NONIDENTICAL SUBUNITS IN α-CRYSTALLIN

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The structural protein - α-crystallin - from the vertebrate lens has shown to be an homogeneous entity as judged by a number of physicochemical criteria. Its molecular weight is about 800,000 (Orekhovich et al.,1955; Bloemendal and ten Cate, 1959; Perry and Koenig, 1961). Strong evidence for a subunit structure was given by Bloemendal et al.,(1962) and confirmed by Björk (1964) and Spector and Katz (1965). In urea, guanidine hydrochloride, 1% sodium dodecyl sulfate (SDS) and at extreme pH values the molecular weight drops to about 25,000. However the question whether the subunits of α-crystallin are identical or whether they differ in amino acid composition has not yet been answered unequivocally. Recently we reported that all subunits are acetylated at the N-terminus (Hoenders and Bloemendal, 1967), while serine was found to be the sole C-terminal amino acid (Schoenmakers et al.,1968). Studies on alkaline polyacrylamide gels in 7 M urea reveals a complicated pattern of bands. On the other hand in acidic gels containing 7 M urea only two strong bands could be observed.

In order to verify the presence of more than one class of polypeptides α-crystallin from calf lenses was subjected to chromatography on SE-Sephadex at pH 3.2 in 0.2 M sodium formate buffer containing 7 M urea. The α-crystallin was prepared as described earlier (Schoenmakers et al.,1968). After development of a liniar sodium chloride gradient in formate-urea buffer two well-resolved peaks were obtained (Fig. 1). Electrophoretic analysis of the pooled peak fractions on polyacrylamide gels at pH 3.0 in 6 M urea revealed their correspondence to the two polypeptide species observed in the urea-dissociated native α-crystallin (Fig. 2, C). After recovery of the protein from the pooled fractions by exhaustive dialysis against water and subsequent lyophylization samples of each peak were subjected to amino acid analysis according to Spackman et al., (1958), in order to ascertain whether the peaks were in fact different polypeptides or merely aggregates of the same basic unit. It is clear from the large differences in their amino acid compositions shown

in Table I that the two peaks constitute different polypeptide species. Most striking are the differences in their relative lysine, serine and proline contents.

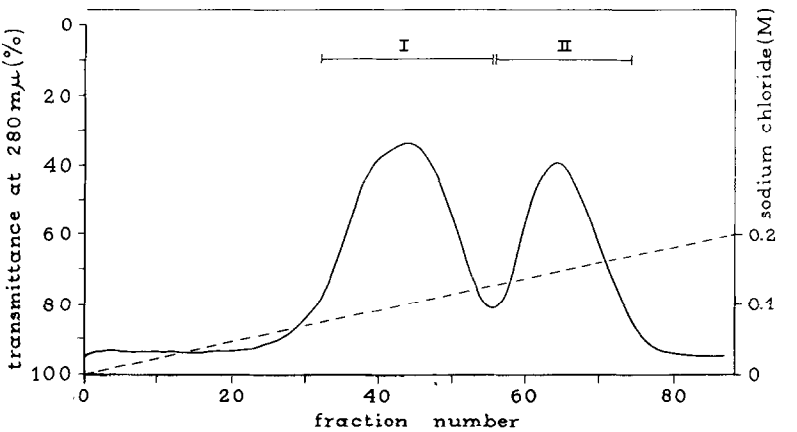

Figure 1. Chromatography of α-crystallin on SE-Sephadex at pH 3.2 in 7 M urea. α-Crystallin (200 mg) was applied to a SE-Sephadex C-50 column (20 x 1.5 cm) equilibrated with 0.2 M sodium formate buffer, pH 3.2, containing 7 M urea. A liniar gradient was applied ranging to 0.2 M NaCl in the same buffer; mixing reservoir 150 ml. Elution was accomplished at room temperature at 15 ml/h. The effluent was monitored at 280 mμ using a LKB Uvicord Absorptiometer. The fraction volume was 2.5 ml.

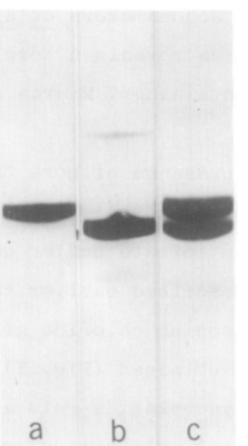

Figure 2. Polyacrylamide gel electrophoresis at pH 3.0 and 6 M urea. The gels were prepared as described elsewhere (Schoenmakers et al.) with 10% acrylamide - 0.2% N,N'methylenebisacrylamide and ammonium persulfate - potassium metabisulfite as the catalyzing system. Samples were applied in 0.25 M formic acid - 6 M urea with the aid of sucrose. After electrophoresis at 5 mA/tube for 90 min the gels were stained with Amido Black. A) Pooled fraction I. B) Pooled fraction II. C) Native α-crystallin at pH 3.0 and 6 M urea. For further details see text and figure 1.

