SEQUENCE OF THE FIRST 68 RESIDUES OF THE αB_2 CHAIN OF BOVINE α-CRYSTALLIN

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SUMMARY

The sequence of the N-terminal cyanogen bromide fragment of the αB_2 chain of bovine α-crystallin was determined. Valuable results were obtained by the use of an extracellular protease from Staphylococcus aureus, cleaving specifically C-terminal of glutamyl residues, and the application of the solid-phase Edman degradation. A 55% sequence homology was observed with the N-terminal region of the αA_2 chain of bovine α-crystallin.

The lens protein α-crystallin has interesting aspects as a model protein for the study of aggregation, differentiation and aging (1, 2, 3). This protein occurs in the native state as an aggregate of 800,000 dalton (4) and has relatively simple building stones, namely two types of polypeptide chains, designated αA and αB(5). These polypeptide chains have molecular weights of about 20.000 and 21.500, respectively (6), and both occur in two electrophoretically distinguishable forms (αA_1 and αA_2, αB_1 and αB_2) which have identical amino acid compositions (6, 7).

The complete primary structure of the major polypeptide chain αA_2 has recently been published (8). The present study established the sequence of the N-terminal cyanogen bromide fragment of the αB_2 chain. It shows a 55% homology with the corresponding region of the αA_2 chain.

Interesting aspects of the applied sequencing techniques are the

succesful use of a recently described protease, which specifically cleaves peptide bonds C-terminal of glutamyl residues (9), and the automatic solid-phase Edman degradation (10) of a homoserine peptide.

MATERIALS AND METHODS

Alpha-crystallin was isolated from aqueous extracts of calf-lens cortex by gel chromatography on a column of Sephadex G-200 (11). The αB_2 chain was prepared from 600 mg α-crystallin by ion-exchange chromatography on a column (30 x 2.5 cm) of DEAE-cellulose (Whatman DE 52). Equilibration and elution of this column was performed at $4^{o}C$ at a flow rate of 35 ml/h with a 2.5 mM Tris-HCl buffer, pH 8.0, containing 6M urea.

Cyanogen bromide cleavage was carried out as described earlier (8). The cyanogen bromide fragments were separated by gel chromatography on a column (100 x 2.5 cm) of Sephadex G-50 fine, equilibrated and eluted with 5% acetic acid at room temperature at a flow rate of 25 ml/h. The effluent was monitored at 280 nm and in a few cases by performing the Folin reaction on an aliquot from every second fraction.

Digestion with trypsin was carried out at room temperature for 30 minutes at pH 8.9, using an enzyme : substrate ratio of 1 : 100 (w/w). Digestion was stopped by the addition of trypsin inhibitor, at a ratio of 1 : 1 over trypsin. Chymotryptic and thermolytic digestions were performed at $37^{o}C$ for 2 hours, at pH 8.9 and 8.6, respectively, using an enzyme : substrate ratio of 1 : 100. A protease cleaving specifically peptide bonds C-terminal of glutamic acid, was isolated from the culture filtrate of Staphylococcus aureus strain V8, as described by Drapeau et al (9). The large tryptic peptide T3 was digested with this enzyme at $37^{o}C$ in 50 mM sodium phosphate

buffer, pH 7.8, using 0.5 mg protease per μmole of peptide. Enzymic digests were fractionated by gel chromatography on a Sephadex G-50 fine column (120 x 1.5 cm), eluted at room temperature with 0.1 M ammonia at a flow rate of 8 ml/h. The effluent was monitored at 230 nm. Fractions of the eluate were further purified by high-voltage paper electrophoresis (pH 6.5) and descending paper chromatography (n-butanol - acetic acid - water - pyridine; 15 : 3 : 12 : 10) (12).

For most peptides the accelerated version of the three stage Edman degradation, as described by Niall and Potts (13), was used. The sequence of the C-terminal homoserine peptide T4 of the N-terminal CNBr fragment was determined by solid-phase Edman degradation. The peptide was activated and attached to triethylenetetramine (TETA) resin according to Horn and Laursen (14). The solid-phase Edman degradation was carried out as described by Laursen (10). Highly purified solvents were used, in order to facilitate gas-chromatographic identification of the phenylthiohydantoin derivatives. Identification of phenylthiohydantoin derivatives of amino acids was performed by thin-layer and gaschromatography as described earlier (8). Histidine and arginine derivatives were determined as described by Summers et al (15). In a few cases the dansyl-Edman technique was used (16). Identification of dansyl amino acids was carried out by thin-layer chromatography on polyamide sheets (17). Asparagine and glutamine were identified directly as their phenylthiohydantoin derivatives.

