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Primary structure of β_s-crystallin from human lens

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The complete primary structure of β_s-crystallin from human lens is reported. The sequence was elucidated by automatic Edman degradation of tryptic and CNBr peptides. The blocked *N*-terminal dipeptide was identified by fast-atom-bombardment mass spectroscopy. The sequence comparison with other members of crystallin family reveals a closer relationship to human γ-crystallin (53% identity) than with βA3/A1 crystallin (37% identity). The structure, evolutionary characteristics and role of β_s-crystallin in lens are discussed.

INTRODUCTION

Crystallins are the main structural proteins of mammalian eye lens. They are grouped into three classes, namely α-, β- and γ-crystallins. The α- and β-crystallins are high-molecular-mass proteins reported to be present as aggregates, whereas γ-crystallin is in monomeric form (Harding & Dilley, 1976). In addition to these components, another low-molecular-mass protein, designated as β_s-crystallin and also referred as γ_s (Bloemendal *et al.*, 1989), has been reported in mammalian lenses (van Dam, 1966). Initially, it was considered as a member of the β-crystallin family. However, recent studies have shown that β_s-crystallin lacks the characteristic *N*-terminal extension of the β-family, is monomeric and has a greater sequence resemblance to γ-crystallin (Quax-Jeuken *et al.*, 1985). β_s-Crystallin has been detected in human, bovine, rabbit, avian, carp and rat lenses (Croft, 1973; McDevitt & Croft, 1977; Bindels *et al.*, 1981; Zigler *et al.*, 1981, 1986; Chang & Chang, 1987; Thomson *et al.*, 1989). The amino acid sequence of the mammalian protein has not been reported so far, except for that deduced from the cDNA sequence for bovine (Quax-Jeuken *et al.*, 1985) β_s-crystallin. In continuation of our earlier studies (Zarina *et al.*, 1987, 1990; Zaidi *et al.*, 1988), we now report the complete amino acid sequence of β_s-crystallin from human cataractous lens. This is the first sequence report for this protein.

EXPERIMENTAL

Human senile mature cataractous lenses were collected from the Anklesaria Hospital and the Rehmattullah Benevolent Eye Hospital, Karachi, Pakistan. Lenses were stored at −70 °C until analysed.

Isolation of proteins

Lenses were decapsulated, homogenized in deionized water at 0 °C and centrifuged at 20000 *g* for 20 min at 4 °C. The supernatant was collected and designated as the water-soluble protein fraction.

Gel chromatography

The fractionation of the water soluble protein fraction (100 mg) was carried out with Sephadex G-50 (Superfine grade) equilibrated and eluted with 5% (v/v) acetic acid.

R.p.-h.p.l.c.

Peak III from Sephadex G-50 chromatography was re-chromatographed using Vydac RP-C_4 (Separation Group, Hesperia, CA, U.S.A.). The column was first equilibrated with 0.1% trifluoroacetic acid (TFA) and samples (1 mg/100 μl) were resolved by using a linear gradient of 0.1% TFA and acetonitrile (0–100%, v/v).

SDS/PAGE

Electrophoresis was performed as described by Laemmli (1970) with 12.5% (w/v) polyacrylamide gels.

Chemical cleavage

The purified fraction of β_s-crystallin was absorbed on a glass-fibre disc and a solution of CNBr in 70% (v/v) formic acid (1 mg/ml) was added to the disc. The cartridge was sealed and the reaction was allowed to proceed for 16 h at 37 °C. The cleaved peptides were then subjected to automated gas-phase sequence analysis (470 A; Applied Biosystems, Foster City, CA, U.S.A.). The amino acid phenylthiohydantoin (PTH) derivatives were identified by an on-line h.p.l.c./PTH analyser (120 A).

The results of cleavage *in situ* were supplemented by a normal digestion procedure (Gross & Witkop, 1961). Peptides were separated by reversed-phase h.p.l.c. (r.p.-h.p.l.c.) on RP-C_2 (LiChrosorb) using a gradient of 0.1% TFA and acetonitrile (0–100%).

Enzymic cleavage

The protein sample (500 μg) was dissolved in 100 μl of aq. 0.1% NH_3 and the pH adjusted to 8.5–8.7 with 0.1% acetic acid (Hirs, 1967). A 10 μl portion of 0.5% tosylphenylalanylchloromethane ('TPCK')-treated trypsin solution was added to the sample and left for 70 min at 35 °C. The reaction was stopped by titration with 0.1% acetic acid to pH 4.0. The whole solution was concentrated *in vacuo* and centrifuged at 3000 *g*. The tryptic cleavage products were subjected to h.p.l.c. on LiChrosorb RP-C_2 using 0.1% TFA and a linear gradient of 0–40% acetonitrile and further purified by rechromatography on RP-C_{18} wherever necessary.

Sequencing

Amino-acid-sequence analysis was performed on a gas-phase sequencer (470 A) using the Edman degradation method (Edman & Begg, 1967; Rodney *et al.*, 1981). PTH amino acids were identified by an on-line h.p.l.c. PTH analyser (120 A). The blocked *N*-terminal peptide was analysed by fast-atom-bombardment m.s. (f.a.b.-m.s.) (Jeol; HX110).

