THE AMINO ACID SEQUENCE OF THE A CHAIN OF HUMAN α-CRYSTALLIN

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1. Introduction

Knowledge of the primary structure of the composing polypeptide chains A and B of the lens protein α-crystallin in man is of interest for at least two reasons. First, a full understanding of the processes involved in cataract formation requires a detailed insight into the structures and properties of normal human lens proteins. Second, the sequences of human lens proteins are an important contribution to the comparative study of vertebrate α-crystallins [1]. The amino acid sequences of the A chains of α-crystallin from cow [2], pig, horse, dog, cat, rabbit, rat and rhesus monkey [3] have shown that comparatively little evolutionary variation occurs in this protein. The primary structure of human A chain differs only slightly from the sequence of monkey A chain, in agreement with the slow rate of evolution of this chain.

2. Materials and methods

Normal adult human lenses and cataractous lenses, of various types, were used in separate experiments. α-Crystallin was obtained from the aqueous lens extracts by gel filtration on Sephadex G-200 [4]. The A chains were isolated by column chromatography in 7 M urea on carboxymethyl cellulose at pH 4.8 [5]. Acrylamide gel electrophoresis was carried out in Tris-glycine buffer, pH 8.5, containing 6 M urea [6]. Aminoethylation, tryptic and thermolytic digestions, and peptide isolation, analysis and sequence determination were carried out as described previously [2,3].

3. Results and discussion

Human α-crystallin eluted as a highly opalescent fraction with the void volume of the Sephadex G-200 column and included the high and lower molecular weight aggregates which are known to be present in both cow and man [7–10]. It was observed that this fraction apparently contained a considerable amount of non-protein material which could not be removed by dialysis. In alkaline urea gels the protein showed a pattern resembling that of other mammalian α-crystallins (fig.1). However, beside the normal A_1, A_2, B_1 and B_2 bands some additional polypeptides were present as already observed by others [9–11]. Studies on bovine α-crystallin have shown that in this species such additional bands, especially pronounced in the adult lens nucleus, represent post-synthetic modifications of the primary gene products A_2 and B_2 [5,12]. α-Crystallin from normal and cataractous lenses showed essentially the same pattern. By means of CM-cellulose column chromatography the A chains were separated from B. The A fraction used for further characterization contained mainly A_2 and a chain which in the cow corresponds to A_2 lacking 22 residues at the C-terminal end [12]. The fingerprint of the tryptic digest of the amino-ethylated A fraction was almost identical to that of the previously studied rhesus monkey A chain [3]. The only difference was that peptide T17b occurred in two electrophoretic forms: neutral and with a charge of + 1. The peptides soluble at pH 6.5 were obtained from such fingerprints and further purified when necessary by electrophoresis at pH 1.9. The peptides T4 and T9, insoluble at pH 6.5, were isolated by gel filtration on

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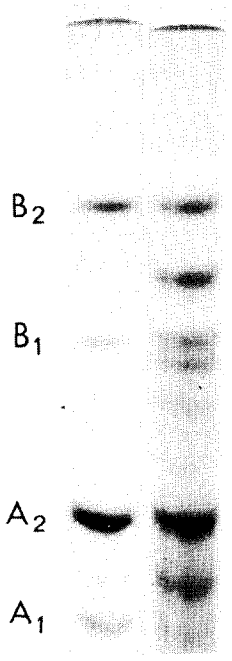

Fig.1. Polyacrylamide gel electrophoresis in 6 M urea at pH 8.5. Left: calf α-crystallin; right: human α-crystallin isolated by gel filtration on Sephadex G-200.

Sephadex G-50 fine in 0.1 M ammonia [2]. The large tryptic peptide T4 was further digested with thermolysin and the resulting small peptides isolated by peptide mapping [3]. The amino acid analyses of all tryptic and thermolytic peptides did not show more than 20% deviation from the integral values corresponding to the compositions of peptides as indicated in fig.2. It turned out that all peptides have compositions identical to the corresponding monkey A chain peptides, apart from T17b, T19 and T20. Peptides T17b, T19 and T20 were therefore isolated in amounts sufficient for sequence analysis, by preparative paper electrophoresis and chromatography. The results of these sequence determinations are indicated in fig.2. Difficulties to obtain sufficient T17b prevented the complete sequence determination of this peptide. However, the deletion of 142-Ser is firmly supported by the presence of only two seryl residues in the peptide, which were placed in positions 132 and 134; no other amino acid was found to replace serine in the amino acid analysis; the presence of tryptophan or tyrosine was excluded by specific stainings of the peptide map; and, most convincingly, prolonged tryptic digestion produced a peptide Gly,Pro,Lys where the same treatment on bovine A chain produces the peptide Ser–Gly–Pro–Lys, due to a chymotrypsin-like cleavage behind 141-Phe [2]. The nature of residue 136-Asx needs some comments. Most of T17b occurs after electrophoresis in a position corresponding to a net charge of +1, which is only possible when Asx is in the amide form. Part of T17b is, however, found in the neutral region, as it is in all previously studied mammals, which should be due to a carboxyl group in residue 136-Asx. Most likely then residue 136 is originally present as asparagine and becomes partially deamidated to aspartic acid during the isolation procedures. It is indeed known that the sequence Asn-Gly is particularly prone to deamidation [13]. The order of all residues in the other tryptic and thermolytic peptides, and of these peptides within the chain, is assumed to be homologous with that in the monkey and bovine A chains [2,3]. Also the distribution of amide and carboxyl groups is assumed to be identical with that in monkey and cow, considering the identical electrophoretic mobility of these chains and their tryptic and thermolytic peptides. The possible bias introduced by this method is discussed elsewhere [3]. As compared to the other known A sequences [2,3] man and monkey share a number of unique substitutions (91 Glu→Asp, 148 Ser→Thr, 155 Ser→Thr) and the deletion of 153-Gly, reflecting their common primate origin. They differ in positions 136, 142, 162 and 168, due to mutations which must have occurred since the divergence of the evolutionary lines leading to man and rhesus monkey, some 40 million years ago [14]. Whether the replacement 136 Asp→Asn in human A chain is really due to mutation is actually doubtful. Such a substitution should make the net charge of the human A chain less negative than those of other mammals. However, the A_2 chain of man moves electrophoretically exactly in the same position as bovine A_2 (fig.1). This can not be due to a substitution elsewhere in the chain which compensates this change in charge at position 136, because apart from T17b all tryptic and thermolytic peptides have the same electrophoretic mobilities as the corresponding bovine peptides. A possible explanation is that all mammals actually possess asparagine in position 136, which is usually rapidly and quantitatively