RESULTS AND DISCUSSION

The αB_2 chain contains two methionyl residues, one of which represents the acetylated N-terminus of the chain (18). Cyanogen bromide cleavage therefore results in free acetyl-homoserine and two larger

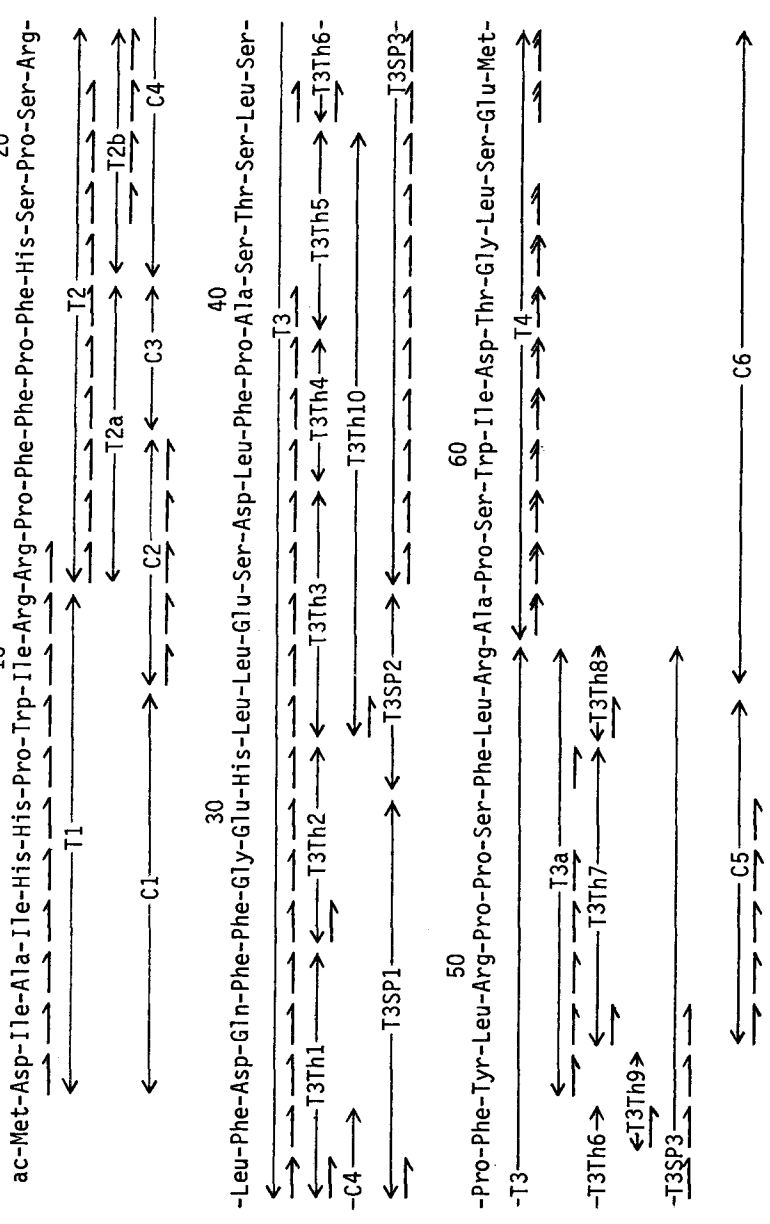

Fig. 1. Proposed sequence of the first 68 residues of the αB_2 chain of bovine α-crystallin. The N-terminal CNBr fragment of αB_2 was digested with trypsin (T) and chymotrypsin (C). T3 was further degraded with thermolysin (Th) and a staphylococcal protease (SP). Sequencing techniques used were: manual Edman (⟶), dansyl-Edman (⟶) and solid-phase Edman (⟶).

Table 1. Amino acid compositions of αB_2-CB1 and its tryptic peptides.

