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Three-dimensional model and quaternary structure of the human eye lens protein γS-crystallin based on β- and γ-crystallin X-ray coordinates and ultracentrifugation

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Abstract

A 3-dimensional model of the human eye lens protein γS-crystallin has been constructed using comparative modeling approaches encoded in the program COMPOSER on the basis of the 3-dimensional structure of γ-crystallin and β-crystallin. The model is biased toward the monomeric γB-crystallin, which is more similar in sequence. Bovine γS-crystallin was shown to be monomeric by analytical ultracentrifugation without any tendency to form assemblies up to concentrations in the millimolar range. The connecting peptide between domains was therefore built assuming an intramolecular association as in the monomeric γ-crystallins. Because the linker has 1 extra residue compared with γB and βB2, the conformation of the connecting peptide was constructed by using a fragment from a protein database. γS-crystallin differs from γB-crystallin mainly in the interface region between domains. The charged residues are generally paired, although in a different way from both β- and γ-crystallins, and may contribute to the different roles of these proteins in the lens.

Keywords: comparative modeling; domain interactions; eye lens protein; γS-crystallin; ultracentrifugation

Transparency of the eye lens is a function of tightly packed structural proteins called crystallins that provide a medium of high refractive index. They are classified into 2 major families, α- and βγ-crystallins, and account for 80–90% of the total soluble proteins in mammalian lenses (Wistow & Piatigorsky, 1988). βγ-Crystallins share a sequence identity of around 30% (Lubsen et al., 1988). β-Crystallins are oligomers composed of basic and acidic subunits that are about 45% related (Berbers et al., 1984; van Rens et al., 1991), whereas γ-crystallins are 21-kDa monomers of around 80% sequence identity (den Dunnen et al., 1986; Hay et al., 1987). β-Crystallin sequences differ from γ-crystallins mainly in having extensions at the N- and C-termini. X-ray analysis of bovine βγ-crystallins has shown that they are built of 4 Greek key motifs organized as 2 similar domains (Blundell et al., 1981; Bax et al., 1990). The 2 families differ in the conformation of their connecting peptides. In β-crystallins, it is extended, whereas in γ-crystallins, it takes a sharp turn. As a result, pairs of domains associate intramolecularly to form monomeric proteins in γ-crystallins, whereas in βB2, the interaction is intermolecular, leading to oligomeric association (Fig. 1). Transplantation of the βB2 connecting peptide into γB-crystallin yields a monomeric protein, showing that specificity for domain organization resides outside the linker sequence (Mayr et al., 1994).

An interesting member of the βγ-crystallin superfamily is γS because it shares features of both β- and γ-crystallins. Recent sequence analyses of bovine, carp, and human γS have shown that it is more closely related to monomeric γ-crystallins (53% sequence identity) compared to β-crystallins (35% identity) (Quax-Jeuken et al., 1985; Chang & Chang, 1987; Zarina et al., 1992). However, whereas the majority of γ-crystallins are deposited in regions of the lens that have a high refractive index and hence have more densely packed protein, γS-crystallin is found mainly in more hydrated regions of the lens (Slingsby, 1985). Human γS-crystallin is a major component of the low