Abbreviations used: r.p.-h.p.l.c., reversed-phase h.p.l.c.; TFA, trifluoroacetic acid; PTH, phenylthiohydantoin; f.a.b.-m.s., fast-atom-bombardment m.s.

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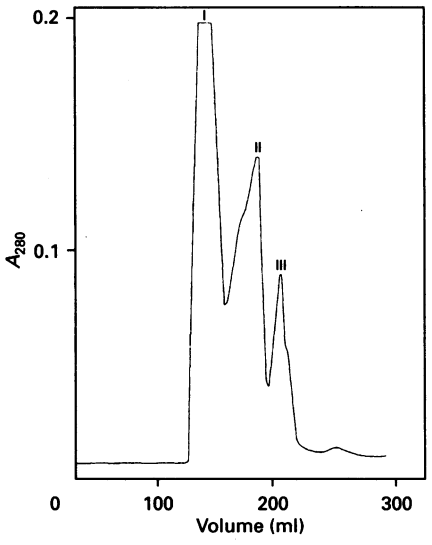

Fig. 1. Elution profile of water-soluble lens crystallins on Sephadex G-50 (superfine grade)

Elution details: column size: 115 cm × 2 cm; eluent 5% acetic acid; flow rate 10 ml/h; the absorbance at 280 nm was recorded.

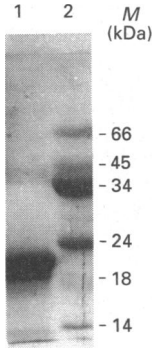

Fig. 2. SDS/PAGE of peak III from Sephadex G-50

From left to right: 1, peak III; 2, molecular-mass (*M*) standards

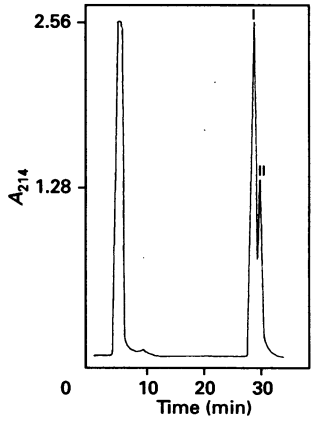

Fig. 3. H.p.l.c. purification of peak III from Sephadex G-50 on RP-C_2

The column was eluted with a 0.1% TFA/acetonitrile gradient. The flow rate was 60 ml/h, and absorbance was recorded at 214 nm.

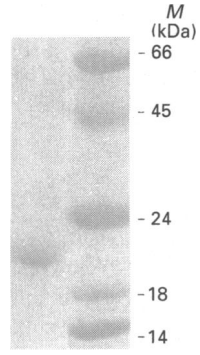

Fig. 4. SDS/PAGE of peak II from RP-C_2

From left to right: 1, peak II; 2, molecular-mass (*M*) standards.

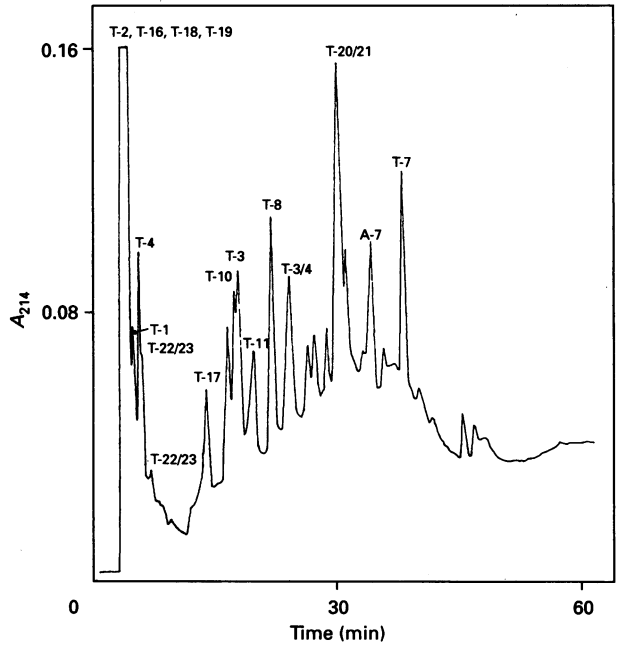

Fig. 5. H.p.l.c. purification of tryptic peptides from β_s-crystallin

The column was eluted with a 0.1% TFA/acetonitrile gradient. The flow rate was 60 ml/h, and absorbance was recorded at 214 nm. T and A represent tryptic and unspecific cleavages respectively.

