reaction mixture allowed to warm to room temperature. After one hour, 172 ml of 0.74 N ethereal butyllithium was added dropwise and the mixture refluxed for 135 min. It was then poured onto solid carbon dioxide covered with ether and worked up in the usual way, yielding 6.9 g (44 %) of 3-formyl-2-thiophenecarboxylic acid having after recrystallization from a benzene-ligroin mixture, the same melting point (130–131°C) and IR-spectrum as an authentic sample.[7]

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Isoelectric Focusing of α-Crystallin Subunits

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α-Crystallin, isolated from the bovine lens by different techniques, such as isoelectric precipitation, zone electrophoresis or gel chromatography, has a molecular weight of about 800 000.[1-4] On treatment with 7 M urea the molecular weight falls to about 25 000, and the urea-treated material can be separated into several bands by polyacrylamide gel electrophoresis.[5] This demonstrates that the α-crystallin molecule is composed of a large number of subunits. Evidence indicating that several of these subunits differ from each other has recently accumulated.[4,6,7] This problem, however, can be unequivocally solved only by the purification of different subunits and the comparison of their properties. The technique of isoelectric focusing in a pH gradient, developed by Svensson and Vesterberg,[8-11] has been applied to the separation of the α-crystallin subunits, and the results obtained will be reported in this communication.

α-Crystallin was prepared from calf lenses by the method of Mok and Waley.[12] In order to remove residual low molecular weight impurities, 50 mg of this material were dissolved in 7 ml of 0.1 M tris buffer, pH 8.0, containing 0.5 M NaCl, and the solution was passed through a column of Sephadex G 200 (4 × 100 cm) equilibrated with the same buffer. The purified sample was then dialysed against distilled water and lyophilised.

Isoelectric focusing was carried out in an LKB 8102 Electrofocusing Column with a total volume of about 450 ml, following the directions given by Haglund.[13] A stepwise sucrose gradient containing 7 M urea was prepared. The carrier ampholytes forming the pH gradient were also supplied by LKB-Produkter AB, Stockholm, Sweden; they covered the pH range 5–8 and were used at a concentration of 1 %. The α-crystallin sample (about 40 mg) was dissolved in one of the middle fractions of the gradient. It was necessary to perform the experiment at 20°C in order to avoid precipitation of urea or sucrose at the bottom of the column, which otherwise

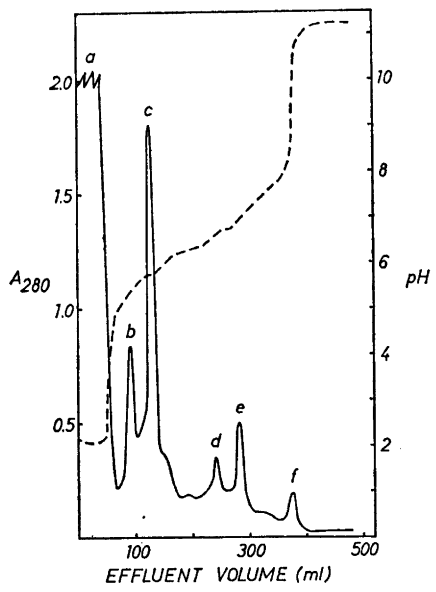

Fig. 1. Isoelectric focusing of α-crystallin subunits in 7 M urea. Solid line, absorbancy at 280 mμ. Broken line, pH.

occurred at lower temperatures. A voltage of 800 V, with the cathode at the bottom of the column, was applied for 62 h; the column was then eluted at a flow rate of 2 ml/min and the ultraviolet absorbancy at 280 mμ and the pH of the effluent were analysed.

The resulting curves are shown in Fig. 1. Four protein-containing fractions, denoted b, c, d, and e, were obtained; fractions a and f did not contain any protein. The recovery was in the order of 65 %. The four fractions were analysed by polyacrylamide gel electrophoresis in 7 M urea (Fig. 2). Fractions b and c showed two main bands, but seemed to have one of these in common. Evidently the separation between these two fractions was incomplete, but most probably a further separation will be realized by the use of a more narrow pH gradient. Fractions d and e, on the other hand, differed from each other and were more homogeneous but nevertheless showed small amounts on impurities.

It is thus evident that isoelectric focusing is a method of great potential value in the purification of α-crystallin subunits in the presence of urea. In addition, the

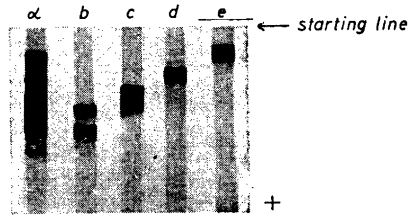

Fig. 2. Polyacrylamide gel electrophoresis of α-crystallin and of the protein fractions from the isoelectric focusing experiment. 0.38 M tris buffer, pH 8.9 + 7 M urea; 180 min at 2 mA/tube. α-Crystallin 100 μg/tube, fractions b—e 50 μg/tube.

method gives information regarding the isoelectric points of the separated fractions. The apparent isoelectric points of the four fractions obtained in this experiment were found to be 5.4, 5.7, 6.6, and 7.0, respectively.

Note added in proof. Similar results have recently been reported by Bloemendal and Schoenmakers.[14]

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