DLOUHÁ, KEIL AND ŠORM[10] purified edestin in buffer containing urea, prepared its *S*-sulfoderivative, and concluded that the 300000 mol. wt. particle was comprised of 6 subunits, each of which consisted of two chains of mol. wt. 23000 and 27000. The solubilization of edestin as described here provides an alternate approach to purification without recourse to the use of urea for solubilization. Even though there is the possibility of artificial micro-heterogeneity induced by a multiplicity of derivatives containing various amounts of alanine, this need not interfere with the process of determining the nature of the subunits and the individual peptide chains.

Department of Chemistry, Tulane University and Seed Protein Pioneering Research Laboratory, Southern Utilization Research and Development Division, Agricultural Research Service, U.S. Dept. of Agriculture, New Orleans, La. (U.S.A.)

ALLEN J. ST. ANGELO
EDITH J. CONKERTON
JOSEPH M. DECHARY
AARON M. ALTSCHUL

1 M. SELA, N. LUPU, A. YARON AND A. BERGER, *Biochim. Biophys. Acta*, 62 (1962) 594.
2 R. R. BECKER AND M. A. STAHMANN, *J. Biol. Chem.*, 204 (1953) 745.
3 C. B. ANFINSEN, M. SELA AND J. P. COOKE, *J. Biol. Chem.*, 237 (1962) 1825.
4 D. A. TEWKSBURY AND M. A. STAHMANN, *Arch. Biochem. Biophys.*, 105 (1964) 527.
5 D. M. STOCKWELL, J. M. DECHARY AND A. M. ALTSCHUL, *Biochim. Biophys. Acta*, 82 (1964) 221.
6 D. BEN-ISHAI AND E. KATCHALSKI, *J. Am. Chem. Soc.*, 74 (1952) 3688.
7 D. A. I. GORING AND P. JOHNSON, *Arch. Biochem. Biophys.*, 56 (1955) 448.
8 W. J. EVANS, W. B. CARNEY, J. M. DECHARY AND A. M. ALTSCHUL, *Arch. Biochem. Biophys.*, 96 (1962) 233.
9 S. MOORE, D. H. SPACKMAN AND W. H. STEIN, *Anal. Chem.*, 30 (1958) 1185.
10 V. DLOUHÁ, B. KEIL AND F. ŠORM, *Collection Czech. Chem. Commun.*, 28 (1963) 2969.

Received January 14th, 1966

Biochim. Biophys. Acta, 121 (1966) 181–183

BBA 23215

Isolation and some properties of bovine α-crystallin

Since the time of MÖRNER[1] the soluble lens proteins have been classified into α-, β- and γ-crystallins. α-Crystallin has attracted special attention.

Various procedures for the purification of this protein have been described in the literature, *e.g.*, isoelectric precipitation, either alone[1] or combined with aethanol fractionation[2], salting out[3], and electrophoresis on starch-blocks[4]. Recently, a new method based on density gradient centrifugation was published[5].

This communication reports a new procedure for the isolation of α-crystallin from bovine lens. By means of immunochemical methods this preparation has been compared with α-crystallins obtained by other methods. Furthermore, the terminal amino acid residues and the ultraviolet absorption spectrum have been determined. The lens extracts were prepared as follows: Immediately after slaughter the eyes were removed and cooled. The lenses were taken out, washed with cold saline, and decapsulated. By gentle stirring for 1.5 h in ice-cold water (3 ml/g wet wt.) the peripheral parts of the lenses were dissolved.

The insoluble albumoid was removed by centrifugation at 78000 × *g* for 30 min and the opalescent solution was lyophilized.

In a typical experiment 1.2 g of the freeze-dried extract, dissolved in 12 ml 0.1 M Tris–HCl buffer (pH 7.3), was applied to the top of a column of Sephadex G-200 (Pharmacia A.B., Uppsala). The columns have a length of 100 cm, an internal diameter of 2.5 cm, and were prepared according to the directions of the manufacturer. As eluant 0.1 M Tris–HCl buffer (pH 7.3), containing 1 M NaCl and a 0.1 mM solution of EDTA, and brought to pH 7.3 with ammonia, was used. For good resolution this column length and buffer strength were found to be essential. The fraction collector used was operated on a time base.

The protein contents of the test tubes were measured by absorbance readings at 280 and 260 mμ in a Beckman DU spectrophotometer. The fractions were collected in the appropriate manner, their volumes were determined, and they were dialyzed and lyophilized.