RESULTS

Fig. 1 shows the elution profile of crude water-soluble lens proteins on Sephadex G-50. SDS/PAGE revealed the presence of low-molecular-mass proteins in fraction III (Fig. 2). Rechromatography of this fraction on r.p.-h.p.l.c. showed the presence of two peaks (Fig. 3). These peaks were found to be pure on SDS/PAGE. The second peak showed a molecular mass of about 21 kDa on SDS/PAGE (Fig. 4). Both the peaks were subjected to Edman degradation. *N*-Terminal analysis of peak I revealed it to be γ-crystallin (Zarina *et al.*, 1990), whereas peak II did not respond to Edman degradation, as β_s-crystallin has a blocked *N*-terminus (van Dam, 1966; Croft, 1973; Zigler *et al.*, 1981). This fraction was subjected to cleavage *in situ* with CNBr, and subsequent sequence analysis confirmed the presence of a blocked *N*-terminus. The fraction (peak II from Fig. 3) was subjected to enzymic and chemical cleavage. Fig. 5 shows the elution profile of tryptic peptides, whereas Fig. 6 shows rechromatography of peptides eluted with injection spikes on

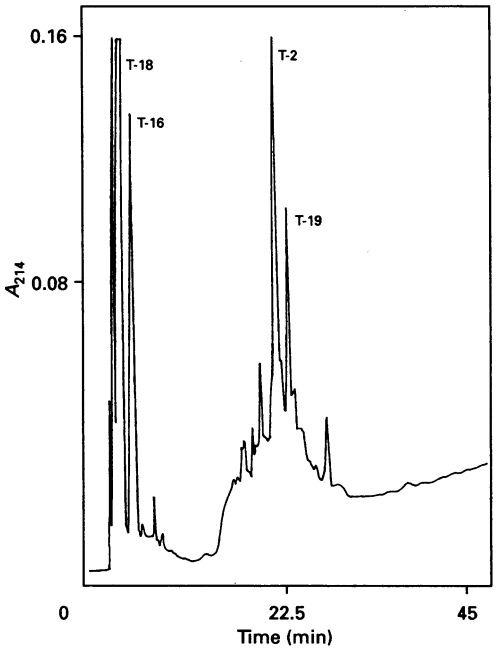

Fig. 6. Rechromatography of tryptic peptides on RP-C_{18}

For details, see the text.

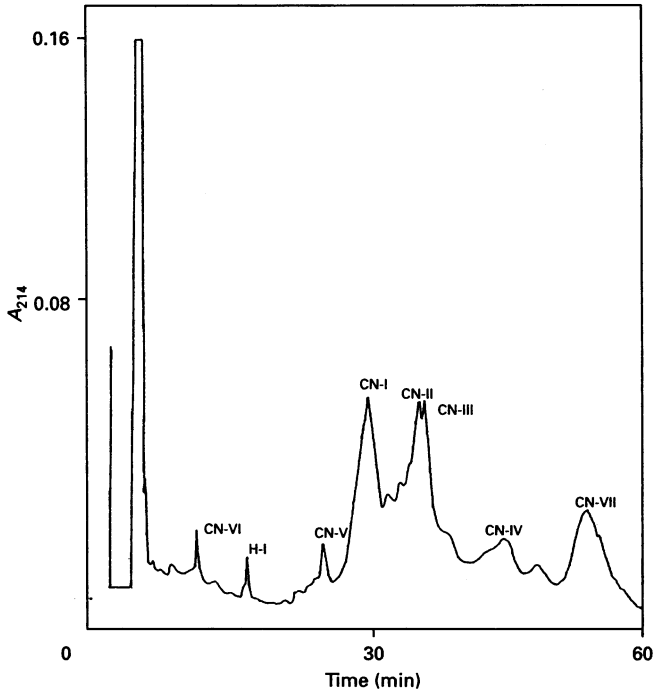

Fig. 8. H.p.l.c. purification of CNBr peptides of β_s-crystallin on RP-C_2

CNBr and H represent CNBr and hydrolytic cleavages respectively. Conditions were the same as those in Fig. 3.

RP-C_{18}. The purified peptides were subjected to automatic gas-phase sequencing. Peptides T 3/4 (7–18) and T 20/21 (158–173) were co-eluted, as polar groups preceding Arg or Lys reduce the rate of hydrolysis, whereas Lys–Pro or Arg–Pro bonds appear to be totally restricted to tryptic cleavage (Kasper, 1975). Multiple forms of the same peptides were also observed, as is expected for tryptophan- and methionine-containing peptides. The blocked peptide (T1) was subjected to f.a.b.-m.s. and showed a protonated

