THE MOLECULAR WEIGHT OF THE A-CHAINS OF α-CRYSTALLIN

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1. Introduction

Ten years ago Bloemendal et al. [1] demonstrated that the lens protein α-crystallin which has a molecular weight of about 800,000 daltons can be dissociated into smaller entities with a molecular weight of approx. 25,000.

This finding was confirmed by several laboratories [2–4]. Schoenmakers and Bloemendal [5] showed that the dissociation products called earlier "subunits" of α-crystallin are in fact polypeptide chains which have been designated αA_1 and αA_2 for the acidic and αB_1 and αB_2 for the basic chains. From ultracentrifugal studies at high dilution it was concluded that the molecular weight of αA_1 and αA_2 drops to about 12,000 after prolonged exposure to 2% sodium dodecyl sulfate, 6 M urea or guanidine HCl [6–8]. This surprisingly low molecular weight for the acidic polypeptide chains has been confirmed by independent ultracentrifugal studies of rabbit α-crystallin [9]. In a note added in proof [10] we stressed already that the value of 12,000 cannot be confirmed by various other techniques.

In the present paper we give an account of the molecular weight determinations by gel filtration and gel electrophoresis carried out in dissociating agents. By means of these methods our "old" value of approx. 20,000 for the acidic chains [11] is consistently obtained. Furthermore, recent sequence studies provided the ultimate proof that 20,000 daltons is the correct value [12].

2. Materials and methods

Alpha crystallin and carboxymethylated α-crystallin were prepared according to Schoenmakers et al. [11]. The separation of the basic and acidic polypeptide chains of α-crystallin has been described earlier [13]. Gel filtration on A5M agarose columns equilibrated with 6 M guanidine HCl was performed according to Fish et al. [14]. Parallel experiments were carried out using Sephadex G-100 as column packing and 6 M urea as dissociating agent. The column dimensions were 70 × 2.2 cm.

Polyacrylamide gel electrophoresis in sodium dodecyl sulfate was performed according to Shapiro et al. [15].

3. Results and discussion

3.1. *Gel filtration on agarose-columns in 6 M guanidine HCl*

Blue dextran, serum albumin, ovalbumin, RNAase and DNP-Ala were used as markers to estimate the molecular weight of carboxymethylated A_1 chains of α-crystallin (CM-αA_1) which had been pretreated in 6 M guanidine HCl for 3 days. The separation pattern of the lens polypeptide and the markers is presented in fig. 1a. From the plot of log molecular weight versus elution position as shown in fig. 1b, the value 21,000 for the molecular weight of CM-αA_1 can be derived.

3.2. *Gel filtration on Sephadex G-100 columns in 6 M urea*

Similar studies as illustrated in fig. 1 have also been carried out by gel filtration on Sephadex G-100 equilibrated in 6 M urea.

In subsequent experiments either carboxymethylated total α-crystallin (CM-α) or CM-αA_1 were separated from serum albumin, ovalbumin and ribonuclease. From the plots of log molecular weight against elution

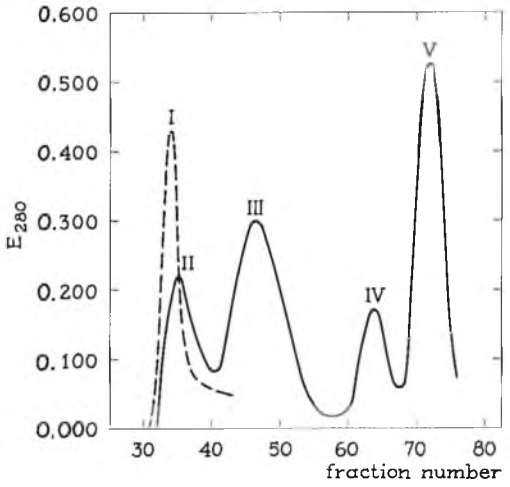

Fig. 1a. Elution profile of separated fractions from a Bio-gel A5M agarose column equilibrated with 6 M guanidine-HCl. Analysis for protein was carried out automatically on a LKB Uvicord at 280 nm. I. Blue dextran (630 nm),II. serum albumin III. ovalbumin, IV. CM-αA_1, V. RNAase.

