THE PRIMARY STRUCTURE OF THE B_2 CHAIN OF HUMAN α-CRYSTALLIN

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1. Introduction

α-Crystallin is one of the major proteins of the mammalian eye lens (for review see [1]). The composing polypeptide chains, A and B, of this structural protein undergo extensive modifications upon ageing, both in the ox [2,3] and, much more pronounced, in man [4,5]. To understand these processes, which may also be relevant to the formation of cataract, it is necessary to know the structures of the A and B chains. The primary structure of human α-crystallin A chain has previously been determined [6]. This letter shows that the human B_2 chain differs only in three positions from the already known bovine B_2 chain [7], thus demonstrating that the B chain, like the homologous A chain, has a very slow rate of evolutionary change. The knowledge of the primary structure of human B_2 chain will facilitate the characterization of the B-like chains which appear upon ageing in the human lens.

2. Materials and methods

α-Crystallin was isolated from aqueous extracts of adult human lenses by gel filtration on Sephadex G-200 or Biogel A-5M [8]. The composing chains were separated by column chromatography in 6 M urea on diethyl-aminoethyl cellulose, at pH 8.0 [9]. The purity of isolated fractions was assessed by isoelectric focusing in 6 M urea [8]. Tryptic, chymotryptic and thermolytic digestions were carried out on native B chain or after cleavage with cyanogen bromide [7]. Isolation of peptides and their analysis and sequence determination was carried out as described previously [10].

3. Results and discussion

Human α-crystallin as isolated by gel-filtration shows a rather complex polypeptide composition [4,5]. After column chromatography of human α-crystallin on DEAE-cellulose in the presence of 6 M urea the fraction was taken, which, on the basis of analysis by isoelectric focusing, contained the polypeptide chain corresponding to the bovine B_2 chain. Detailed results of our structural analyses of this chain will be given elsewhere [11].

Peptides resulting from digestions of the human B_2 chain or its cyanogen bromide fragments with trypsin, thermolysin and chymotrypsin were isolated from peptide maps. Amino acid analyses [11] showed these peptides to be identical in composition, within 20% deviation from integral values, to the corresponding bovine peptides, apart from the peptides containing residues 40, 61 and 152 (fig.1). Where the bovine B_2 chain contains alanine, isoleucine and alanine in these positions, respectively, the human chain showed apparently the presence of threonine, phenylalanine and valine, respectively. The substitutions 61 Ile→Phe and 152 Ala→Val were confirmed by dansyl-Edman degradation.