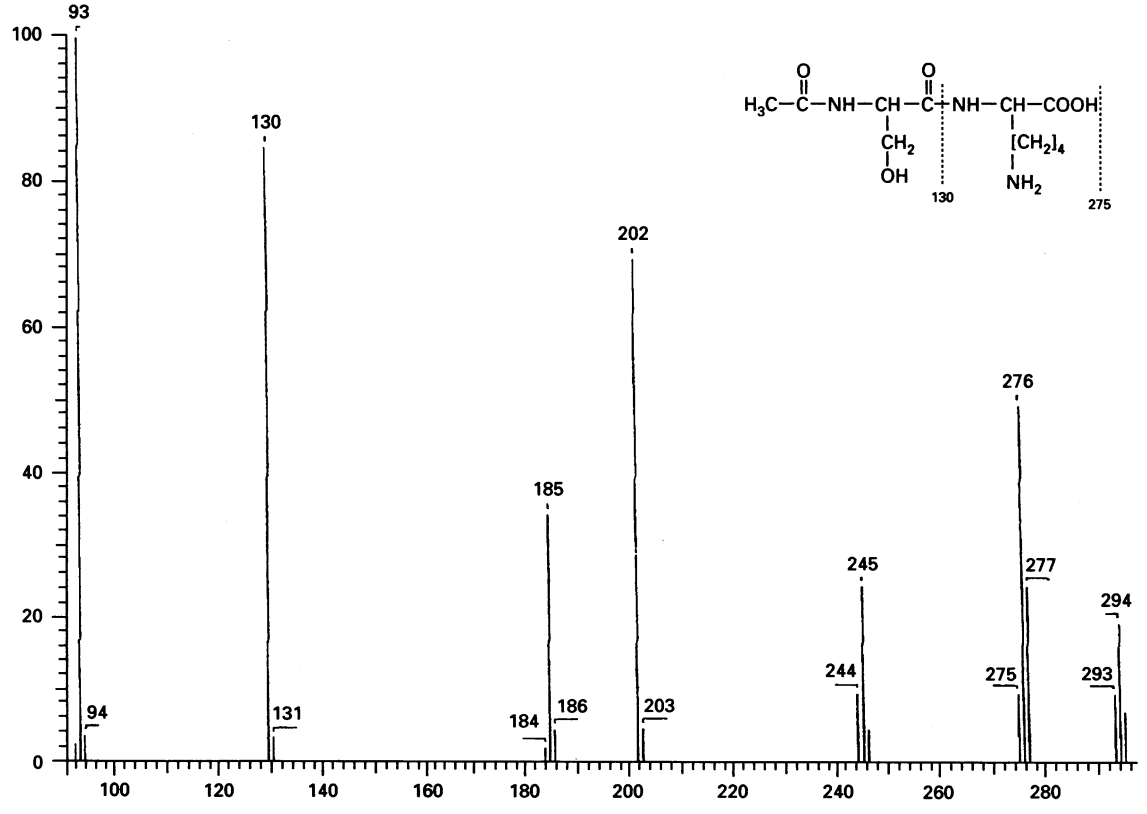

Fig. 7. F.a.b.-m.s. spectra of peptide T1

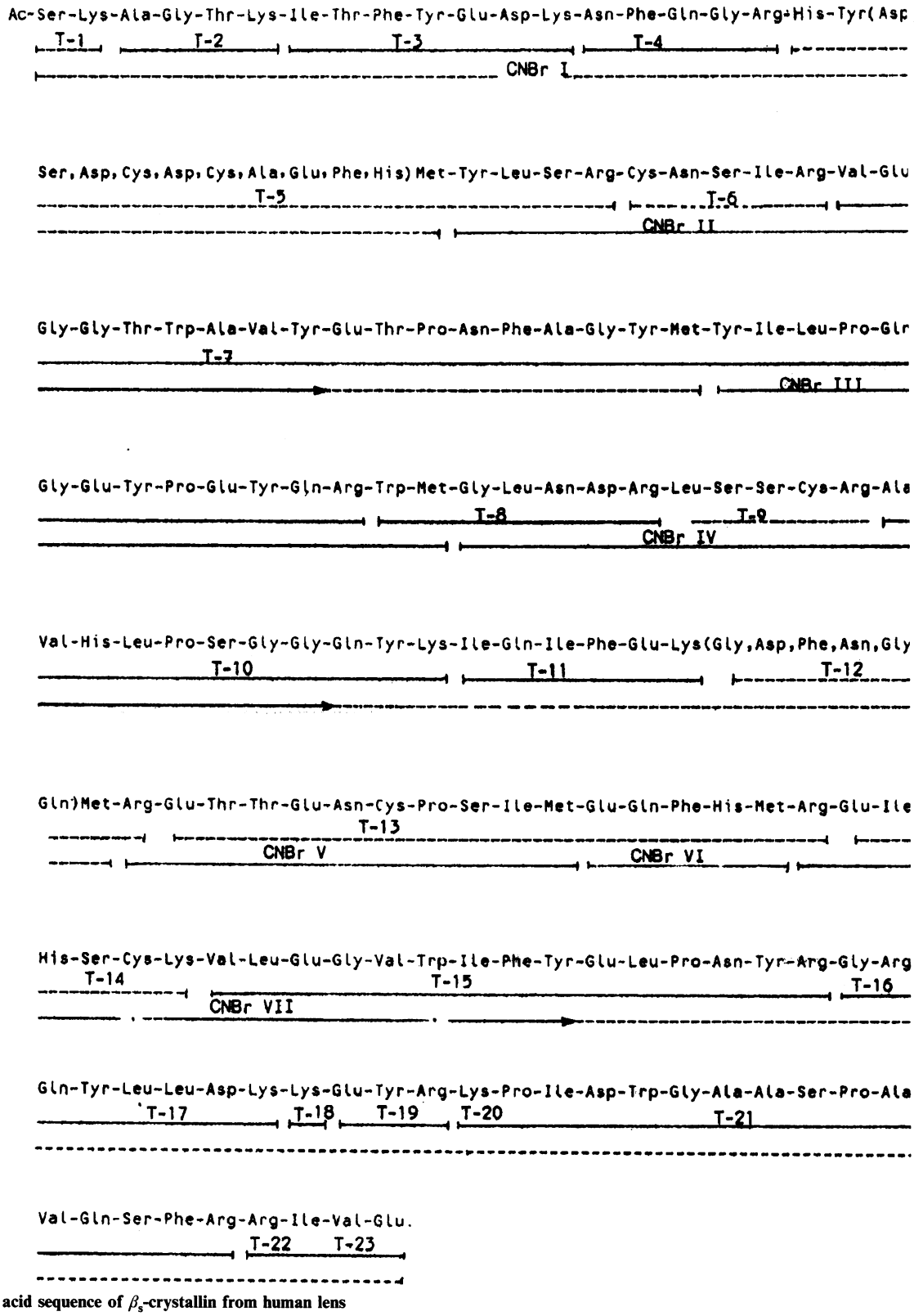

Fig. 9. Amino acid sequence of β_s-crystallin from human lens

Sequences were determined by gas-phase sequence analysis (——). The broken line indicates amino acid composition. Sequenced residues are connected by hyphens (-). Residues which have not been identified reliably but placed on the basis of amino acid composition and sequence similarity to other β_s-crystallins are connected by commas and given in parenthesis. The arrows indicate the position to which sequence analysis was carried out, and the dot represents residues which were placed on the basis of sequence similarity. T and CNBr denote tryptic and CNBr peptides respectively.

molecular ion peak at m/z 276 (Fig. 7) representing the presence of *N*-acetylated serine followed by lysine, as has been reported by Quax-Jeuken *et al.* (1985) for bovine β_s-crystallin on the basis of cDNA studies. Fig. 8 shows the separation profile of CNBr peptides; seven peptides were resolved. Of these, CNBr 1 had a blocked *N*-terminus, and thus could not be sequenced. CNBr 2, 3, 4, 5, 6 and 7 were sequenced on gas-phase sequencer. Although CNBr is specific for cleavage of methionine residues, some hydrolytic cleavage was also observed at the Trp–Ala position (46–47). Fig. 9 presents the primary structure of β_s-crystallin. A few residues which were not reliably identified but could be placed by sequence identity with other β_s-crystallins on the basis of amino acid analysis are given in parenthesis.

DISCUSSION

Primary structure and evolutionary relationship

