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THE STRUCTURE OF THE ACIDIC POLYPEPTIDE CHAINS FROM α-CRYSTALLIN. AMINO ACID COMPOSITION, PEPTIDE MAPPING, AND N-TERMINUS

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Carefully performed amino acid analyses of the acidic polypeptides from the bovine eye lens protein α-crystallin were in agreement with the recently found molecular weight of about 12,000 for these chains. The proposed amino acid composition is: (Asp_{10}, Thr_3, $Ser_{15(14)}$, Glu_{11}, Pro_7, Gly_6, $Ala_{4(3)}$, Val_6, *Met*, Ile_5, Leu_8, Tyr_3, Phe_8, Lys_4, His_4, Arg_8, *Cys*, *Try*).

On tryptic digestion both polypeptide chains yielded a rather small number of ninhydrin-positive spots on peptide maps; 15 strong and 4 very light spots for A-1 and 14 strong and 4 light spots for A-2.

After blocking the ε-amino groups of the lysine residues by trifluoro acetylation, the acidic chains, again, were submitted to tryptic digestion. Both chains, A-1 and A-2, appeared to contain the same N-terminus up to the first arginine residue. In connection with recent findings, its amino acid sequence is proposed to be:

acetyl-Met-Asp-Ile-Ala-Ile-Gln-His-Pro-Try-Phe-Lys-Arg.

Alpha-crystallin, one of the characteristic proteins of the bovine eye lens, has a molecular weight of about 820,000 (1, 2, 3). It is composed of two main kinds of polypeptide chains, the acidic A and the basic B chains (4, 5). In contrast to the long generally accepted value of 20,000 to 25,000, the molecular weight of these polypeptide chains appeared to be about 11,000 to 12,000 (6, 7). About two-thirds of the polypeptide chains belong to the A type. The separation of the A chains into two polypeptides, A-1 and A-2, has recently been reported (5). Considerable attention has been paid to the N-terminal sequence of both A and B chains (8, 9, 10, 11). Very recently Corran and Waley (12) communicated the sequence of the tryptic N-terminal peptides obtained from the A and the B chains. According to these authors, the C-terminal amino acid of the tryptic N-terminal peptide from the A chains is lysine.

In the present investigation the lysine residues of both A chains have been blocked with the trifluoroacetyl group in order to obtain a larger tryptic N-terminal peptide, up to the first arginine residue. This N-terminal peptide could be isolated and identified.

Carefully performed amino acid analyses, including tryptophan and cysteine, of the acidic chains of α-crystallin revealed values which are in good agreement with the recently found molecular weight of about 11,500 for the polypeptide chains. By means of peptide mapping after tryptic digestion, a number of strong spots almost in accordance with the theoretical number was obtained.

MATERIALS AND METHODS

Isolation of α-Crystallin

Pure α-crystallin was prepared from the cortex of calf eye lenses as previously described (13).

Separation of A Chain Fractions

After alkylation of the thiol groups with the aid of iodo acetic acid (14) the polypeptide fractions were separated by ion-exchange chromatography on sulphoethyl Sephadex C-50 in 6 M urea at pH 3.2 (4) and pH 5.5 (5). An alternative procedure was the separation of the A and B chain fractions without previous alkylation but in the presence of 0.1% 2-mercaptoethanol (unpublished results).

Blocking and De-blocking of ε-Amino Groups

Using S-ethyl trifluorothioacetate the ε-amino groups in the side chain of lysine residues were trifluoroacetylated at pH 10 (15). After tryptic hydrolysis the trifluoroacetyl groups were removed at 0° with 1 M piperidine (15).

Tryptic Digestion of Modified A Chain Preparations

Trypsin was pretreated with 1/16 N HCl at 37° over night in order to suppress chymotryptic activity (16). In most cases a trypsine preparation was used which had been treated with TPCK (L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone) (17).

The tryptic degradation of the modified polypeptide preparations was performed in water at pH 8.0. A substrate:enzyme ratio of 1:50 (w/w) was used.

Liquid Film Continuous-flow Electrophoresis

The apparatus according to Hannig (18), type "Elphor", manufactured by Bender & Holbein, Munich, Germany, was used (19). The electrode chambers were filled with Tris-HCl buffer, pH 8.3, containing 29.1 g Tris per l. The separation chamber was filled with nine times diluted buffer. Electrophoresis was performed at a voltage of 2900 V, giving a current of 80 mA, at 4°. An amount of 100 mg of tryptic peptide mixture, dissolved in 3 ml buffer solution, was supplied during one hour. The buffer pump rate was 50 ml/hr. The N-terminal peptide was detected by adding Ehrlich reagent; one ml of a solution containing 1 g of p-dimethylaminobenzaldehyde in 95 ml acetone and 5 ml concentrated HCl was added to 0.5 ml of the fractions.