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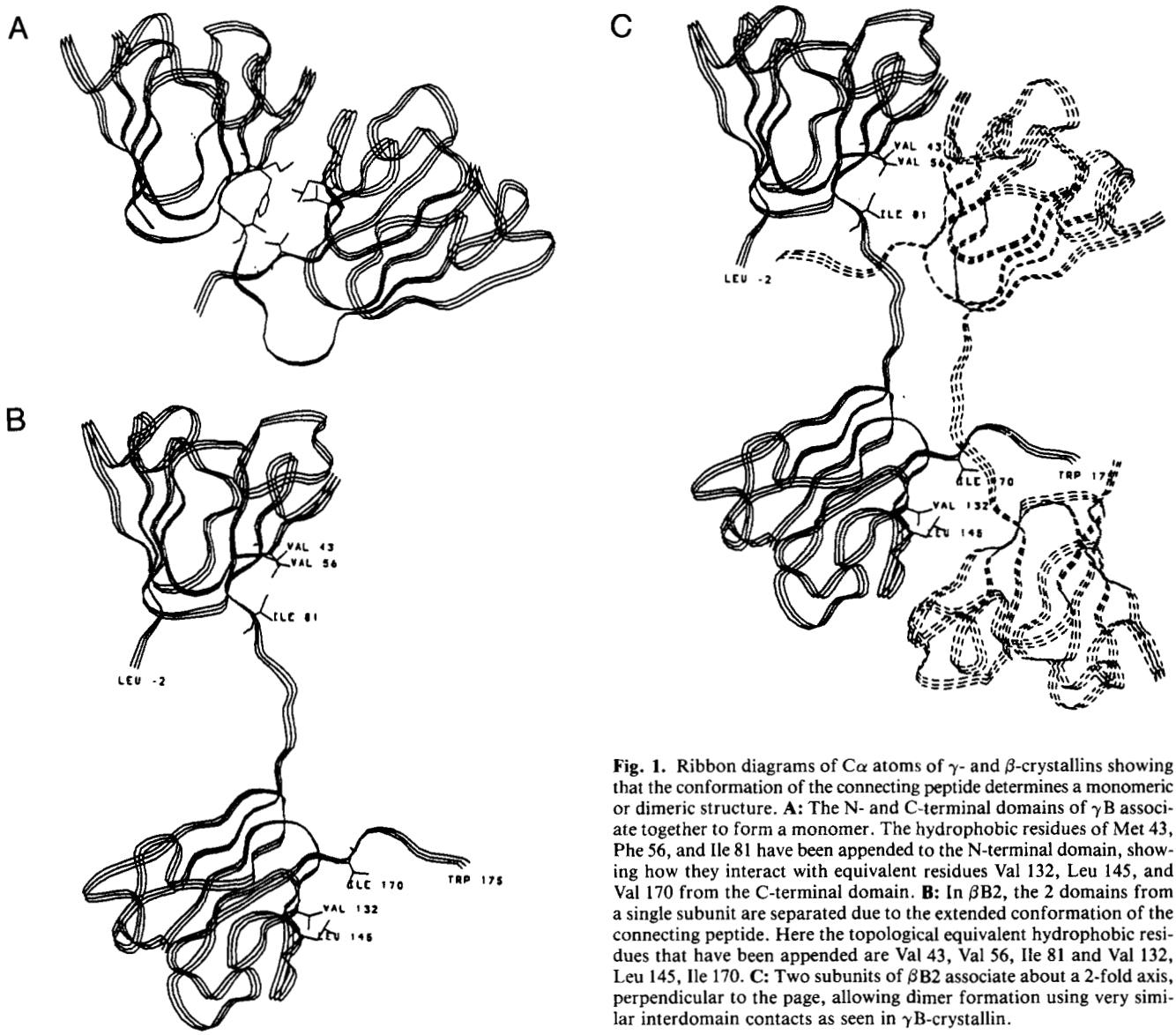

Fig. 1. Ribbon diagrams of Cα atoms of γ- and β-crystallins showing that the conformation of the connecting peptide determines a monomeric or dimeric structure. **A:** The N- and C-terminal domains of γB associate together to form a monomer. The hydrophobic residues of Met 43, Phe 56, and Ile 81 have been appended to the N-terminal domain, showing how they interact with equivalent residues Val 132, Leu 145, and Val 170 from the C-terminal domain. **B:** In βB2, the 2 domains from a single subunit are separated due to the extended conformation of the connecting peptide. Here the topological equivalent hydrophobic residues that have been appended are Val 43, Val 56, Ile 81 and Val 132, Leu 145, Ile 170. **C:** Two subunits of βB2 associate about a 2-fold axis, perpendicular to the page, allowing dimer formation using very similar interdomain contacts as seen in γB-crystallin.

molecular weight crystallins in human lens, and it is well known that these molecules are either degraded or become crosslinked in cataract (Harding & Crabbe, 1984).