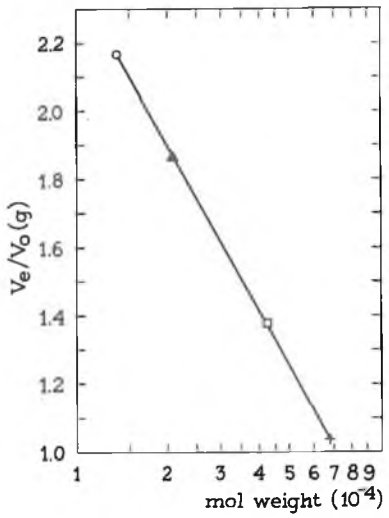

Fig. 1b. Graphical interpretation of the elution data from fig. 1a. ○ RNAase; ▲ CM-αA_1; □ ovalbumin; + serum albumin.

Table 1

Molecular weight estimates of α-crystallin A chains by gel filtration on Sephadex G-100 urea columns.

Polypeptide	CM-α	CM-αA_1	CM-αA_2
Mol. weight	17,500	19,000	18,500

The values are averages of three experiments.

volume as given in fig. 2 the values of the molecular weights listed in table 1 are calculated.

The ultracentrifugal experiments carried out earlier [6] revealed that whereas the further drop in molecular weight from 20,000 to about 12,000 occurs in the A-chains, the B-chains are almost unaffected by prolonged urea treatment. Hence we treated CM-αA_2 with 6 M urea for 3 days and mixed it with unincubated B-chains. When applied to the Sephadex G-100 urea column the mixture emerges as a single symmetrical peak (fig. 3a). For comparison mixtures of CM-αA_2, CM-αB and ribonuclease which has a molecular weight of approx. 13,000 daltons were chromatographed under identical conditions. The position of CM-αA_2 which is shown here is clearly in front of ribonuclease (fig. 3b). These results clearly show that CM-αA_2 and CM-αB have molecular weights of the same order of magnitude.

3.3. *Gel electrophoresis in polyacrylamide containing SDS*

CM-αA_1 was incubated in 2% SDS at 37° for 17 hr or boiled for 5 min. This sample was subjected to electrophoresis in 0.1% SDS together with untreated CM-αA_1, trypsin and ribonuclease.

The band patterns are shown in fig. 4a. It appears that long incubation or heating in SDS does not affect the electrophoretic mobility of CM-αA_1. When the R_f-values are plotted versus the log of the molecular weight a value of 20,000 for CM-αA_1 can be derived (fig. 4b). Similar results were obtained when polyacrylamide gels were used containing 8 M urea and 0.1% SDS. From the observations described here it appears that a correlation between ultracentrifugal data and gel electrophoretic or chromatographic data may lead to conflicting results when highly hydrophobic proteins like the polypeptides of α-crystallin are under investigation.

It has earlier been demonstrated by Schoenmakers et al. [11] that the C-terminal serine occurs in a ratio of one residue per 23,000 daltons in both acidic poly-

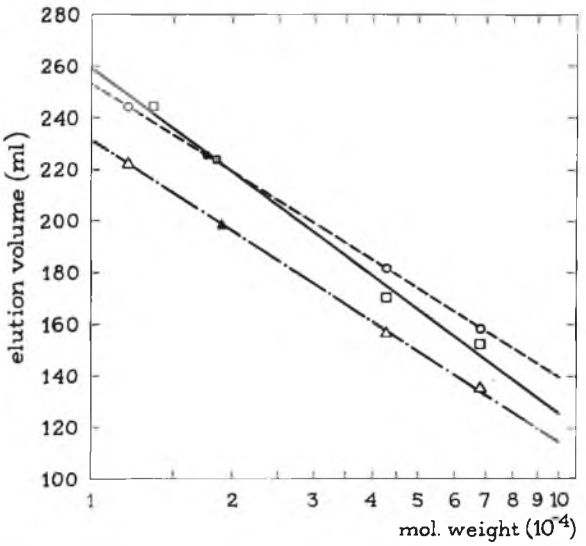

Fig. 2. Graphical interpretation of elution data of separated fractions from a Sephadex G-100 column equilibrated with 6 M urea–0.05 M phosphate buffer at pH 7.6.
– – ○ – –serum albumin, ovalbumin, cytochrome c and CM-α;
–.– △ –.– serum albumin, ovalbumin, cytochrome c and CM-αA_1; – □ – serum albumin, ovalbumin, ribonuclease and CM-αA_2.

peptides. This chemical criterion seemed to support the present results. However we have recently shown that the C-terminus has the sequence Pro–Ser–Ser which apparently inhibits the cleavage of the penultimate Ser residue by carboxypeptidase A [12]. Otherwise one Ser per 11,500 should be found. In a different chemical way, i.e. by gas chromatographic determination of the acetyl group content of α-crystallin, values in favor of a molecular weight of about 12,000 have been reported [7]. Surprisingly the less exact method of Ludowieg and Dorfman [16] yield the value one acetyl group per 20,000 g of α-crystallin [17].