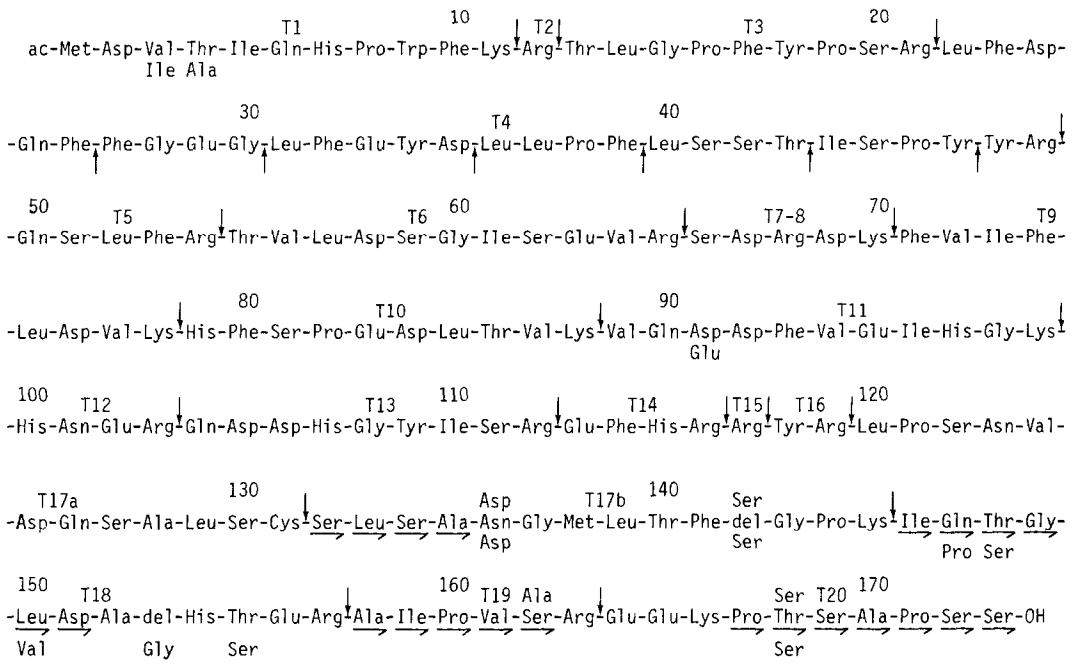

Fig.2. Amino acid sequence of the A chain of human α-crystallin. Residues which are different in bovine and monkey A chains are given below and above the human sequence, respectively. The points of tryptic (↓) and thermolytic (↑) cleavage are indicated. Most residues are placed by homology with the bovine [2] and monkey [3] A chains, on the basis of amino acid compositions of tryptic and thermolytic peptides. The order of some critical residues was determined by dansyl-Edman degradation (→). The sequence shown actually represents the A_2 chain, which contains two amides in T17a, whereas A_1 contains one amide in T17a. The difference at position 136 is subject to some reservation, as discussed in the text.

deamidated during the isolation procedures, but in man remains partially in the amidated form. However, we have as yet not obtained any experimental evidence to support this assumption. The net effect of the amino acid substitutions between cow and human A chains is especially an increase in threonine content from 2.9% to 5.2%, a fact which had already been observed from the amino acid composition of total human α-crystallin [9,11,15]. It should be noted that the A chain isolated from cateractous lenses appeared to be completely identical to that of normal lenses. However, both in normal and in cataractous lenses, and most pronounced in the so-called urea-soluble protein, A chains are present which show definite abnormalities in their sequences, probably due to post synthetic modifications, and which are presently under investigation.

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