Using the computer graphics program FRODO (Jones, 1978), bovine γS-crystallin has already been modeled based on the coordinates of bovine γB-crystallin (Quax-Jeuken et al., 1985). Prediction of tertiary structures of proteins can now be carried out employing rule-based approaches when several related structures are known. We have built a model of human γS-crystallin (Zarina et al., 1992) using the program COMPOSER (Sutcliffe et al., 1987a, 1987b; Blundell et al., 1988) based on the coordinates of both bovine γB- and bovine βB2-crystallins. We have performed ultracentrifugation studies on bovine γS-, γB-, and βB2-crystallin in order to determine the quaternary organization of γS-crystallin. Three-dimensional models of the different members of the βγ-crystallin superfamily will aid our understanding of their role in providing optical acuity and maintaining lens transparency.

Results and discussion

Quaternary structure

In order to determine the state of association of γS, analytical ultracentrifugation was applied using γB and βB2 for comparison. Sedimentation velocity experiments at 44,000 rev/min showed that all 3 proteins are homogeneous, without any trailing due to aggregation and/or dissociation. The sedimentation coefficients are indicative of identical molecular weights (around 20,000) for γS and γB, whereas βB2 clearly represents a dimer in the range beyond 40,000 (Table 1), in agreement with previous determinations. High-speed sedimentation equilibria under meniscus depletion conditions (≤20,000 rev/min) confirm this result. For all 3 systems, linearization in the ln c versus r^2 diagram is possible over the whole concentration range from 0.01 to ~4 mg/mL (correlation coefficient ≥0.9997) (Fig. 2). Thus, we conclude that both γS and γB are monomeric and closely similar regarding their domain organization.

Table 1. *Sedimentation analysis of* γS-, γB-, and βB2-crystallins[a]

Crystallin	$M_{1,calc}$ (Da)	$s^{\circ}_{20,w}$ (S)	$D^{\circ}_{20,w}$ (F)	M_W (kDa)	$M_{s,D}$ (kDa)	f/f_o	Hydration[b] (g/g)
γS	20,839	2.30 ± 0.07	10.8 ± 0.5	20.6 ± 0.9	19.1 ± 1.0	1.14	0.38
γB	20,966	2.23 ± 0.08	9.5 ± 0.5	20.1 ± 1.1	21.1 ± 1.2	1.13	0.35
βB2	23,280	4.23 ± 0.05	7.6 ± 0.9	46.7 ± 1.9	50.0 ± 4.0	1.07	~0.2

[a] The results for γS and γB were obtained in a 6-hole rotor under identical conditions. Limits of error include deviations in UV scans at 280, 286, and 295 nm, respectively. M_1, M_W, $M_{s,D}$: calculated molecular mass of the polypeptide chain, weight average molecular mass from sedimentation equilibrium, molecular mass from sedimentation velocity ($s^{\circ}_{20,w}$), and quasielastic light scattering ($D^{\circ}_{20,w}$). f/f_o calculated from $s^{\circ}_{20,w}$ and M_W.
[b] Determined from Oncley diagrams (Oncley, 1941), assuming a spherical shape for the domains and an elongated shape for the bilobal monomers.

Although γS has a similar molecular mass as γB (see above), it appears to show a difference in its frictional properties as judged by gel filtration behavior (cf. Bindels et al., 1981; Thomson et al., 1989). This could be due to differences in shape or hydration because differences in the state of association can be excluded (Table 1). However, the close similarity of both the *s*-values and the frictional ratios show that differences between the 2 proteins regarding their hydration are insignificant within the limits of detection (Table 1).