Fig. 1 shows that in the elution pattern of 1.2 g of bovine lens cortex extract 6 peaks could be demonstrated. Agar- and immunoelectrophoretic analysis of the fractions present in these peaks revealed that the first peak contains α-crystallin with a trace of a β-crystallin. Peaks 2, 3 and 4 contain β-crystallins and Peak 5 includes pre-α-, some β- and the γ-crystallins. Peak 6 contains dialysable products of low molecular weight, such as peptides, amino acids and nucleotides. Essentially the same

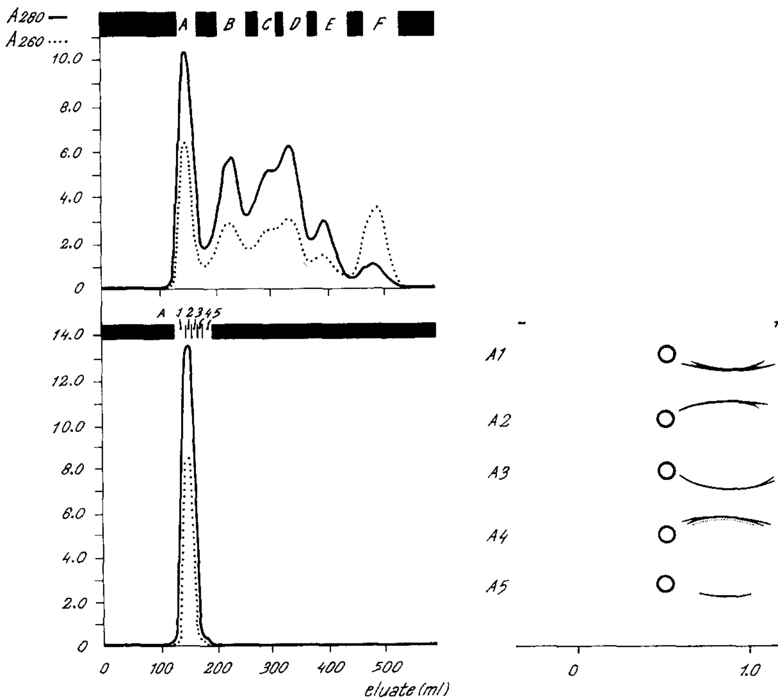

Fig. 1. (Upper) Gel filtration of extract from bovine lens cortex on Sephadex G-200; A, B, C, D, E and F indicate fractions used for further analysis. (Lower) Rechromatography of Peak A. For experimental conditions see text.

Fig. 2. Immunoelectrophoresis of the Fractions (A_1–A_5) after refiltration. Barbiturate buffer (pH 8.6, I = 0.05), 100 min, 6 V/cm. Rabbit antiserum against extract of lens cortex in the troughs. Relative mobilities (0–1.0) are indicated according to WIEME[11].

pattern was obtained when a column with an internal diameter of 4.5 cm was loaded with 8 g of the same extract.

The eluates of the first peak were rechromatographed on the same column. The top of the peak emerged after the same effluent volume as in the first elution procedure (Fig. 1).

The contents of the tubes were collected as indicated in the figure and investigated by immunoelectrophoresis (Fig. 2). In Fraction A only the α-crystallin lines were found, and no contamination by other lens proteins could be detected.

The phenomenon of the two almost concentric arcs, as seen in Fig. 2, has been encountered after immunoelectrophoresis of total lens extracts and of α-crystallins prepared by other methods. It indicates immunochemical heterogeneity of the α-crystallin, on which a more detailed study will be published later.

We have also compared immunochemically some α-crystallin preparations obtained by different fractionation procedures. The results are presented in Fig. 3. The preparations, obtained by salting out[3] and by starch block electrophoresis[4], respectively, contain a slight contamination of a faster component (pre-α-crystallin).