High-Voltage Paper Electrophoresis of the N-Terminal Peptide

Electrophoresis on Whatman 3 MM paper was carried out in a cooled-plate apparatus (Savant Instruments, Inc., Hicksville, New York) at 1100 V and 60 mA for 90 minutes with pyridine, acetic acid and water (100:10:890, by vol.) at pH 6.5. In some cases electrophoresis at 1500 V and 60 mA for 1 hour with 1.64 M formic acid was carried out. Detection of tryptophan-containing material was done by spraying with Ehrlich reagent. Moreover, peptides were detected by spraying the paper sheets with ninhydrin reagent (20). Extraction of tryptophan-containing peptide was performed with 30% acetic acid and 1 M pyridine.

Thin-Layer Chromatography

Final purification of the N-terminal peptide was achieved by thin-layer chromatography on DC Fertigplatten Kieselgel F_{254}, 20 × 20 cm (E. Merck AG, Darmstadt; No. 5715). Ascending development of the chromatogram with pyridine-n-butanol-acetic acid-water (40:68:14:25, by vol) required about 6 hours for 17 cm migration of the solvent front. After evaporation of the mobile phase (30 minutes at 80°), the plates were stained at the extremities by spraying with Ehrlich reagent and the Sakaguchi reagent (20). The desired peptide zone was scraped from the plates and the peptide extracted from the silica gel with 30% acetic acid and 1 M pyridine. After centrifugation of the suspensions the supernatant solutions were evaporated.

Peptide Mapping

Ten mg of salt-free polypeptides were suspended in 1 ml of 0.1 M NH_4HCO_3 and 0.2 mg trypsine was added. Incubation at 37° was performed for 18 hours. Thereafter the solution was lyophilized. Electrophoresis on Whatman 3 MM paper was carried out in a Savant high voltage apparatus at 4000 V and 50 mA for 90 minutes. The buffer used was pyridine, acetic acid, water (200:8:2792) at pH 6.4. After electrophoresis the sheets were dried at 90° for 30 minutes. Thereafter ascending chromatography was carried out in butanol, pyridine, acetic acid, water (75:50:16:60). Peptides

were detected by spraying the paper with a ninhydrin solution (0.1 g ninhydrin in 100 ml acetone containing 1 ml pyridine and 1 ml glacial acetic acid). The colour became visible after drying at 80° for 25 minutes.

Tryptophan and Tyrosine Determinations

Solutions containing about 0.4 mg of peptide per ml of 0.1 N NaOH were submitted to ultraviolet spectral analysis in a Zeiss RPQ 20A recording spectrophotometer. The accurate peptide content was determined according to Waddell (21) while the tyrosine and tryptophan content was obtained according to the method of Bencze and Schmid (22).

Amino Acid Analysis

Peptide samples were hydrolysed in 6 N HCl (Merck, Suprapur) at 110° for 22 hours in evacuated sealed Pyrex tubes. The subsequent analyses were carried out with a Phoenix amino acid analyser, equipped with micro-cuvettes (optical path 10 and 15 mm) and an Infotronics model CRS-10AB2 integrator. The apparatus was automated as described by Gerding (23). Calibration runs were carried out with Beckman amino acid calibration mixtures, containing 2.5 μmoles/ml of each amino acid. Some peptide preparations were hydrolysed during varying times in order to determine the following correction factors for certain amino acids: Thr 3%, Ser 10%, Val 5%, Ile 2%. Cysteine was determined as cysteic acid.

RESULTS AND DISCUSSION

Amino Acid Composition

Hitherto the number of amino acid residues in the acidic polypeptide chains of α-crystallin could not be given with absolute accuracy (4), e.g. a value of 21,500 for the molecular weight of the chains was assumed. We have, however, recently shown (6, 7) that the molecular weight of the polypeptide chains composing α-crystallin is approximately 12,000.

