FOR THE RECORD

Amino acid sequence of human lens β B2-crystallin

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The proteins in the lens nucleus are as old as the organism, making the lens a useful model in which to study growth and aging. Furthermore, modification and cross-linking of the lens proteins are of interest because they are thought to be important in cataract formation. The mammalian lens contains three families of structural proteins, the α -, β -, and γ -crystallins. Of these, the β -crystallins are the most heterogeneous and the least well characterized. The primary sequences of only two human β -crystallins, β A3/A1 and β B3 (partial sequence), have been determined from their genomic DNA sequences (Hogg et al., 1986; Aarts et al., 1989a). Although the amino acid sequence of the principal component of the β -crystallins, β B2, formerly called β Bp, is known for bovine, rat and mouse, only a small section of the human gene sequence has been reported (Driessen et al., 1981; Inana et al., 1982; Aarts et al., 1989b; Chambers & Russell, 1991). A possible role of β B2 in cataractogenesis has been demonstrated by detection of a protein polymer in human cataractous lenses, formed by a Ca^{2+} -dependent transglutaminase mediated cross-link (Lorand et al., 1981). Further studies with rabbit lenses showed that this cross-link could be produced with β -crystallin (not with α - or γ -crystallin) and was recognized by antiserum to bovine β Bp (Velasco & Lorand, 1987). To identify the nature of those cross-links and others that may be present in cataractous lenses, the amino acid sequence of β B2 is necessary. We have sequenced human β B2 by mass spectrometric analysis of trypsin and endoproteinase Glu-C-generated peptides using homology to the bovine sequence as a reference.

The water-soluble portion of a normal human lens was fractionated into α -, β -, and γ -crystallins by Sephadex G-200 gel filtration chromatography. β B2 was isolated from the β -crystallin fraction by reversed-phase high performance liquid chromatography (HPLC) on a C_4 column using a water/acetonitrile (0.1% trifluoroacetic acid

[TFA]) gradient. Enzymatic digestion for 4 h with a 1:50 enzyme-to-substrate ratio of trypsin or endoproteinase Glu-C produced peptides that were separated by C_{18} reversed-phase HPLC. The molecular weights of the peptides in the fractions were determined by fast atom bombardment mass spectrometry (FABMS). Enzymes of specific activity were used to create peptides of an appropriate size for analysis by FABMS and to limit sequence possibilities for the masses found. Nonspecific chymotryptic activity was reduced by the use of N-tosyl-L-phenylalanyl chloromethyl ketone (TPCK)-treated trypsin, so that cleavage was seen almost exclusively C-terminally to Lys and Arg. However, as in the bovine β B2 tryptic digest, additional cleavage after Tyr 111 and Tyr 163 was seen (Smith et al., 1991). Endoproteinase Glu-C cleaves specifically C-terminally to Glu, but can also cleave after Asp if the protein is digested in phosphate buffer at pH 7.8. This approach was used to get additional cleavage since some of the expected peptides were too large to be easily seen by FABMS. Figure 1 shows the proposed sequence for human β B2 and the masses of the peptides found by FABMS in the tryptic digest (solid arrows) and the Glu-C digest (dashed arrows).

The masses of the tryptic peptides found by FABMS were compared with those of a tryptic digest of bovine β B2 (Smith et al., 1991). Fourteen of the 19 peptides from human β B2 had the same mass and HPLC retention as the bovine β B2 peptides. The sequences of these peptides were assumed to be the same as the bovine. Peptides with masses that did not correspond to the bovine sequence were further examined. These peptides included residues 1–17, 108–120, 121–139, 172–187, and 198–204. In most cases, a peptide with the same HPLC retention time but a mass different than the bovine peptide was present in the mass spectrum. The mass difference gave some indication of which amino acid varied in the human peptide. Additionally, overlap between the trypsin and Glu-C digests gave further indication of a residue change and its approximate location.

The molecular ion peak (MH^+ 1,849) corresponded to the N-acetylated peptide (1–17) with a mass 10 Da less

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Fig. 1. Amino acid sequence of human β B2 crystallin. The positions that differ from the bovine β B2 sequence are shown in outline. The masses of the peptides found by FABMS in a tryptic digest (solid arrows) and an endoproteinase Glu-C digest (dashed arrows) are indicated.