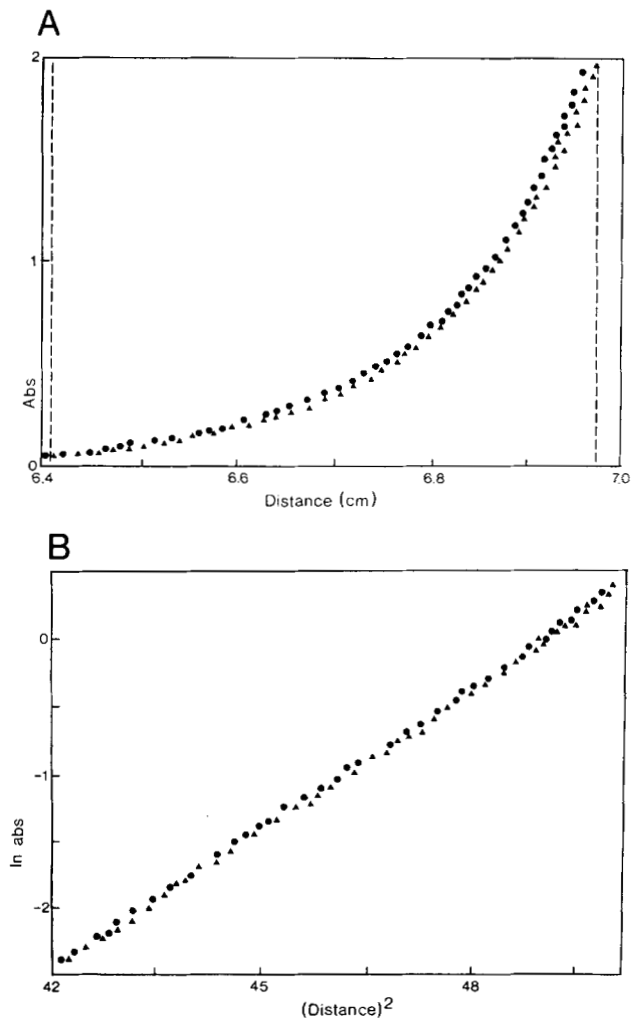

Fig. 2. Meniscus-depletion sedimentation equilibrium of γS- and γ-crystallins at 20,000 rev/min in 50 mM sodium phosphate buffer, pH 7.0, and 25 °C. Initial protein concentrations: 1.8 mg/mL for γS (●) and 2.0 mg/mL for γB (▲). **A:** Radial distribution scanned at 286 nm. **B:** Linearization of absorbance profile A.

Connecting peptide

The alignment of the sequences of human γS-, bovine γB-, and bovine βB2-crystallins is shown in Figure 3. Structural features are indicated at individual residue positions by the program JOY (Overington et al., 1990). Superposition of the Cα backbone of the COMPOSER model of human γS-crystallin and bovine γB-crystallin shows that they are very similar, with the exception that γS-crystallin has an additional residue (Gly 85A) in the connecting peptide and has a short N-terminal extension (Fig. 4A). The model building shows that the human γS sequence can be built with an intramolecular linker of good stereochemistry, although with a different conformation from γB-crystallin (Fig. 4B).

N- and C-terminal extensions

The oligomeric β-crystallins are characterized by the presence of sequence extensions compared to the γ-crystallins: basic β-crystallins have long N-terminal extensions and a shorter C-terminal extension, whereas acidic β-crystallins have only an N-terminal extension, having lost the short C-terminal extension of γ-crystallins (Berbers et al., 1984; Peterson & Piatigorsky, 1986). γS-crystallins have a short, blocked N-terminal arm of 4 residues and are truncated at the C-terminus in the same place as acidic β-crystallin subunits. The C-terminal 2 residues of the high-resolution X-ray structure of γB-crystallin have high temperature factors, indicating flexibility (Najmudin et al., 1993). The N-terminal 4 residues of human γS-crystallin were modeled so that they did not interact with the body of the protein. This is consistent with the lack of definition of the N-terminal arm of βB2 in the X-ray structure of the I222 form of βB2 (Bax et al., 1990) and the mobile character of the βB2 arms as determined by NMR spectroscopy (Carver et al., 1993). The model of γS-crystallin has a small flexible N-terminal extension and has lost a small, relatively flexible C-terminal extension.