Applying the method of Bencze and Schmid (22) about 1 mole of tryptophan and about 3 moles of tyrosine per 12,000 g of polypeptide were determined (Table 1). The tryptophan value deviates from the previous finding (5), but it is in good agreement with the recently determined molecular weight values for the polypeptide chains. The reason for this discrepancy is presumably the choice of methods for the tryptophan determination. The same holds for the determination of cysteine. On the other hand, the values earlier found for methionine could be confirmed to be 1 mole per approximately 12,000 g.

Careful automatic amino acid analyses of several A, A1, and A2 polypeptide chain preparations have now been carried out and the

TABLE 1.

Tryptophan and tyrosine content of acidic polypeptide chain preparations

Preparation	Molar ratio Tyr:Try	Mole% Try	Mole% Tyr
A	3.3	1.0	3.4
A1	2.6	1.1	2.9
A2	3.45	1.0	3.2

TABLE 2.

Number of amino acid residues in the acidic polypeptide chains comprising α-crystallin

Amino acid	Polypeptide chain		Integral number
	A1	A2	
Asp	9.6	9.8	10
Thr*	2.6	2.7	3
Ser*	14.7	14.9	15 (14)
Glu	10.6	11.0	11
Pro	7.0	7.2	7
Gly	5.8	6.1	6
Ala	3.5	3.7	4 (3)
Val	5.8	6.0	6
Met	1.0	1.0	1
Ile	5.0	4.9	5
Leu	8.0	8.3	8
Tyr	3.4	3.3	3
Phe	8.2	8.3	8
Lys	4.2	4.2	4
His	4.0	4.0	4
Arg	7.8	8.0	8
Cys†	1.0	1.1	1
Try†	1.0	1.1	1
Σ residues	103.2	105.6	105 (103)
Σ residue weights	11,620	11,857	11,971 (11,813)

Fixed numbers of amino acid residues are in boldface type.

* In view of an overlap of Thr and Ser, the small peak (Thr) will be found to be too low whereas the large peak (Ser) will be found to be too high.

† Determined separately.

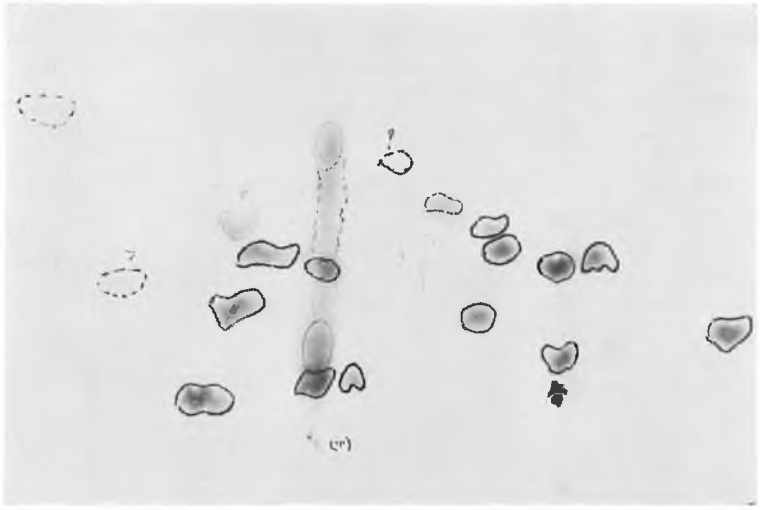

FIGURE 1.
Peptide map of the tryptic digest of polypeptide chain A-1 (for experimental conditions, see text).

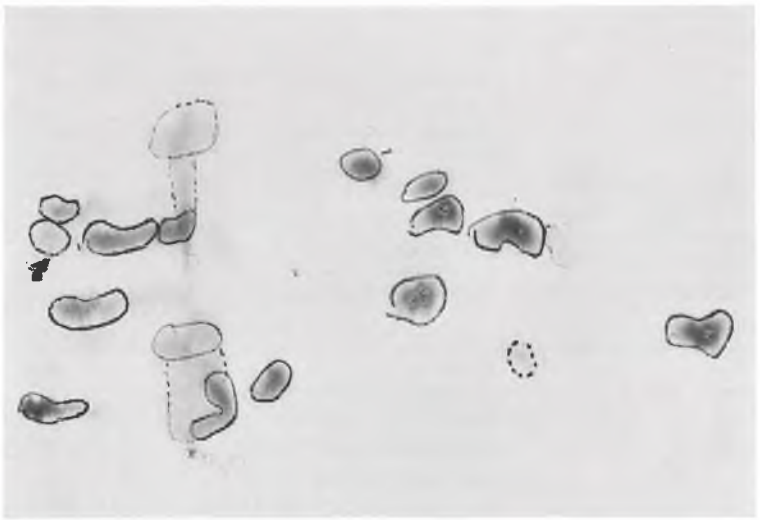

FIGURE 2.
Peptide map of the tryptic digest of polypeptide chain A-2 (for experimental conditions, see text). The arrow indicates the "difference" peptides.

number of the amino acid residues has been calculated taking into account the molecular weight of the chains. Examples of these calculations as well as the decision with respect to the integral numbers of amino acid residues in the acidic polypeptide chains are given in Table 2.