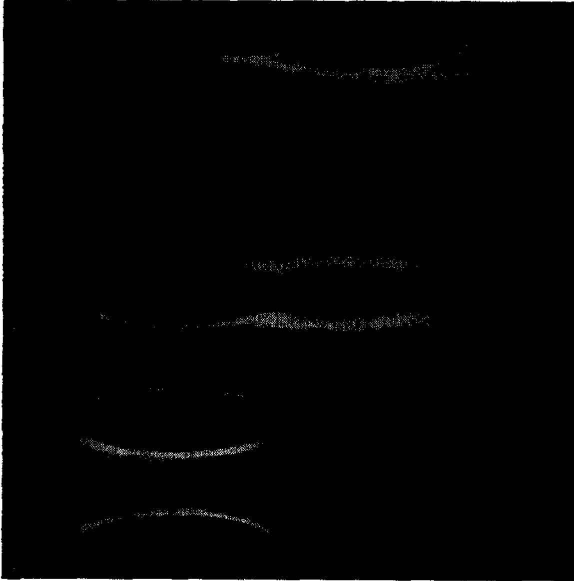

Fig. 3. Immunoelectrophoresis of lens cortex extract (a) and of α-crystallins from lens cortex prepared by salting out[3] (b), isoelectric precipitation[2] (c), starch-block electrophoresis[4] (d) and repeated gel filtration (e). Anode on the left. Rabbit antiserum against lens cortex extract in the troughs. Barbiturate buffer (pH 8.6, $I = 0.05$), 120 min at 6 V/cm, protein concn. 2 %.

The preparation obtained by isoelectric precipitation[2] also includes other lens proteins. However, α-crystallin prepared by gel filtration was immunologically homogeneous. The same results could be obtained with lens extracts from calves, horses and pigs.

The ultraviolet absorption spectrum was determined in a Beckman DU spectrophotometer. A maximum at 278 mμ was observed and an $E_{1\,\text{cm}}^{1\,\%}$ value at 280 mμ of 9.44 was calculated with the aid of dry-weight determinations. The ratio between the absorbance values at 280 and 260 mμ was 1.55.

Analysis of the N- and C-terminal residues was carried out by the fluorodinitrobenzene and carboxypeptidase methods, respectively, as described by FRAENKEL-CONRAT, HARRIS AND LEVY[6].

In agreement with BLOEMENDAL AND TEN CATE[4], FIRFAROVA[7], and HAEHNEL[8], we found glutamic acid to be the N-terminal group.

For the determination of the C-terminal amino acid residues (Procedure 3), products of the reaction at different time intervals ranging from 0.5 to 23 h were identified and estimated by treatment with FDNB.

Per 48000 g of anhydrous protein (uncorrected for losses), serine was identified to an extent of 0.8–1.0 mole, while glycine and glutamic acid did not exceed 0.08 mole.

Valine, alanine, leucine and threonine were present in still smaller amounts (0.04–0.03 mole). The value of 48000 corresponds with the molecular weight of the subunits of α-crystallin, as determined by BJÖRK[9] and by SPECTOR AND KATZ[10] by ultracentrifugal analysis.

Since α-crystallin has a mol. wt. of about 1 million[10] it can be tentatively concluded that this protein is composed of 20–25 subunits, each subunit having a C-terminal serine residue.

Our thanks are due to Mrs. M. G. J. VAN DAM, Mrs. M. GANZEMAN and Miss T. OPPELAAR for their skilful assistance. This work was supported by grants from the Dutch Organization for Pure Scientific Research (Z.W.O.).

Laboratory for Medical Anatomy and Embryology, State University, Utrecht (The Netherlands)

A. F. VAN DAM
G. TEN CATE

1 C. T. MÖRNER, *Z. Physiol. Chem.*, 18 (1894) 61.
2 J. FRANÇOIS, M. RABAEY AND R. J. WIEME, *A.M.A. Arch. Ophthalmol.*, 53 (1955) 481.
3 V. N. OREKHOVICH, K. F. FIRFAROVA AND V. O. SHPIKITER, *Ukr. Biokhim. Zh.*, 27 (1955) 355.
4 H. BLOEMENDAL AND G. TEN CATE, *Arch. Biochem. Biophys.*, 84 (1959) 512.
5 H. BLOEMENDAL, W. S. BONT, J. F. JONGKIND AND J. H. WISSE, *Biochim. Biophys. Acta*, 82 (1964) 191.
6 H. FRAENKEL-CONRAT, J. I. HARRIS AND A. L. LEVY, in D. GLICK, *Methods of Biochemical Analysis*, Vol. 2, Interscience, New York, 1955, p. 359.
7 K. F. FIRFAROVA, *Biokhimiya*, 23 (1958) 129.
8 R. HÄHNEL, *Arch. Ophthalmol.*, 163 (1961) 283.
9 I. BJÖRK, *Exptl. Eye Res.*, 3 (1964) 1.
10 A. SPECTOR AND E. KATZ, *J. Biol. Chem.*, 240 (1965) 1979.
11 R. J. WIEME, *Thesis*, University of Ghent, 1959.

Received January 14th, 1966