Anomalous results have been observed by others [18] when using the analytical ultracentrifuge to measure molecular weights in SDS. Barnett and Spragg [19] ascribe these anomalies to the rather arbitrarily chosen value of the partial specific volume. In case of the polypeptide chain of yeast hexokinase it appeared that enhancement of proteolysis takes place by the use of SDS [20].

That proteolytic activity in lens crystallin preparations is a possible cause of the anomalies which may occur with molecular weight estimations cannot completely be ruled out, as the presence of proteases in soluble lens proteins has clearly been demonstrated previously [21, 22].

The present results which are in accordance with gel electrophoretic experiments of Spector et al. [23] together with our sequence studies [12] leave no doubt that the molecular weight of the α-crystallin A chains is approx. 20,000 daltons.

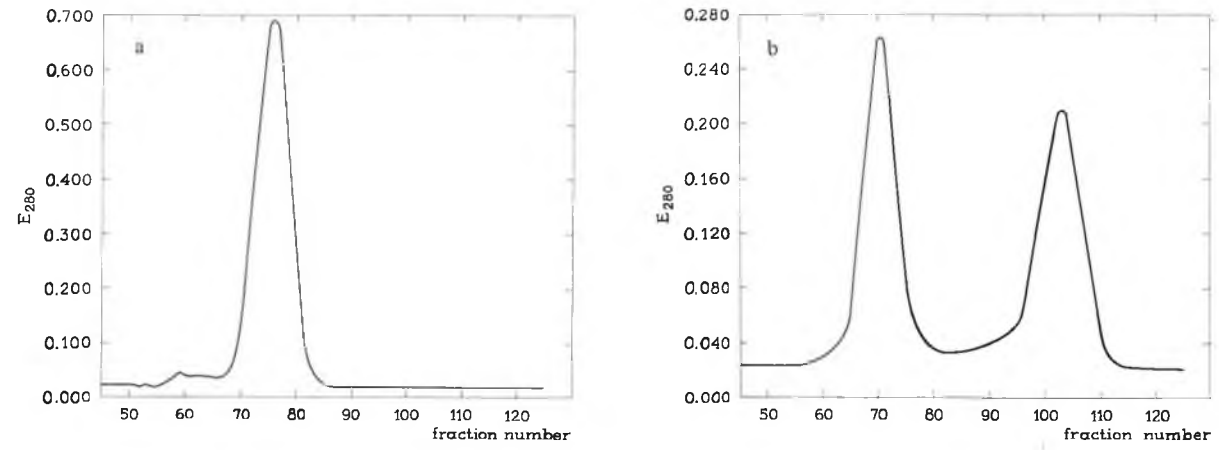

Fig. 3. Gel filtration on G-100 urea columns of CM-αA_2 a) after incubation in 6 M urea for 3 days mixed with untreated CM-αB; b) after incubation in 6 M urea for 3 days mixed with ribonuclease.

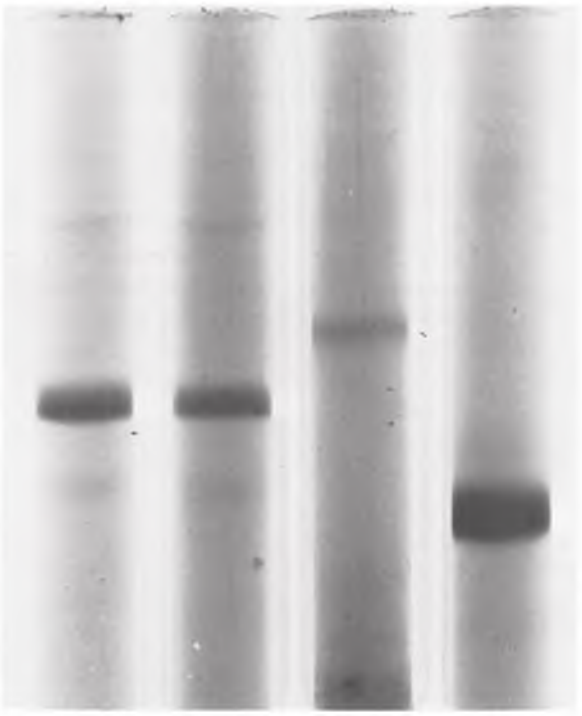

Fig. 4a. Electrophoresis on polyacrylamide-SDS gels. 1) CM-αA_1 incubated in 2% SDS for 17 hr at 37°; 2) CM-αA_1 untreated; 3) trypsin; 4) ribonuclease. The electrophoretic experiment was performed according to Shapiro et al. [15] with the modification of double cross linking of the gels.

