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BOVINE α-CRYSTALLIN: SEQUENCE OF THE C-TERMINAL
CYANOGEN BROMIDE FRAGMENT OF THE αA CHAIN

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1. Introduction

α-Crystallin is one of the water-soluble proteins of vertebrate lenses. It has recently been shown that this protein is composed of aggregates differing only in size [1]. The average molecular weight is about 800,000 daltons. The individual aggregates which can be dissociated by high concentration of urea or in 1% sodium dodecyl sulfate, are composed of αA (acidic) and αB (basic) polypeptide chains in a ratio of approx. 2:1. Both αA and αB chains occur in two electrophoretically distinguishable forms, which are otherwise very similar [2]. The two forms of the αA chain, αA_1 and αA_2, have identical amino acid compositions [2]. The αA_1 chain is thought to be derived *in vivo* from the αA_2 chain by deamidation [3,4]. Partial sequences of the αA chain have been published: the N-terminal 11 residues [5], and 28 residues around the cysteine residue [6]. The C-terminal amino acid is serine [2]. The molecular weight of the αA chain is reported to be approx. 20,000 daltons [2, 7], although a lower molecular weight (12,000 daltons) has also been proposed [8]. The αA chain is one of the very few polypeptides for which the messenger-RNA has been isolated and translated in *in vitro* [9, 10] and *in vivo* [11] systems. Further knowledge of the primary structure of the αA chain was highly desirable in order to characterize the products made under the direction of the isolated messenger.

2. Materials and methods

α-Crystallin was prepared from aqueous extracts of calf lenses by $ZnSO_4$ precipitation, followed by either gel filtration on Sephadex G-200 [12] or preparative zonal centrifugation [1]. αA and αB chains were separated on SE-Sephadex [2]. Cyanogen bromide cleavage (100 mg cyanogen bromide per 100 mg protein) was performed in 70% formic acid for 18 hr. The cyanogen bromide (CB) fragments were separated by either gel filtration on Sephadex G-75 Fine in 6 M urea and 0.5% formic acid, or high voltage paper electrophoresis in pyridine—acetic acid—water (25:1:225, v/v/v, pH 6.5).

Tryptic and chymotryptic digestion was carried out at an enzyme:protein ratio of 2:100 in 0.1 M NH_4HCO_3, pH 8.9, at 37° for 3 hr and 18 hr, respectively. Peptic digestion was carried out in 0.01 M HCl overnight at room temp., using 0.3 mg pepsin per μmole of peptide. Thermolytic digestion was performed in 0.2 M ammonium acetate, pH 8.5, for 15 hr at 37°, using 0.5 mg thermolysin per μmole of peptide. Peptides were purified either by Aminex A-5 column chromatography with pyridine—acetate buffers [13], or by paper electrophoresis (pH 6.5) and chromatography (butanol— acetic acid—water—pyridine, 15:3:12:10, by vol) [14]. Amino acid analyses were performed on a Beckman Multichrom amino acid analyzer, using a single column system.

Amino acid sequences were determined by the dansyl-Edman technique [15], or according to a direct Edman method [16]. Dansyl amino acids were identified by polyamide thin-layer chromatography [17]. PTH amino acids were identified by gas chromatography [18], or silica gel thin-layer chromatography [19, 20]. Assignment of amide groups was

based on electrophoretic mobility of peptides. Carboxypeptidase A digestion was carried out as described earlier [2].

3. Results and discussion

Since the αA_1 and αA_2 chains are apparently identical, apart from an amide group, they were not separated for the work described in this paper.

Two fragments were isolated from cyanogen bromide treated αA chain, accounting together for the complete amino acid composition of the αA chain, minus the N-terminal methionine (table 1). This result clearly indicates that the αA chain contains approx. 177 residues, corresponding to a molecular weight of about 20,000 daltons.

The larger fragment, CB1, and the smaller fragment, CB2, were found to have aspartic acid and leucine, respectively, as N-terminal residues. This is in agreement with the findings of Schoenmakers et al. [12] that after cyanogen bromide cleavage of α-crystallin leucine and aspartic acid appear as N-terminal residues. The αA chain contains two methionine residues of which the N-terminal acetyl-methionine is followed by aspartic acid [21], and the other, present in the cysteine peptide sequenced by Corran and Waley [6], by leucine. CB2 therefore represents the C-terminal part of the αA chain.

Four tryptic and several chymotryptic peptides were obtained from CB2 (table 2). Peptide T2 was further hydrolysed with pepsin, and peptide T4 with thermolysin.

Sequence determination of the various peptides was performed as indicated in fig. 1. The sequence Lys–Pro in T4 makes clear why no tryptic hydrolysis occurs at this lysine residue. The chymotryptic peptides provided sufficient evidence to align the tryptic peptides, resulting in the proposed sequence of CB2 as shown in fig. 1.