The amide assignment in fig.1 is based on the electrophoretic mobility at pH 6.5 of peptides, apart from residues 25, 26, 108, 109 and 110, where the amides are placed by homology with the bovine B_2 chain [7]. The human B_2 chain is NH_2-terminally blocked, probably by an acetyl group as in the bovine α-crystallin chains. Although serine has been reported, on the basis of carboxypeptidase digestions, to be the COOH-terminal residue of human B chains [12], we have to conclude that human B_2, like bovine B_2, has COOH-terminal lysine.
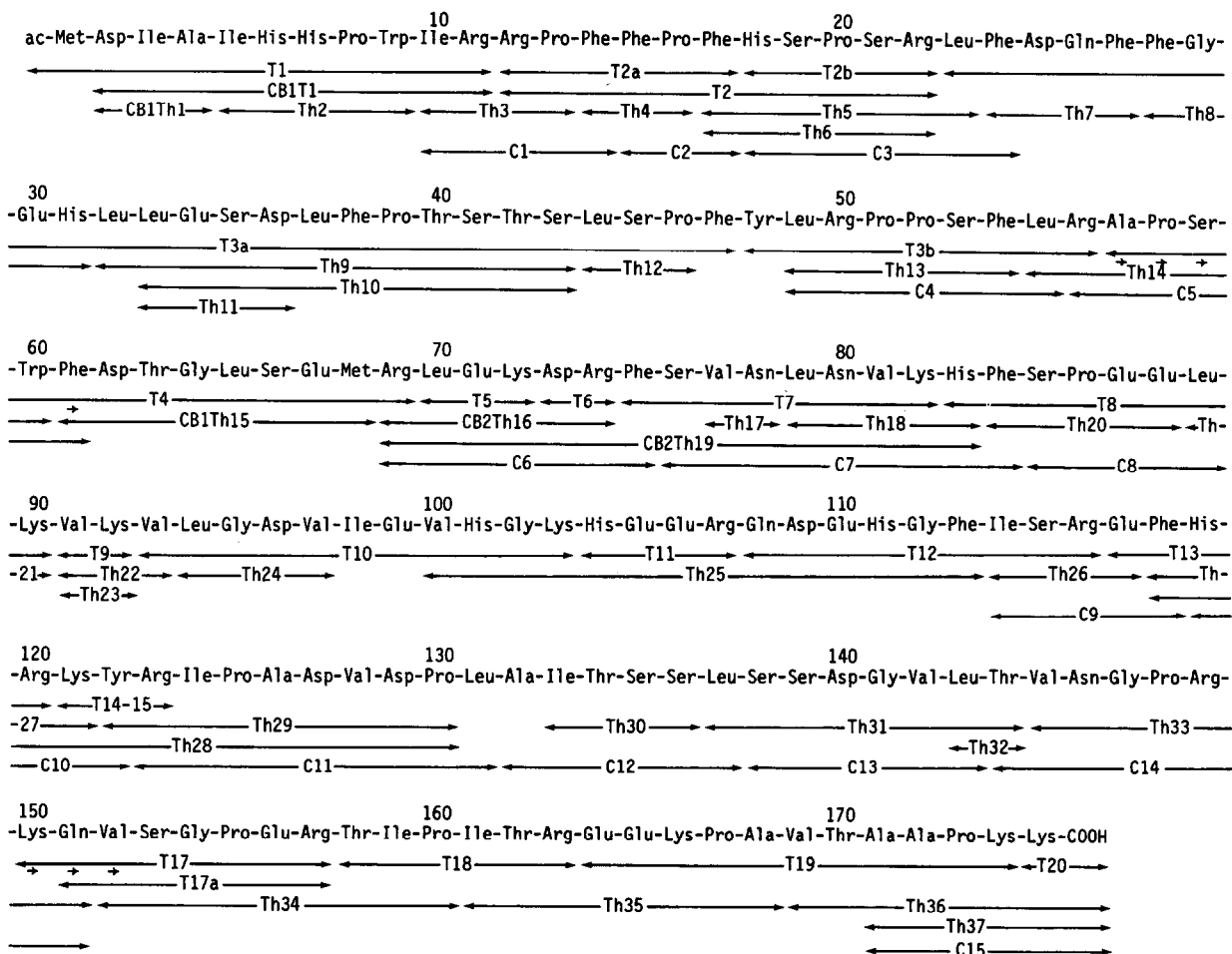
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                              10                                      20
ac-Met-Asp-Ile-Ala-Ile-His-His-Pro-Trp-Ile-Arg-Arg-Pro-Phe-Phe-Pro-Phe-His-Ser-Pro-Ser-Arg-Leu-Phe-Asp-Gln-Phe-Phe-Gly-
  ◄───────────────T1───────────────►  ◄───T2a───► ◄──T2b───► ◄──
       ◄──────CB1T1──────────►        ◄────────T2────────►
  ◄─CB1Th1─► ◄──────Th2──────► ◄───Th3───► ◄─Th4─► ◄──────Th5──────► ◄────Th7───► ◄──Th8─
                                              ◄──────Th6──────►
            ◄──────C1──────► ◄───C2──► ◄──────C3──────►