β_s-Crystallin is a monomeric protein present in the low-molecular-mass fraction of the lens (Bindels *et al.*, 1981; Zigler *et al.*, 1981, 1986; Thomson *et al.*, 1989). The molecular mass of β_s-crystallin has been a matter of controversy for the last two decades. van Dam (1966), on the basis of amino acid composition and sedimentation analysis, reported it to be 28400 Da, whereas Bloemendal & Zweers (1976) estimated it to be 22000 Da by SDS/PAGE. Bindels *et al.* (1981) found a similar discrepancy obtaining a molecular mass of 22000 Da by SDS/PAGE and 28500 Da by sedimentation-equilibrium analysis. Quax-Jeuken *et al.* (1985) found the molecular mass of bovine β_s-crystallin to be 20773 Da on the basis of cDNA-derived sequence. According to our studies, based on its primary structure, the molecular mass of human β_s-crystallin is 20891 Da, which is very close to the one reported by Quax-Jeuken *et al.* (1985).

Table 1. Percentage identity between $\beta\gamma$-crystallins as an index of evolutionary diversification

References: [a]Hogg *et al.* (1986); [b]Meakin *et al.* (1987); [c]Quax-Jeuken *et al.* (1985).

	$H\beta_s$	$H\beta A_3/A_1$	$H\gamma 1\phi$	$H\gamma 2\phi$	$H\gamma 5$	$H\gamma 4$	$H\gamma 3$	$H\gamma 1$–2	$B\beta_s$
$H\beta_s$	100								
$H\beta A_3/A_1$[a]	37	100							
$H\gamma 1\phi$[b]	49	32	100						
$H\gamma 2\phi$[b]	51	35	89	100					
$H\gamma 5$[b]	50	33	70	69	100				
$H\gamma 4$[b]	50	35	81	84	71	100			
$H\gamma 3$[b]	53	38	74	73	74	71	100		
$H\gamma 1$–2[b]	53	34	71	70	73	72	79	100	
$B\beta_s$[c]	93	38	50	51	51	49	53	53	100

Table 2. Percentage identity of β_s-crystallin with other proteins

References: [a]Summers *et al.* (1986); [b]Croft (1972); [c]Driessen *et al.* (1981); [d]Quax-Jeuken *et al.* (1985); [e]Ingolia & Craig (1982); [f]Czarnecka *et al.* (1985); [g]Rosen *et al.* (1985).

Crystallins	Identity (%)	Non-crystallins	Identity (%)
Rat[a]		*Myxococcus xanthus* protein S[d]	20
γ1–1	51		
γ1–2	52		
γ2–1	50	Soybean heat-shock protein[e]	18
γ2–2	51		
γ3–1	50		
γ4–1	50	*Drosophila* heat-shock protein 22[f]	14
Calf γ2[b]	52	*Dictyostelium* heat-shock protein (large)[g]	15
Bovine βB_P[c]	34		