From the C-terminal sequence Ala–Pro–Ser–Ser one might expect two serine residues to be released

Table 1
Amino acid compositions of the αA chain of bovine α-crystallin and its cyanogen bromide fragments.

	αA chain	CB1	CB2
Lys	7.2 (7)	5.2 (5)	1.9 (2)
His	7.0 (7)	5.8 (6)	1.0 (1)
Arg	13.1 (13)	11.0 (11)	2.0 (2)
Asp	16.3 (16)	14.3 (14)	1.1 (1)
Thr	5.0 (5)	3.9 (4)	1.0 (1)
Ser	23.3[a](23)	15.6 (16)	7.8 (8)
Glu	17.8 (18)	14.6 (15)	3.1 (3)
Pro	12.4 (12)	7.7 (8)	5.0 (5)
Gly	10.9 (11)	7.8 (8)	3.1 (3)
Ala	6.9 (7)	3.7 (4)	3.1 (3)
Cys	(1)[b]	(1)	
Val	10.0[c](10)	8.0 (8)	2.0 (2)
Met	1.8 (2)	(1)	
Ile	9.1[c](9)	6.9 (7)	1.9 (2)
Leu	14.4 (14)	12.9 (13)	1.0 (1)
Tyr	6.1 (6)	5.9 (6)	
Phe	13.9 (14)	12.9 (13)	1.0 (1)
Trp	(2)[b]	(2)	
Total	177	142	35

Data are given as molar ratios, with nearest integral values in parentheses. a) Extrapolated to zero time of hydrolysis. b) Values taken from the literature [2, 23]. c) Determined after 72 hr hydrolysis.

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Leu-Thr-Phe-Ser-Gly-Pro-Lys-Ile-Pro-Ser-Gly-Val-Asp-Ala-Gly-His-Ser-Glu-
├─→─────────T1───────────┤├─→──→──→──→───T2──→──→──→─────────→─
├───C1──────┤├───→──→──→──→──────────────C2─────────────────────
                               ├──────────T2P1────────────┤├─→────────T2P2──→─
-Arg-Ala-Ile-Pro-Val-Ser-Arg-Glu-Glu-Lys-Pro-Ser-Ser-Ala-Pro-Ser-Ser-OH
─────→─┤├─→──→───T3──→──→──→─┤├─→──→──→──→──T4──→─→──→─←──←──←
────┤├─→─────────────────────C3────────────────
─────→─┤                        ├──→───T4Th1──→──→─┤├─→──T4Th2──→──→──┤
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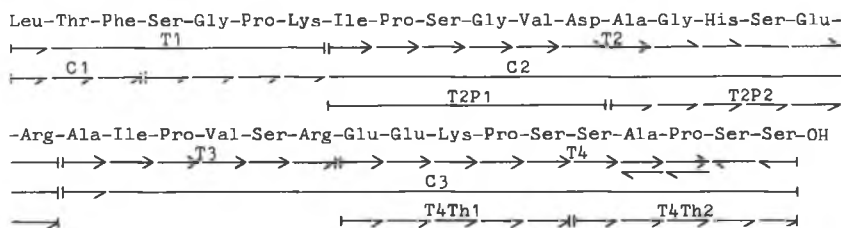
Fig. 1. Proposed amino acid sequence of the C-terminal cyanogen bromide fragment of the αA chain. The sequences of tryptic (T), chymotryptic (C), peptic (P) and thermolytic (Th) peptides were determined by the dansyl-Edman technique (→), the direct Edman method (→), or both methods (→), and by carboxypeptidase C degradation (←) [25].

Table 2
Amino acid compositions of tryptic (T) and chymotryptic (C) peptides from αA-CB2.

	T1	T2	T3	T4	Total	C1	C2	C3	Total
Lys	1.0			1.1	2		1.0	1.1	2
His		1.0			1		0.9		1
Arg		0.9	1.0		2		1.0	1.1	2
Asp		1.1			1		1.2		1
Thr	1.0				1	0.9			1
Ser	1.0	2.0	1.0	3.6	8		2.9	4.5	8
Glu		1.1		2.0	3		1.1	2.3	3
Pro	1.1	1.0	1.0	2.2	5		1.7	3.0	5
Gly	1.0	2.1			3		3.0		3
Ala		1.1	1.0	1.1	3		1.1	2.0	3
Val		1.1	1.0		2		1.0	1.1	2
Ile		0.9	1.0		2		1.0	1.1	2
Leu	0.8				1	1.0			1
Phe	1.1				1	1.1			1
Total					35				35

from the αA chain upon carboxypeptidase A treatment, whereas it has been reported [2] that never more than one residue of serine is in fact released. We therefore incubated both peptide T4 and carboxymethyl-αA with carboxypeptidase A. Again, only 0.84 and 0.97 equivalents of serine, respectively, were released after 8 hr incubation. The presence of proline in the third position from the C-terminus thus apparently prevents the release of serine from the penultimate position. A similar situation is observed in tobacco mosaic virus protein, where carboxypeptidase A releases only threonine from the C-terminal sequence Pro—Ala—Thr [22].