TABLE I. Amino acid analysis of the polypeptide fractions derived from α-crystallin.

Amino acid	Amino acid composition (moles/100 moles)		
	I	II	α-crystallin**
Lysine	4.2	5.7	4.5
Histidine	4.0	4.6	4.4
Arginine	8.0	8.5	7.8
Aspartic acid	9.7	7.8	9.3
Threonine	3.0	3.8	3.2
Serine	11.4	8.9	10.6
Glutamic acid	10.6	11.1	11.1
Proline	6.0	10.1	7.6
Glycine	6.2	5.0	6.0
Alanine	3.8	5.1	4.0
½Cystine*	1.0	0	0.7
Valine	5.9	5.5	5.8
Methionine	1.0	1.0	1.1
Isoleucine	5.1	5.3	5.2
Leucine	8.7	9.1	8.4
Tyrosine	2.3	0.9	2.9
Phenylalanine	8.8	7.9	7.9

*) Determined as cysteic acid on performic acid-treated samples and corrected for 10% loss.

**) Amino acid composition of α-crystallin as reported by Wisse et al., (1965).

Another indication of subunit heterogeneity was the ability of the slow-moving polypeptide band (Fig. 2, A) to yield dimers by disulfide bridge formation (Fig. 3, A). As expected from the amino acid composition of the fast-moving polypeptide band (Fig. 2, B) - which lacks sulfhydryl groups - dimerization was not observed with this peptide (Fig. 3, B). Pretreatment of the native α-crystallin with iodoacetate in the presence of 1% SDS at pH 8.5 abolished this phenomenon. To ascertain whether the transition in polypeptide bands was caused by disulfide bond formation the protein fraction containing the dimers (Fig. 3, A) was reduced for 2 hours at 37° with 0.2 M 2-mercaptoethanol at pH 8.5 in 0.2 M Tris-HCl buffer containing 2% SDS, followed by alkylation with 1.0 molar excess of iodoacetate for 2 hours at 25° in the dark. The reaction mixture was precipitated with four volumes 20% trichloroacetic acid in 50% ethanol. The centrifuged precipitate was washed twice with the same solvent and finally washed with dry ether to remove excess reactants. The S-carboxymethylated protein was then subjected to polyacryl amide gel electrophoresis at pH 3.0 in 6 M urea. As is illustrated in figure 3, C dimers could not be detected after reduction and alkylation of this protein. In contrast such treatment apparently had no effect on the electrophoretic be-

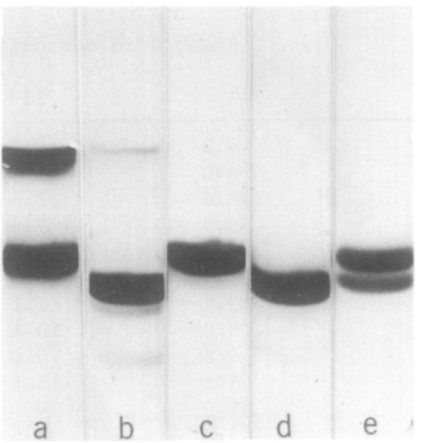

Figure 3. Polyacrylamide gel electrophoresis at pH 3.0 and 6 M urea. The patterns shown are: A) and B) Pooled fraction I and II respectively, after exhaustive dialysis against water. C) and D) The same fractions as figured out in A and B after reduction with 2-mercaptoethanol in 2% SDS at pH 8.5 and alkylation with iodoacetate. E) S-carboxymethylated α-crystallin. For further details see text.

haviour of the fast-moving polypeptide band, which is in accordance with the observation that in this protein cysteine is absent (compare fig. 2, B and 3, D).

The findings that α-crystallin can be dissociated into polypeptide species with different amino acid sequences ranges this structural protein from vertebrate lens to the class of high molecular weight proteins with different subunit architecture (Schachman, 1963; Klotz, 1967).

Summary

Homogeneous preparation of calf lens α-crystallin have been dissociated into subunits with 6 M urea. The dissociated protein was resolved into two distinct polypeptides by chromatography on SE-Sephadex at pH 3 in 7 M urea. It has been demonstrated that the two polypeptide species have different electrophoretic mobilities and amino acid compositions. Differences could be demonstrated between the two types of polypeptides with regard to their ability to dimerize by formation of disulfide bonds.

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