The domain cores

βγ-Crystallin structure is based on 2 similar domains each composed of 2 Greek key motifs (Blundell et al., 1981; Bax et al.,

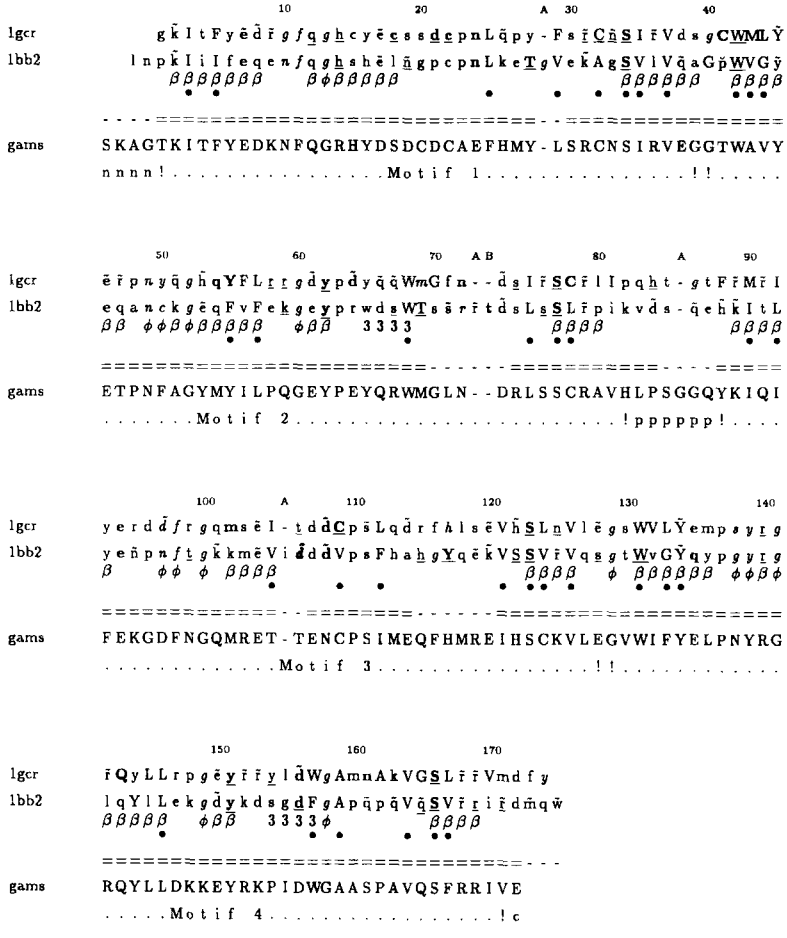

Fig. 3. Structural alignment of the sequences of bovine γB (1gcr), bovine βB2 (1bb2), and human γS (gams) derived by the program JOY. The key to the JOY alignment is: solvent inaccessible, uppercase letters; solvent accessible, lowercase letters; positive φ, italic; *cis*-peptide, breve ˘; hydrogen bond to other side chain, tilde ˜; hydrogen bond to main-chain amide, bold; hydrogen bond to main-chain carbonyl, underline. The solid circles indicate residues that are inaccessible in the X-ray-derived coordinates of γB and βB2. The double row of dashed lines underneath the 1bb2 sequence represents the SCRs and the single dashed lines indicate the variable loop regions. The topological features are indicated underneath the gams sequence: n, the N-terminal extension; ! indicates the borders of motifs 1, 2, 3, and 4; p, the connecting peptide; c, the c-terminal extension. The numbering is based on γB.

1990). The N- and C-terminal domains are each stabilized by a hydrophobic core, and the 2 domains interact through a hydrophobic interface about an approximate 2-fold axis (Fig. 1). The residues in the cores of the N- and C-terminal domains indicate that the γS structure is more closely related to γ-crystallin than β-crystallin; core residues 5, 28, 32, 35, 57, 65, 78, 92, 106, 109, 116, 127, and 157 are identical in γS and γB, yet different in βB2, and residues 44 and 133 are bulky in γS and γB, whereas they are glycines in βB2. By contrast, only Leu 75 in human and bovine γS is the same in βB2- rather than γB-crystallin.

Interdomain contact

In the crystal structures of βγ-crystallins, the N- and C-terminal domains interact closely about a hydrophobic contact, maintaining a similar pseudo-2-fold contact regardless of whether they are from the same polypeptide or different subunits (Fig. 1). In γB-crystallin, the residues involved are Met 43, Phe 56, Ile 81, Val 132, Leu 145, and Val 170, and in human γS, these are Ala, Ile, Val, Ile, Leu, and Ile. In the model of γS, the side chain of Ile 56 has to combine the packing roles of Met 43 and Phe 56 of γB (Fig. 4C), indicating that the hydrophobic contribution to interdomain packing is reduced in γS compared with γB.