than the bovine sequence. A 10-Da difference could be due to the change of a Pro residue to Ser, which requires only a single base change in the DNA (Lee & Rahbar, 1991). The bovine sequence contains Pro at residues 11, 13, and 16. C-terminal sequencing of the tryptic peptide by carboxypeptidases B and Y indicated that residue 13 is Ser in human β B2. Translation of a partial DNA sequence of the human β B2 gene corresponding to residues 96–149 showed amino acid differences, as compared with bovine, at residues 109 (Ile in place of Thr) and 123 (Ile in place of Val) (Aarts et al., 1987). These sequence changes are supported by the tryptic and Glu-C peptide molecular weight maps. A 39-Da mass difference in the peptide (172–187) was the result of two changes in the sequence. N-terminal Edman sequencing of the first six residues determined that at position 174, Gly is replaced by Ser. This change accounted for 30 Da. C-terminal sequencing by carboxypeptidases B and Y showed that the region 181–187 was the same as the bovine sequence, therefore a 9-Da change in the region 178–180 required that residue 180 be His, rather than Gln (Lee & Rahbar, 1991). Finally, carboxypeptidase subdigestion and linked-scan mass spectrometry confirmed the remaining sequence change as Asn 204 rather than Ser. Phosphorylation at Ser 203, which was reported using radiolabeled phosphate in bovine β B2 (Kleiman et al., 1988), was not observed in human β B2.

The calculated molecular weight of the human β B2 sequence we have proposed is 23,291 Da. Ion-spray mass spectrometric analysis gave a mass of 23,293 Da, which is within 0.01% of the calculated value. Peptide molecular weight mapping would not detect residue substitutions that conserve the peptide mass, such as between Ile and Leu or Gln and Lys, but the specificity of trypsin would differentiate between Gln and Lys. Also, the possibility exists that a peptide could have the same overall amino acid composition, and therefore mass, as the bovine peptide but a different primary sequence. However,

scrambling of residue sequences between similar species is uncommon.

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References

- Aarts, H.J.M., Den Dunnen, J.T., Lubsen, N.H., & Schoenmakers, J.G.G. (1987). Linkage between the β B2 and β B3 crystallin genes in man and rat: A remnant of an ancient β -crystallin gene cluster. *Gene* 59, 127–135.
- Aarts, H.J.M., Jacobs, E.H.M., van Willigen, G., Lubsen, N.H., & Schoenmakers, J.G.G. (1989a). Different evolution rates within the lens-specific β -crystallin gene family. *J. Mol. Evol.* 28, 313–321.
- Aarts, H.J.M., Lubsen, N.H., & Schoenmakers, J.G. (1989b). Crystallin gene expression during rat lens development. *Eur. J. Biochem.* 183, 31–36.
- Chambers, C. & Russell, P. (1991). Deletion mutation in an eye lens β -crystallin. *J. Biol. Chem.* 266, 6742–6746.
- Driessen, H.P.C., Herbrink, P., Bloemendal, H., & de Jong, W.W. (1981). Primary structure of the bovine β -crystallin Bp chain. *Eur. J. Biochem.* 121, 83–91.
- Hogg, D., Tsui, L.-C., Gorin, M., & Breitman, M.L. (1986). Characterization of the human β -crystallin gene Hu β A3/A1 reveals ancestral relationships among the β -crystallin superfamily. *J. Biol. Chem.* 261, 12420–12427.
- Inana, G., Shinohara, T., Maizel, J.V., Jr., & Piatigorsky, J. (1982). Evolution and diversity of the crystallins. *J. Biol. Chem.* 257, 9064–9071.
- Kleiman, N.J., Chiesa, R., Gawinowicz Kolks, M.A., & Spector, A. (1988). Phosphorylation of β -crystallin B2 (β Bp) in the bovine lens. *J. Biol. Chem.* 263, 14978–14983.
- Lee, T.D. & Rahbar, S. (1991). The mass spectral analysis of hemoglobin variants. In *Mass Spectrometry of Peptides* (D.M. Desiderio, Ed.), pp. 257–274. CRC Press, Boca Raton, Florida.
- Lorand, L., Hsu, L.K.H., Siefring, G.E., Jr., & Rafferty, N.S. (1981). Lens transglutaminase and cataract formation. *Proc. Natl. Acad. Sci. USA* 78, 1356–1360.
- Smith, J.B., Miesbauer, L.R., Leeds, J., Smith, D.L., Loo, J.A., Smith, R.D., & Edmonds, C.G. (1991). Mass spectrometric analysis of lens β -crystallins. *Int. J. Mass Spectrom. Ion Proc.* 111, 229–245.
- Velasco, P.T. & Lorand, L. (1987). Acceptor–donor relationships in the transglutaminase-mediated cross-linking of lens β -crystallin subunits. *Biochemistry* 26, 4629–4634.