β_s-Crystallin has been shown to be present in amphibian, avian and mammalian lenses (Croft, 1973; McDevitt & Croft, 1977; Bindels *et al.*, 1981; Zigler *et al.*, 1981, 1986; Chang & Chang, 1987; Thomson *et al.*, 1989). However, the sequence data derived from cDNA have only been obtained for the bovine (Quax-Jeuken *et al.*, 1985) and carp (Chang & Chang, 1987) proteins. Table 1 shows a comparison of the amino acid sequence of human β_s-crystallin with other known sequences of β- and γ-crystallins. Alignment of the β_s-crystallin sequence with the cDNA-deduced γ-crystallin sequence from human shows 49–53% identity (82–88 exchanges), whereas with human βA3/A1-crystallin the identity is only 37% (150 exchanges). A comparison of β_s-crystallin with the bovine one shows more than 93% identity. The observed exchanges include 10 Phe → Tyr, 28 Asp → Glu, 51 Arg → Thr, 63 Arg → Gln, 71 His → Arg, 88 Ser → Pro, 95 Leu → Ile, 108 His → Arg, 113 Asp → Asn, 126 Val → Ile, 135 Ala → Val and 160 Val → Ile. An important feature of β-crystallin is the presence of *N*-terminal arms or extensions (Berbers *et al.*, 1984) which has been reported to play a role in aggregation (Slingsby & Bateman, 1990). Human β_s-crystallin, like the bovine β_s-crystallin (Quax-Jeuken *et al.*, 1985) is a monomer possessing a short *N*-terminal arm of four residues against 12–58 residues in other members of the β-crystallin family (Quax-Jeuken *et al.*, 1985; Wistow & Piatigorsky, 1988). The carp β_s-crystallin sequence (Chang & Chang, 1987), however, lacks the *N*-terminal arm of bovine and human β_s-crystallin and resembles γ-crystallins in this respect.

Among other proteins, heat-shock proteins from *Glycine max* (soybean), *Drosophila* (fruitfly) and *Dictyostelium* (slime mould) and protein S from *Myxococcus xanthus* exhibit structural similarity with human β_s-crystallin. The percent identity in each case is presented in Table 2. Heat-shock protein from soybean has an identity of 18% with human β_s-crystallin. It has striking similarity to α-crystallins sequences, suggesting a possible similarity in function (Ingolia & Craig, 1982). Human and bovine β_s-crystallin show 20% and 17.1% identity respectively with protein S. Quax-Jeuken *et al.* (1985) have constructed a phylogenetic tree for the $\beta\gamma$-crystallin superfamily having Protein S at its roots, and concluded that bovine β_s is related more to γ- than to β-crystallin. Our sequence studies on human β_s-crystallin also support this relationship.

The involvement of γ-crystallins in opacification has long been suspected because they are located in the lens nucleus and have been shown to be absent (Croft, 1973) or depressed during aging and cataractogenesis (Harding & Dilley, 1976; Siezen *et al.*, 1985). As β_s- and γ-crystallin have a similar character, it would be more appropriate to examine the role of β_s-crystallin in cataract formation. Furthermore, it is well known that synthesis of γ-crystallin decreases sharply during aging, in contrast with β_s-crystallin, which increases with age in both bovine and human lenses (Zigler *et al.*, 1981). Further studies are required to evaluate the role of β_s- as well as γ-crystallin in human cataractogenesis.

Tertiary structure of β_s-crystallin

Amino-acid-sequence analysis has shown a considerable identity between β- and γ-crystallins (Driessen *et al.*, 1981; Inana *et al.*, 1982); a major difference is the presence of *N*- and *C*-terminal arms in β-crystallins (Wistow & Piatigorsky, 1988). The three-dimensional structure of bovine γII (now γB) has revealed a