Charged residues, characteristic of either β-crystallins or γ-crystallins, surround the hydrophobic interface. The intramolecular interdomain contact of γ-crystallins has arginines at positions 58 and 79 and their topological equivalents at 147 and 168 in the C-terminal domain. In β-crystallins, glutamates are at positions 58 and 147 and, due to the symmetry of the domain pairing, they make intermolecular salt bridges with arginines 168 and 79, respectively (Bax et al., 1990; Lapatto et al., 1991). γS is different from both β- and γ-crystallins in having a proline at position 58. γS-crystallin is β-like in having an aspartate at position 147 and, in the model, this makes an ion pair with arginine 79, which would strengthen interdomain interactions. This probably compensates for the reduction in interdomain hydrophobic contact because γS and γB were indistinguishable in quaternary structure (Table 1).

Charge distribution

In γB-crystallin, most acidic and basic side chains are involved in ion pairs, and this may reduce their interactions with water (Blundell et al., 1981; Wistow et al., 1983). γB-crystallin has a higher than average number of salt bridges compared to other proteins, and this may be related to the unusually high percentage of arginines compared with lysines (Barlow & Thornton, 1983). Each domain has a balance of both negative and positive charges, although there are more charges in the C-terminal domain (Summers et al., 1986). γS-crystallins have a similar number of charged groups but are slightly more acidic. The model shows that human γS-crystallin has an even distribu-