  30                              40                                 50
-Glu-His-Leu-Leu-Glu-Ser-Asp-Leu-Phe-Pro-Thr-Ser-Thr-Ser-Leu-Ser-Pro-Phe-Tyr-Leu-Arg-Pro-Pro-Ser-Phe-Leu-Arg-Ala-Pro-Ser-
──────────────T3a──────────────►  ◄──────────T3b──────────► ◄──
►◄──────────────Th9──────────────► ◄─Th12─► ◄──────Th13──────► ◄──Th14──►
     ◄──────Th10──────►                     ◄──────C4──────► ◄──C5──
  ◄───Th11───►

  60                              70                                 80
-Trp-Phe-Asp-Thr-Gly-Leu-Ser-Glu-Met-Arg-Leu-Glu-Lys-Asp-Arg-Phe-Ser-Val-Asn-Leu-Asn-Val-Lys-His-Phe-Ser-Pro-Glu-Glu-Leu-
─►◄──────T4──────► ◄─T5─► ◄─T6─► ◄──────T7──────► ◄──────T8──────
─►◄───CB1Th15───► ◄───CB2Th16───► ◄─Th17─► ◄───Th18───► ◄───Th20───► ◄─Th─
                   ◄──────CB2Th19──────►
          ◄──────C6──────► ◄──────C7──────► ◄──────C8──────►

  90                              100                                110
-Lys-Val-Lys-Val-Leu-Gly-Asp-Val-Ile-Glu-Val-His-Gly-Lys-His-Glu-Glu-Arg-Gln-Asp-Glu-His-Gly-Phe-Ile-Ser-Arg-Glu-Phe-His-
─►◄─T9─►◄──────T10──────► ◄───T11───► ◄───T12───► ◄──T13─
-21►◄─Th22─►◄───Th24───►  ◄──────Th25──────► ◄──Th26─►◄─Th─
  ◄─Th23─►                            ◄──────C9──────►

  120                             130                                140
-Arg-Lys-Tyr-Arg-Ile-Pro-Ala-Asp-Val-Asp-Pro-Leu-Ala-Ile-Thr-Ser-Ser-Leu-Ser-Ser-Asp-Gly-Val-Leu-Thr-Val-Asn-Gly-Pro-Arg-
─►◄─T14-15─►
-27─►◄──────Th29──────► ◄──Th30─► ◄──────Th31──────► ◄──Th33─
──────Th28──────►                        ◄─Th32─►
──C10──►◄──────C11──────► ◄──────C12──────► ◄──C13──► ◄──────C14──────►

  150                             160                                170
-Lys-Gln-Val-Ser-Gly-Pro-Glu-Arg-Thr-Ile-Pro-Ile-Thr-Arg-Glu-Glu-Lys-Pro-Ala-Val-Thr-Ala-Ala-Pro-Lys-Lys-COOH
◄──T17──► ◄──────T18──────► ◄──────T19──────► ◄─T20─►
  ◄──────T17a──────►
◄──────Th34──────► ◄──────Th35──────► ◄──────Th36──────►
                                      ◄──────Th37──────►
                                      ◄──────C15──────►
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Fig.1. Proposed amino acid sequence of the B_2 chain of human α-crystallin. Residues are aligned by homology with the known sequence of bovine B_2 chain [7] on the basis of amino acid compositions of tryptic (T), thermolytic (Th) and chymotryptic (C) peptides. Some peptides were obtained from B_1 chain treated with cyanogen bromide, resulting in the fragments CB1 and CB2. Some steps of dansyl-Edman degradation (→) were performed on T4 and T17. This sequence differs from bovine B_2 by the substitution 40 Ala→Thr, 61 Ile→Phe and 152 Ala→Val.

The fact that human B_2 and bovine B_2 chains differ in 3 out of 175 positions, whereas human and bovine A chains differ in 10 out of 173 positions [6], indicates that the rate of evolution of the α-crystallin B chain is at least as slow as that of the A chain [13]. The A and B chains show 57% homology [7] and supposedly fulfill comparable roles in the α-crystallin aggregate which they build up together. A more detailed comparison of the rates and patterns of evolution of the α-crystallin A and B chains might well reveal interesting aspects of evolutionary mechanisms in proteins.

Acknowledgements

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