Peptide Mapping

After digestion with TPCK-treated trypsin, high-voltage paper electrophoresis, and paper chromatography, both acidic polypeptide chains A-1 and A-2 reproducibly yielded 14–15 strong spots in several experiments after spraying with ninhydrin (Figs. 1 and 2). This number is in reasonable agreement with the sum of the lysine and arginine content (Table 2). However, A-1 and A-2 do not yield completely identical peptide maps. More particularly, A-1 reveals a peptide which is absent in A-2 and A-2 contains a peptide lacking in A-1. Work is in progress to elucidate the amino acid composition of the ninhydrin-positive spots.

After spraying the paper sheets with Ehrlich reagent, the N-terminal tryptic peptide could be detected because of the presence of tryptophan

(11, 12). For both chains, only one spot at the same place was found. Therefore the conclusion is justified that both acidic chains contain the same N-terminal sequence up to the first lysine residue.

N-terminal Sequence

The A chain fraction of α-crystallin as obtained by ion-exchange chromatography on SE-Sephadex C-50 was treated with S-ethyl trifluorothioacetate in order to block the side chain amino groups of the lysine residues. After this step, enzymatic digestion with trypsin was performed. By means of carrier-free electrophoresis a partial fractionation of the tryptic oligopeptides was obtained. Examination for the presence of tryptophan using Ehrlich reagent revealed that only a limited number of the fractions obtained were positive. After pooling and freeze-drying of these fractions, high-voltage paper electrophoresis at pH 6.5 was applied. Only one Try-containing zone could be observed (peptide maps of this material also yielded only one Try-positive spot; unpublished results). From these findings the conclusion had to be drawn that the acidic chains A-1 and A-2, which are identical in their amino acid composition, (5) contain the same N-terminal tryptic peptide. After extraction from the paper sheet and freeze-drying, the Try-containing peptide was submitted to further purification on thin-layer plates. By the use of 14 plates, an amount sufficient for further investigation was obtained. The amino acid analysis of the purified N-terminal Try-containing tryptic peptide (Table 3) revealed the composition: (Asp, Glu, Pro, Ala, Met, Ile_2, Phe, Lys, His, Arg). Taking into account the tryptophan content of the A chains, the destruction of tryptophan by acid hydrolysis, and the results of the sequence analysis as given by Corran and Waley (12), it can be concluded that the sequence of the amino acid residues in the N-terminal peptide of the A chains is: acetyl-Met-Asp-Ile-Ala-Ile-Gln-His-Pro-Try-Phe-Lys-Arg.

Additional evidence for the sequence at the C-terminus of this peptide was obtained in the following way: After removal of the trifluoroacetyl group at the ε-amino group of the lysine residue, tryptic digestion was applied. The reaction mixture obtained was submitted to high-voltage paper electrophoresis with formic acid as electrolyte as well as to amino acid analysis of the unhydrolysed material. In both cases only arginine and no other free amino acids were found.

TABLE 3.

*Amino acid analysis of the lysine-blocked N-terminal tryptic peptide**

Asp	0.91	Ile	1.90
Glu	0.97	Phe	0.85
Pro	0.94	Lys	1.00
Ala	1.12	His	1.00
Met	0.69	Arg	1.03

* Values relative to lysine.

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NOTE ADDED IN PROOF

The finding of a number of weak ninhydrin-positive spots in addition to the strongly stained tryptic peptides may implicate the occurrence of more than two closely related acidic polypeptides. Some evidence for this complexity has recently been obtained (24, 25). Furthermore it has to be emphasized that the molecular weight of 12,000, as measured in the ultracentrifuge, cannot be confirmed by the following techniques: Electrophoresis in dodecyl sulphate containing polyacrylamide gels; gel filtration on columns equilibrated with SDS or high concentrations of urea or guanidine hydrochloride (26).

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