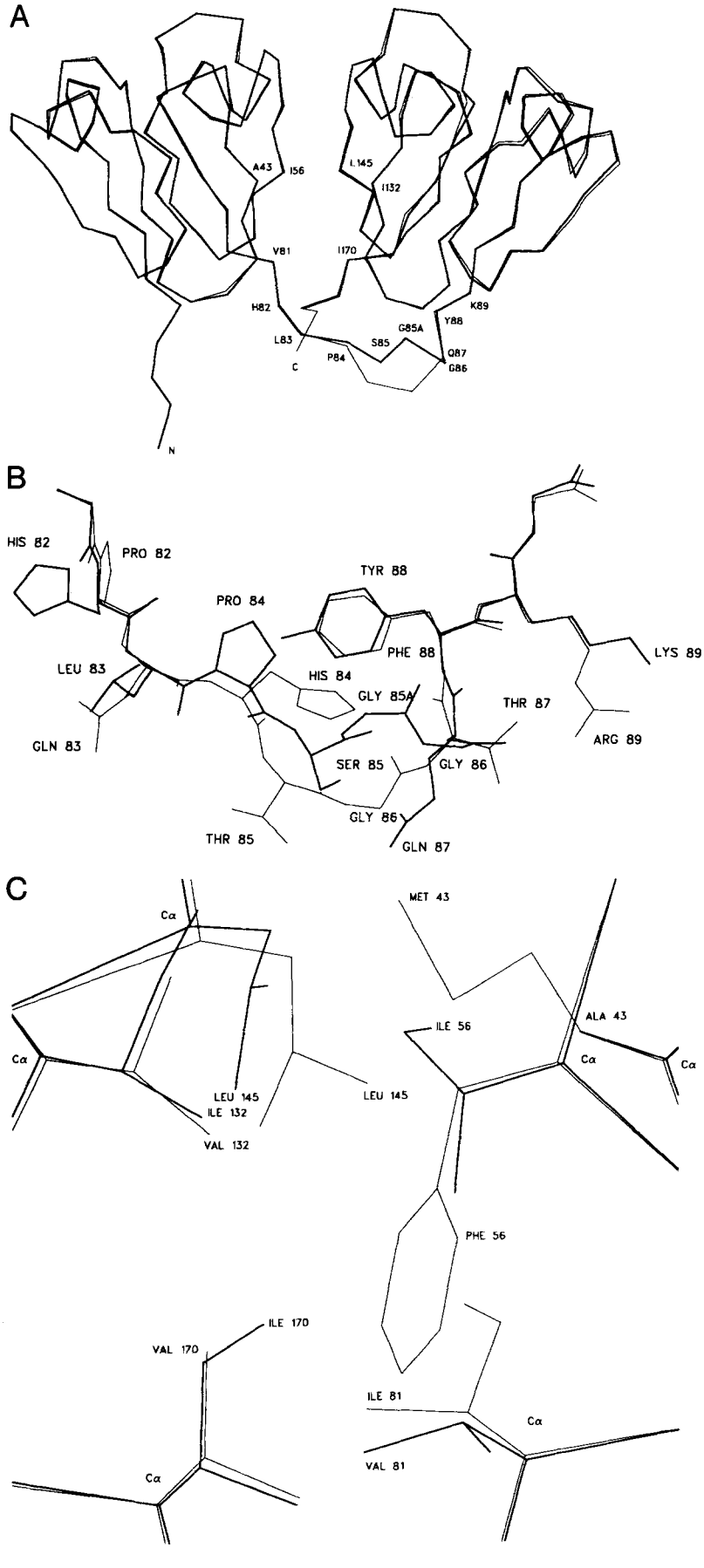

Fig. 4. Comparison of the COMPOSER-derived model of human γS (bold line) with bovine γB. **A:** Superposition of Cα coordinates of human γS and bovine γB structures showing the location of residues highlighted in 4B and 4C. **B:** Detail of the side-chain and main-chain positions of the connecting peptide conformations. **C:** Hydrophobic interdomain region.

tion of surface ion pairs, although different in detail from γB-crystallin. For example, in the C-terminal domain, Arg 91, Arg 99, Arg 115, Arg 147, and Lys 163 in γB are replaced by glutamine, asparagine, glutamine, aspartate, and alanine, respectively, in γS, and Arg 103, Lys 125, Lys 148, and Lys 149 in γS are replaced by serine, asparagine, proline, and glycine, respectively, in γB. The distribution of residue type is different in that γS-crystallins have more lysines and glutamates compared with γB-crystallin. The 22 positions in γB that have acidic side chains are generally conserved in γS-crystallins, although many aspartates have been replaced with glutamates. These differences in charge distribution may be related to the different locations of these proteins in the lens. γ-Crystallins are found mainly in the central densely packed core region of the lens, whereas γS-crystallin occurs in the more hydrated outer region: this has led to the notion that their different interactions with protein and solvent may contribute to the overall refractive index (Slingsby, 1985). However, the estimated hydration gained from ultracentrifugal analysis is not sufficiently accurate to prove increased water binding.

Sulfur content

Human and bovine γS-crystallins have 6 cysteines and 6 methionines, which is less than in γB (7 each), but is still a high proportion of sulfur-containing amino acids for a globular protein of this size. Four of the cysteines (22, 32, 78, and 109) are in common with γB, but only two of the methionines are conserved (69 and 102), reflecting the high surface accessibility of methionine in this family. There is a cluster of cysteines (18, 22, and 78) in the N-terminal domain of γB that can reversibly form an unstable disulfide bridge between cysteines 18 and 22 (Najmudin et al., 1993), and this may play a protective role in the lens. In γS-crystallin, the side chain of cysteine 20 has the highest accessibility (28 Å^2) of all the cysteines and, unlike cysteine 18 of γB, cannot safely oxidize with Cys 22. Various studies have indicated that in human senile cataract, protein sulfur groups are oxidized (Truscott & Augusteyn, 1977; Anderson & Spector, 1978; Spector et al., 1979; van Haard et al., 1980; Harding, 1991), emphasizing the importance of the reduced form for maintenance of transparency.

Methods

Modeling of human γS-crystallin was based on coordinates of bovine γB-crystallin (Brookhaven Protein Data Bank code: 1GCR) and βB2 (2BB2) determined at 1.5 Å and 2.2 Å resolution, respectively. Because the sequence identity between γS and 1GCR is 51.7% compared with 34.5% between γS and βB2, the 3-dimensional structure of γS is expected to be closer to the γ-crystallin. Use of both structures, but with a bias toward the closest homologue, should yield the more accurate model (Srinivasan & Blundell, 1993). The relationship between percent sequence identity and the RMS deviation (RMSD) based on 3-dimensional structures (Chothia & Lesk, 1986; Hubbard & Blundell, 1987) suggests that 2 proteins with 57% sequence identity corresponds to RMSD values in the range of 0.5–1.5 Å (Chelvanayagam et al., 1994). The RMSD between 1GCR and the γS model is 0.43 Å for 172 topologically equivalent Cα atoms, and lies close to the lower limit of the expected range.

