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One-step preparation of the polypeptide chains of α -crystallin

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SUMMARY

A simple ion-exchange chromatographic procedure is described for the preparation of the polypeptides of α -crystallin. All the four polypeptides as found by polyacrylamide electrophoresis can be isolated in one single step.

The fractionation of the polypeptide chains of α -crystallin has been described earlier (refs. 1-4 and A. Spector, personal communication). In order to obtain a satisfactory separation several steps were required in the older procedures. We have now succeeded in separating the basic and acidic chains in a single chromatographic procedure, yielding sufficient material for studies on the primary structure of the individual polypeptides. The method is recommended for its simplicity.

The procedure which is carried out entirely in the cold room ($\pm 4^\circ$) is as follows: The column (Pharmacia K 29-45) is packed with DEAE-cellulose (1.5 cm $\times$ 15 cm), pretreated according to the instructions of the manufacturer (Serva, Heidelberg). In contrast to various other DEAE-cellulose ion-exchange preparations tried this material gives the desired results. The ion exchanger is equilibrated with 0.01 M Tris-HCl (pH 7.6) containing 6 M urea and 0.01% dithiothreitol (starting buffer). Before use the urea solution is washed through a mixed-bed ion exchanger in order to remove isocyanate ions. It can be concluded that the urea is freed from isocyanate as the test according to Marier and Rose⁵ is negative. Dithiothreitol is used in order to avoid the occurrence of dimers of the acidic chains. The column is loaded with approximately 300 mg of purified α -crystallin⁶, dissolved in 4 ml starting buffer. Elution is achieved by means of a linear gradient from 0.01 to 0.15 M Tris-HCl in 6 M urea and 0.01% dithiothreitol at pH 7.6. The total volume of the eluant is 500 ml and the elution rate 20 ml/h. The location of the eluted fraction is either directly recorded by means of transmission measurement in a Uvicord II or determined by measuring the absorbance at 280 nm of the separated fractions in a Zeiss spectrophotometer. Fig. 1 shows a typical elution curve. Leon⁷ applying a stepwise gradient for elution obtained less clear-cut separations. The peaks are pooled as indicated.

Fig. 1. Chromatography of α -crystallin on a DEAE-cellulose column in 6 M urea. Elution with a gradient as described in the text. The range between B1 and A2 chains contained material, not yet identified unequivocally.

Fig. 2. Polyacrylamide gel electrophoresis of polypeptide chains of α -crystallin. 10% gels are used. Electrophoresis was performed at pH 8.9 in 6 M urea. About 50 μ g of peptide was applied on top of the gel. From left to right: α -crystallin, B2, B1, unidentified, unidentified, A2, A1, α -crystallin.

The "acidic" fractions containing the polypeptide chains with isoelectric points below pH 6.5 are dialyzed directly against distilled water. The "basic" fractions with an isoelectric point higher than pH 6.5 are dialyzed against acidified water after lowering the pH to 5.0 by addition of formic acid. The fractions are dialyzed 2 times for 15 min, 30 min and 16 h, respectively. This procedure is necessary in order to decrease the urea concentration quickly to less than 3 M. Below 3 M urea the polypeptides reaggregate and remain in the dialysis bag. After dialysis the material is lyophilized. Electrophoresis on polyacrylamide gels in 6 M urea at pH 8.9 reveals that the separated fractions are homogeneous (Fig. 2). The protein eluted in the first two peaks (Fig. 1) belongs to the group of the basic constituents designated B chains. The other two peaks belong to the acidic polypeptides designated A chains⁸.

Further investigations on the nature of the different polypeptides are in progress.

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Biochim. Biophys. Acta, 243 (1971) 149–151