The first seven residues of CB2 are the last ones of the 28-residue cysteine peptide [6]. The sequence of the C-terminal 56 residues of the αA chain is thus known.

Remarkable features of the αA-CB2 fragment are the high content of proline, making any significant α-helix structure in this part of the molecule unlikely, and the relative scarcity of hydrophobic residues. It seems that, like in the αB chain [23], the hydrophobic residues are accumulated in the N-terminal part of the chain. The N-terminal 11 residues of the αA and αB chains show a striking homology [5, 23]. Like the αA

chain, the αB chain is split into two fragments after cyanogen bromide treatment [23]. However, whereas the N-terminal fragment of αB (81 residues) is shorter than the C-terminal fragment (110 residues), the opposite is the case in the αA chain. This indicates that the internal methionine residue does not occupy a homologous position in αA and αB.

The complete amino acid sequence of bovine γ-crystallin has recently been published [24]. No part of this sequence is apparently homologous with any of the known sequences of the αA chain. A close phylogenetic relationship between the α- and γ-crystallins seems therefore unlikely.

Acknowledgements

References

[1] H. Bloemendal, T. Berns. A. Zweers, H. Hoenders and E.L. Benedetti, European J. Biochem. 24 (1972) 401.

[2] J.G.G. Schoenmakers, J.J.T. Gerding and H. Bloemendal European J. Biochem. 11 (1969) 472.

[3] W.G. Palmer and J. Papaconstantinou, Proc. Natl. Acad. Sci. U.S. 64 (1969) 404.

[4] H. Bloemendal, A.J.M. Berns, F. van der Ouderaa and and W.W. de Jong, Exp. Eye Res. 14 (1972) 80.

[5] P.H. Corran and S.G. Waley, Biochem. J. 115 (1969) 789.

[6] P.H. Corran and S.G. Waley, Biochem. J. 124 (1971) 61.

[7] A. Spector, L.-K. Li, R.C. Augusteyn, A. Schneider and T. Freund, Biochem. J. 124 (1971) 337.

[8] K. de Groot, H.J. Hoenders, J.J.T. Gerding and H. Bloemendal, Biochem. Biophys. Acta 207 (1970) 202.

[9] A.J.M. Berns, G.J.A.M. Strous and H. Bloemendal, Nature New Biology 236 (1972) 7.

[10] M.B. Mathews, M. Osborn, A.J.M. Berns and H. Bloemendal, Nature New Biology 236 (1972) 5.

[11] A.J.M. Berns, M. van Kraaikamp, H. Bloemendal and C.D. Lane, Proc. Natl. Acad. Sci. U.S. 69 (1972) 1606.

[12] J.G.G. Schoenmakers, H.J. Hoenders and H. Bloemendal, Exp. Eye Res. 7 (1968) 172.

[13] R.T. Jones, in: Methods of Biochemical Analysis, Vol. 18, ed. D. Glick (John Wiley & Sons, New York, 1970) p. 205.

[14] S.G. Waley and J. Watson, Biochem. J. 55 (1953) 328.

[15] W.R. Gray and J.F. Smith, Anal Biochem. 33 (1970) 36.

[16] H.D. Niall and J.T. Potts, in: Peptides, Chemistry and Biochemistry, eds. B. Weinstein and S. Lande (Dekker, New York, 1970) p. 215.

[17] K.R. Woods and K.-T. Wang, Biochim. Biophys. Acta 133 (1967) 369.

[18] J.J. Pisano and T.J. Bronzert, J. Biol. Chem. 244 (1969) 5597.

[19] J.O. Jeppson and J. Sjöquist, Anal Biochem. 18 (1967) 264.

[20] G. Pataki, Dünnschichtchromatographie in der Amino-säure- und Peptid-Chemie (De Gruyter, Berlin, 1966) p. 181.

[21] H.J. Hoenders and H. Bloemendal, Biochim. Biophys. Acta 147 (1967) 183.

[22] F.A. Anderer, Adv. Prot. Chem. 18 (1963) 1.

[23] R.C. Augusteyn and A. Spector, Biochem. J. 124 (1971) 345.

[24] L.R. Croft, Biochem. J. 128 (1972) 961.

[25] H. Tschesche and S. Kupfer, European J. Biochem. 26 (1972) 33.