Although the tertiary structures of corresponding domains in 1GCR and 2BB2 are similar, the relative orientations and positions of the domains in the monomer of γ-crystallin and the subunit of β-crystallin are different (Fig. 1). Hence, the corresponding domains of 1GCR and 2BB2 were superimposed separately and the 2 domains of γS were first modeled independently. The domains were then linked by modeling the connecting peptide with the domains in the same orientation as 1GCR. COMPOSER (Blundell et al., 1988), available in SYBYL (Tripos, Inc.), was used to build the individual domains of γS-crystallin (Sutcliffe et al., 1987a). The framework region was defined as a mean structure derived from 1GCR and 2BB2 weighted by the square of sequence identity. The structurally conserved regions (SCRs) that define the framework are indicated in Figure 3. The last SCR of the first domain extends into the connecting peptide (position 84) because this part of the linker is conserved in both β- and γ-crystallin. To model the SCRs of γS, the corresponding SCR from 1GCR or 2BB2 was superimposed on the framework depending on the local sequence identity. The side chains were modeled in the same orientation as in equivalent positions of known structures if the sequence is conserved; otherwise, the conformation is based on a number of rules relating the amino acid type with the local secondary structure (Sutcliffe et al., 1987b). Loops that link contiguous SCRs are modeled by searching for segments from known protein structures and "melded" to the model structure. The connecting peptide between domains of γS-crystallin was based on a fragment (residues 55–58 of the A chain of porcine kallikrein, 2KAI) identified from a distance-based constraint search from a database of unrelated proteins; the equivalent SCR of both 1GCR and 2BB2 is 1 residue less.

The initial model was energy minimized using AMBER force field (Weiner et al., 1984) incorporated in SYBYL. Each energy minimization cycle consisted of 20 cycles of Simplex and a further 100 cycles of the Powell algorithm. During initial cycles of energy minimization, the backbone atoms were fixed, and the electrostatic energy term was not considered in order to relieve short contacts and to correct geometry, particularly at the "anchor" regions. In subsequent cycles, all the atoms were allowed to move and electrostatic terms were included. A distance-dependent dielectric constant with a distance cutoff of 9 Å and an ϵ value of 4 was used. The stereochemistry of the final model was checked using the program PROCHECK (Laskowski et al., 1993).

γB-crystallin was isolated by gel filtration followed by ion-exchange chromatography on a MonoS (Pharmacia) column as described in Tardieu et al. (1992). βB2-crystallin was isolated under dissociating conditions and refolded as described in Slingsby and Bateman (1990). γS-crystallin was isolated by gel filtration on Superose HR (Slingsby & Bateman, 1990) and then further purified by ion-exchange chromatography on a Mono Q column equilibrated with 0.05 M Tris-HCl, pH 8.5. Each purified protein was equilibrated into water before lyophilization.

Sedimentation experiments were performed in a Beckman Model E analytical ultracentrifuge using UV scanning at 280 and 230 nm. Double-sector cells with sapphire windows were used in an AuG rotor. $s_{20,w}$-Values were obtained from sedimentation velocity runs at 44,000 rev/min, correcting for temperature and water viscosity. Sedimentation equilibria at 16,000 and 20,000 rev/min, 20 °C, made use of the meniscus depletion technique (Yphantis, 1964) using scanning wavelengths 280, 286,

and 295 nm. Weight-average molecular weights were evaluated from ln c versus r^2 plots, making use of a computer program developed by G. Böhm (University of Regensburg). The partial specific volume of the proteins was calculated from the amino acid composition. Proteins were dissolved in 50 mM sodium phosphate buffer, pH 7.0, in the absence and presence of 0.1 M NaCl. In order to determine the initial concentrations, solutions were filtered and absorbance measured at 280 nm.

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