be drawn at present as to the electrophoretical homogeneity of Fraction III.

The ultracentrifuge patterns are shown in Fig. 3. Fraction I and III sediment as single components, but Fraction II shows considerable heterogeneity. In addition to the main peak it contains two smaller components, one with higher and the other with lower sedimentation velocity.

The sedimentation coefficients of Fraction I, which was both electrophoretically and ultracentrifugationally homogeneous, were measured at different concentrations in 0.1 M phosphate buffer (pH 6.9). By extrapolation of these values to infinite dilution a value for $S_{20,w}$ of 18,3 $\pm$ 0.2 was found.

Fraction I is the only fraction that is known to be homogeneous as judged from both electrophoresis and ultracentrifugation. Fraction III is homogeneous in the ultracentrifuge, but its electrophoretical homogeneity is uncertain. Fraction II is quite inhomogeneous. However, since the subsidiary components of Fraction II have sedimentation and electrophoretic characteristics distinctly different from those possessed by Fractions I and III, it is apparent that the three fractions can be completely separated from each other by the zone-electrophoretical method.

On the basis of the similarity of the zone-electrophoretic pattern to the paper-electrophoretic picture[9,10], and the agreement of the calculated sedimentation coefficient with that reported in the literature[11], it is concluded that Fraction I corresponds to α-crystallin, whereas Fractions II and III correspond to the β-, γ-crystallin complex.

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