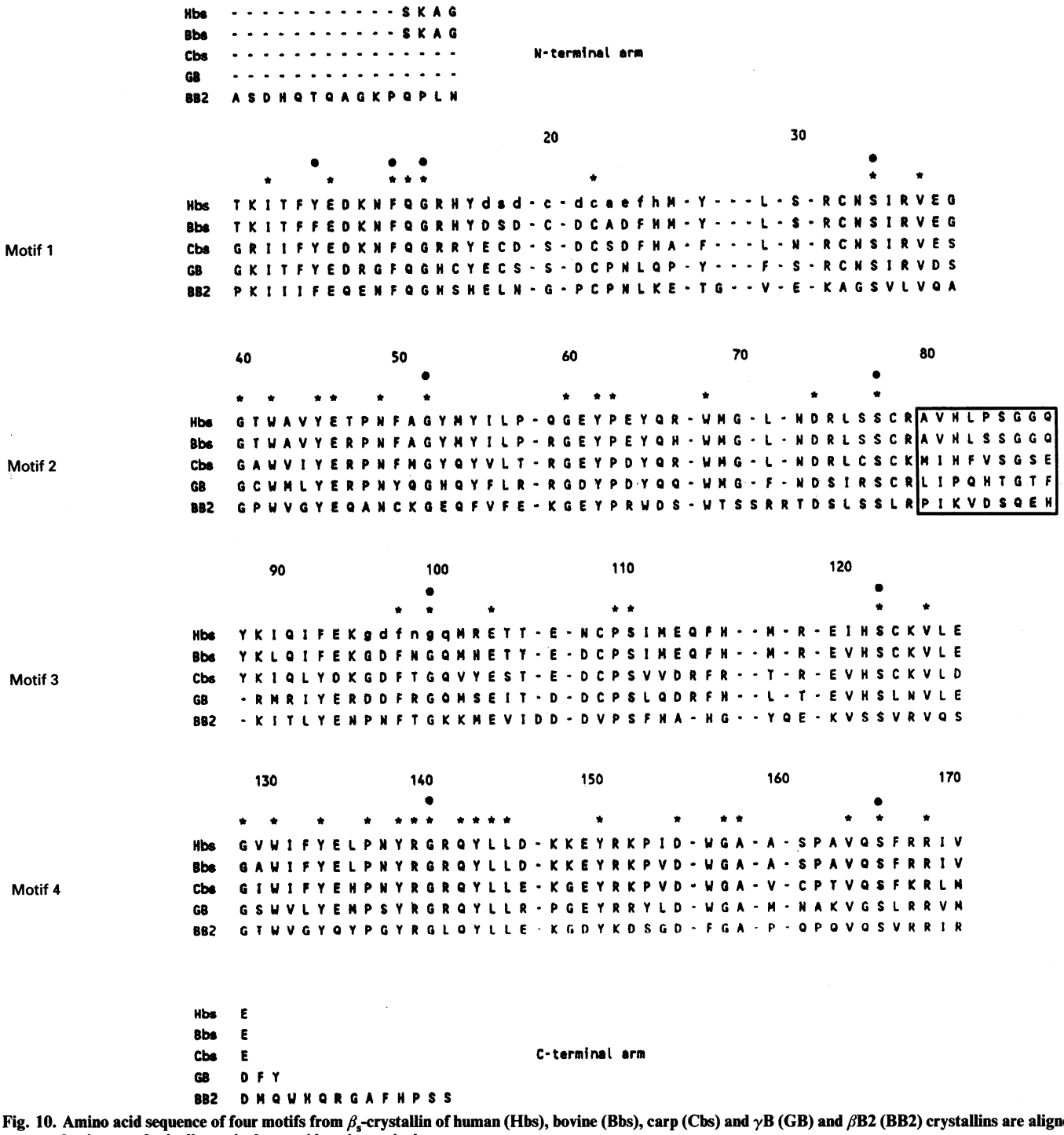

Fig. 10. Amino acid sequence of four motifs from β_s-crystallin of human (Hbs), bovine (Bbs), carp (Cbs) and γB (GB) and βB2 (BB2) crystallins are aligned having topologically equivalent residues in vertical rows

Motifs 1,3 and 2,4 are grouped together to depict their close similarity. Protein sequence is shown in single-letter code. Amino acid residues which could not be definitely placed in the sequence are given in lower-case letters. Gaps are introduced to optimize alignment. The numbering is based on the sequence of γB-crystallin. Structurally conserved residues are shown by an asterix (*). Solid circles indicate residues required for maintaining tertiary structure (Wistow *et al.*, 1981). The connector peptide is boxed.

four-fold internally repeating sequence corresponding to four 'Greek key' motifs arranged in two compact domains (Blundell *et al.*, 1981). The polypeptide contains some conserved residues (Fig. 10) which are required for a fold-over and for locking each motif. β-Crystallins also reportedly possess these amino acids, suggesting a similar tertiary fold (Wistow *et al.*, 1981; Inana *et al.*, 1983; Slingsby *et al.*, 1988). However, a recent study on βB2 crystallin (formerly βBp) has shown that its two domains are not as close together as in γ-crystallin, and, additionally, the contacts between the domains are largely intermolecular, favouring the formation of monomeric, dimeric and higher aggregate forms (Bax *et al.*, 1990). In contrast, the contacts in γ-crystallins are predominently intramolecular. Another difference is the presence of a Gly residue at position 86 in γ-crystallin. This important feature allows a sharp turn in the molecule, making interaction between the domains possible. This amino acid has not been

detected in this position in the β-crystallins so far studied. These contain an extended connecting peptide with bulky polar side-chain amino acids. However, the present study shows that the connecting peptide does possess Gly at position 86 (Fig. 10), and a comparison with cDNA-derived sequences of the same protein in the cow (Quax-Jeuken *et al.*, 1985) and the carp (Chang & Chang, 1987) also show the same amino acid at position 86. This suggests a γ-crystallin-like interaction between the domains.

In the light of our findings, we feel that neither β_s nor γF (as suggested by Quax-Jeuken *et al.*, 1985) is the correct nomenclature for this protein. A comparison of amino acid sequence of human β_s shows 37% identity with β-crystallin and 53% with γ-crystallin. However, despite greater identity with γ-crystallin, especially at conserved residues, the sequence variation is twice that observed within the family members of γ-crystallin which ranges between 21 and 29%. This suggests that the protein might belong to a group of its own. However, with the limited data available, it seems appropriate at present to classify them as γ-like crystallins.