	T1	T2	T3	T4	Sum	αB_2-CB1
His	1.9	1.0	1.1		4	4.0 (4)
Arg	1.2	2.0	2.1		5	5.2 (5)
Asp	1.0		2.0	1.2	4	4.1 (4)
Thr			0.9	0.9	2	2.0 (2)
Ser		1.8	4.6	1.8	9	8.3 (9)
Glu			3.0	1.0	4	3.9 (4)
Pro	1.1	3.2	3.9	1.0	9	8.9 (9)
Gly			1.1	1.1	2	2.2 (2)
Ala	1.1		1.1	1.1	3	3.3 (3)
Met*				0.9	1	0.8 (1)
Ile	2.7			1.0	4	3.9 (4)
Leu			6.8	1.0	8	8.0 (8)
Tyr			0.7		1	1.0 (1)
Phe		3.0	5.6		9	8.8 (9)
Trp	+			+	2	2 (2)
	10	11	34	12	67	67

*determined as homoserine

fragments. These fragments were easily separated by gel chromatography on Sephadex G-50 fine. The amino acid compositions of the N-terminal CNBr fragment (αB_2-CB1) and its four tryptic peptides are shown in Table 1. When the tryptic digestion was continued for 4 h, instead of the usual ½ h, three additional peptides (T2a, T2b and T3a) were obtained due to chymotrypsin-like activity of the trypsin preparation. The accumulated evidence for the sequence of CB1 is given in Fig. 1. The sequence of T1 and the first residue of T2 was obtained by degradation of total CB1. T2 was sequenced through 10 steps, leaving the C-terminal arginine by subtraction. The sequence of T3 could be established by direct degradation up to Ala-18. Moreover Leu-22 could also be identified unambiguously. The peptide fragment C-terminal of Glu-12 was isolated after digestion of T3 with the staphylococcal protease and could be sequenced up to the N-terminal Tyr-Leu sequence of T3a. The sequence of T3a was determined with the dansyl-Edman technique. The presence of homoserine in T4 indicates that it is the C-terminal peptide of αB_2-CB1. After activation with trifluoroacetic acid it was

possible to attach this peptide to TETA resin. Solid-phase sequence determination revealed its complete structure; only Ser-10 had to be placed by subtraction. The phenylthiohydantoin of homoserine was cleaved off from the resin and moved on thin-layer chromatography slightly above the phenylthiohydantoin of serine.

Solid-phase sequence determination is particularly useful for homoserine peptides, since these are liable to extraction into the organic phase during manual Edman degradation, which seriously decreases the repetitive yields. The more polar arginine peptides and lysine peptides modified by 4-sulfophenylisothiocyanate (19) are much less affected by such extraction. The repetitive yield of T4 during solid-phase degradation appeared to be 85%, which is as good or better than the values obtained during manual Edman degradation of T3, a peptide with a polar C-terminal part.

The alignment of the tryptic peptides was deduced from the following data. The direct sequencing of CB1 shows that T2 follows T1, which is confirmed by the sequence of C2. The position of T4 must be C-terminal since it contains the only homoserine residue. This places T3 penultimate in the alignment, which is in agreement with the compositions of C4 and C6. In the tryptic digest of total αB_2 the N-terminal acetyl-methionine is found attached to peptide T1. The sequence of αB_2-CB1, together with the acetyl-methionine, therefore accounts for the first 68 residues of the αB_2 chain.

N-terminal sequences of αB_2 have previously been published by others (7, 20). The sequence proposed by Corran and Waley (20) for the first 11 residues of αB deviates from ours in the absence of His-6. This might be due to the fact that this sequence was mainly deduced from qualitative paper-chromatographic amino acid analyses of the N-terminal tryptic peptide T1 of αB and the thermolytic products of T1. The sequence of the first 18 residues of αB_2 as given by Augusteyn and

Spector (7) differs in not less than 4 positions from ours. Their sequence study was performed by using the automatic sequence procedure according to Edman and Begg (21) on αB_2-CB1, without supporting evidence from sequences and amino-acid analyses of tryptic peptides. Comparison of the sequence of the N-terminal 68 residues of αB_2 with the corresponding part of bovine αA_2 (8) reveals a 55% homology. This indicates that the genes for αA_2 and αB_2 must have originated by duplication of a common ancestral gene. The striking observation has been made that, despite the homology between αA_2 and αB_2 and their similarity in size, the synthesis of αA_2 is directed by an m-RNA which is approximately twice as long as the m-RNA for αB_2 (22).

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