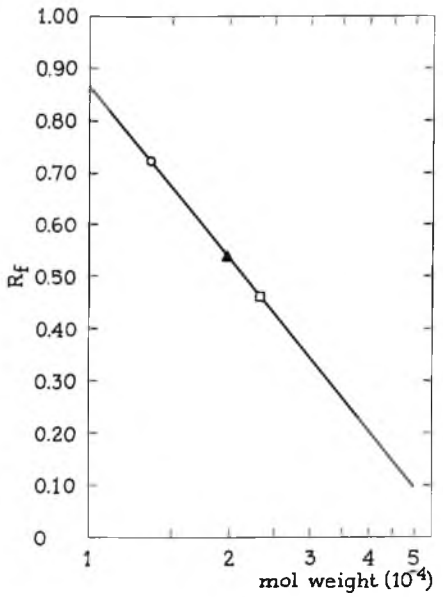

Fig. 4b. Graphical interpretation of the electrophoretic migration data of the different proteins shown in fig. 4a. ○ RNase; ▲ CM-αA_1; □ trypsin.

Acknowledgements

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References

[1] H. Bloemendal, W.S. Bont, J.F. Jongkind and J.H. Wisse, Exptl. Eye Res. 1 (1962) 300.
[2] I. Björk, Exptl. Eye Res. 3 (1964) 1.
[3] A. Spector and E.P. Katz, J. Biol. Chem. 240 (1965) 1979.
[4] S.G. Waley, Biochem. J. 96 (1965) 722.
[5] J.G.G. Schoenmakers and H. Bloemendal, Nature 220 (1968) 790.
[6] H.J. Hoenders, K. de Groot, J.J.T. Gerding and H. Bloemendal, Biochim. Biophys. Acta 188 (1969) 162.
[7] K. de Groot, H.J. Hoenders, J.J.T. Gerding and H. Bloemendal, Biochim. Biophys. Acta 207 (1970) 202.
[8] A.E. Leon, K. de Groot and H. Bloemendal, Exptl. Eye Res. 10 (1970) 75.
[9] A. Manalaysay, California Institute of Technology, personal communication.
[10] A.E. Leon, J.J.T. Gerding, K. de Groot, H.J. Hoenders and H. Bloemendal, Int. J. Protein Res III (1971) 19.
[11] J.G.G. Schoenmakers, J.J.T. Gerding and H. Bloemendal, European J. Biochem. 11 (1969) 472.
[12] F. van der Ouderaa, W.W. de Jong and H. Bloemendal, FEBS Letters 28 (1972) 77.
[13] J.G.G. Schoenmakers and H. Bloemendal, Biochem. Biophys. Res. Commun. 31 (1968) 257.
[14] W.W. Fish, G. Mann and C. Tanford, J. Biol. Chem. 244 (1969) 4989.
[15] A.L. Shapiro, E. Vinuela and J.V. Maizel Jr., Biochem. Biophys. Res. Commun. 28 (1967) 815.
[16] J. Ludowieg and A. Dorfman, Biochim. Biophys. Acta 38 (1960) 212.
[17] H.J. Hoenders, J.G.G. Schoenmakers, J.J.T. Gerding and G.I. Tesser, Exptl. Eye Res. 7 (1968) 291.
[18] E.A. Carusi and R.L. Sinsheimer, J. Mol.Biol. 7 (1963) 388.

[19] W. Barnett and S.P. Spragg, Nature New Biol. 234 (1971) 191.
[20] J.R. Pringle, Biochem. Biophys. Res. Commun 39 (1970) 46.
[21] S.G. Waley and R. van Heyningen, Biochem. J. 83 (1962) 274.
[22] H. Bloemendal, W.S. Bont, J.F. Jongkind and J.H. Wisse, Biochim. Biophys. Acta 82 (1964) 191.
[23] A. Spector, L.K. Li, R.C. Augustey, A. Schneider and T. Freund, Biochem. J. 124 (1971) 337.