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REFERENCES

Bax, B., Lapatto, R., Nalini, V., Driessen, H., Lindley, P. F., Mahadevan, D., Blundell, T. L. & Slingsby, C. (1990) Nature (London) **347**, 776–780

Berbers, G. A. M., Hoekman, W. A., Bloemendal, H., de Jong, W. W., Kleinschmidt, T. & Braunitzer, G. (1984) Eur. J. Biochem. **139**, 467–479

Bindels, J. G., Koppers, A. & Hoenders, H. J. (1981) Exp. Eye Res. **33**, 333–343

Bloemendal, H. & Zweers, A. (1976) Doc. Ophthalmol. Proc. Ser. **8**, 91–104

Bloemendal, H., Piatigorsky, J. & Spector, A. (1989) Exp. Eye Res. **48**, 465–466

Blundell, T., Lindley, P., Miller, L., Moss, D., Slingsby, C., Tickle, I., Turnell, B. & Wistow, G. (1981) Nature (London) **289**, 771–777

Chang, T. & Chang, W.-C. (1987) Biochim. Biophys. Acta **910**, 89–92

Croft, L. R. (1972) Biochem. J. **128**, 961–970

Croft, L. R. (1973) Ciba Found. Symp. **19**, 207–226

Czarnecka, E., Gurley, W. B., Nagao, R. T., Mosquera, L. A. & Key, J. A. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 3726–3730

Driessen, H. P. C., Herbrink, P., Bloemendal, H. & de Jong, W. W. (1981) Eur. J. Biochem. **121**, 83–91

Edman, P. & Begg, G. (1967) Eur. J. Biochem. **1**, 80–91

Gross, E. & Witkop, B. (1961) J. Am. Chem. Soc. **83**, 1510–1511

Harding, J. J. & Dilley, K. J. (1976) Exp. Eye Res. **22**, 1–73

Hirs, C. H. W. (1967) Methods Enzymol. **11**, 216–222

Hogg, D., Tsui, L.-C., Gorin, M. & Breitman, M. L. (1986) J. Biol. Chem. **261**, 12420–12427

Inana, G., Shinohara, T., Maizel, J. V. & Piatigorsky, J. (1982) J. Biol. Chem. **257**, 9064–9071

Inana, G., Piatigorsky, J., Norman, B., Slingsby, C. & Blundell, T. (1983) Nature (London) **302**, 310–315

Ingolia, T. D. & Craig, E. A. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 2360–2364

Kasper, C. B. (1975) in Protein Sequence Determination (Needleman, S. B., ed.), pp. 114–158, Springer-Verlag, Berlin and Heidelberg

Laemmli, U. K. (1970) Nature (London) **277**, 680–685

McDevitt, D. S. & Croft, L. R. (1977) Exp. Eye Res. **25**, 473–481

Meakin, S. O., Du, R. P., Tsui, L.-C. & Breitman, M. L. (1987) Mol. Cell. Biol. **7**, 2671–2679

Quax-Jeuken, Y., Driessen, H., Leunissen, J., Quax, W., de Jong, W. W. & Bloemendal, H. (1985) EMBO J. **4**, 2597–2602

Rodney, M. H., Hunkapiller, M. W., Hood, L. E. & Dreyer, W. J. (1981) J. Biol. Chem. **256**, 7990–7997

Rosen, E., Sivertsen, A., Firtel, R. A., Wheeler, S. & Loomis, W. F. (1985) in Changes in Eukaryotic Gene Expression in Response to Environmental Stress (Atkinson, B. G. & Walden, D. B., ed.), pp. 257–278, Academic Press, Orlando

Siezen, R. J., Fisch, M. R., Slingsby, C. & Benedek, G. B. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 1701–1705

Slingsby, C. & Bateman, O. A. (1990) Biochemistry **29**, 6593–6599

Slingsby, C., Driessen, H. P. C., Mahadevan, D., Bax, B. & Blundell, T. (1988) Exp. Eye Res. **46**, 375–403

Summers, L. J., Slingsby, C., Blundell, T., den Dunnen, J. T., Moormann, R. J. M. & Schoenmakers, J. G. G. (1986) Exp. Eye Res. **43**, 77–92

Thomson, J. A., Siezen, R. J., Kaplan, E. D., Messmer, M. & Chakrabarti, B. (1989) Curr. Eye Res. **8**, 139–149

van Dam, A. F. (1966) Exp. Eye Res. **5**, 255–266

Wistow, G. & Piatigorsky, J. (1988) Annu. Rev. Biochem. **57**, 479–504

Wistow, G., Slingsby, C., Blundell, T., Driessen, H. P. C., de Jong, W. W. & Bloemendal, H. (1981) FEBS Lett. **133**, 9–16

Zaidi, Z. H., Zarina, S. & Wania, J. H. (1988) in Protein Structure–Function Relationship (Zaidi, Z. H., ed), pp. 337–344, Elsevier, Amsterdam

Zarina, S., Wania, J. H. & Zaidi, Z. H. (1987) J. Pak. Med. Assoc. **37**, 67–70

Zarina, S., Abbasi, A., Wania, J. H. & Zaidi, Z. H. (1990) in Proc. Natl. Chem. Conf. 2nd (Kazmi, A., Naqvi, R. R., Zaidi, Z. H. & Atta-ur-Rahman, eds.), pp. 154–157, B.C.C. & T. Press, Karachi

Zigler, J. S., Jr., Horwitz, J. & Kinoshita, J. H. (1981) Exp. Eye Res. **32**, 21–30

Zigler, J. S., Jr., Russell, P., Horwitz, J., Reddy, V. N. & Kinoshita, J. H. (1986) Curr. Eye Res. **5**, 395–401

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