The X-ray Crystal Structure of Human γS-crystallin C-terminal Domain*

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γS-crystallin is a major human lens protein found in the outer region of the eye lens, where the refractive index is low. Because crystallins are not renewed they acquire post-translational modifications that may perturb stability and solubility. In common with other members of the βγ-crystallin superfamily, γS-crystallin comprises two similar β-sheet domains. The crystal structure of the C-terminal domain of human γS-crystallin has been solved at 2.4 Å resolution. The structure shows that in the in vitro expressed protein, the buried cysteines remain reduced. The backbone conformation of the “tyrosine corner” differs from that of other βγ-crystallins because of deviation from the consensus sequence. The two C-terminal domains in the asymmetric unit are organized about a slightly distorted 2-fold axis to form a dimer with similar geometry to full-length γ-crystallins because of deviation from the consensus sequence. The two C-terminal domains in the asymmetric unit are organized about a slightly distorted 2-fold axis to form a dimer with similar geometry to full-length two-domain family members. Two glutamines found in lattice contacts may be important for short range intermolecular interactions in the lens. An asparagine known to be deamidated in human cataract is located in a highly ordered structural region.

The lens crystallins are protein molecules that need to last a lifetime, because they are found in cells that have no protein synthetic or degradation machinery (1). The leading senile cataract hypothesis is that aged non-native crystallin molecules overwhelm the binding capacity of the small heat shock protein α-crystallin (also found in the lens), resulting in aggregation and formation of light scattering centers (2). Detailed molecular information from selected crystallin domains and proteins is needed to model their unfolding and characterize their likely ensemble biophysical properties, particularly the early unfolding intermediates that have been hypothesized to bind to α-crystallin (3). As a first step toward providing detailed molecular information on a major human lens crystallin involved in cataract, we have solved the x-ray crystal structure of the C-terminal domain from human γS-crystallin.

The crystallins are a well studied family of proteins for which there are several three-dimensional structures (4) as well as thermodynamic and kinetic data on folding/unfolding (5–7). The polypeptides of the 13-member βγ-crystallin superfamily each comprise similar ~10-kDa N- and C-terminal domains that are themselves formed from two symmetrically organized Greek key motifs. In all cases, the N- and C-terminal domains pair about a similar pseudo-2-fold axis, with the domains in monomeric γ-crystallins being covalently connected, whereas domain swapping can lead to dimerization in β-crystallins (8).

There are seven genes coding for γ-crystallins in vertebrate lenses (9), and they consist of the closely related γA-γF family and the more distantly related but more conserved γS-crystallin. The expression patterns of the family of γ-crystallins appear to be correlated with the formation of the decreasing refractive index gradient from the center to the cortex of the adult lens (4, 10). The propensity of certain γ-crystallins to easily form a concentrated phase (11), such as the high Tc γ-crystallins that are enriched in the core region of the lens, probably reflects their “attractive” interactions (12). γS-crystallin, located in the low refractive index outer regions of the lens, is characterized by more repulsive intermolecular interactions (13). The molecular basis for the stability of these long-lived structural proteins, along with their solution intermolecular interactions that govern solubility and phase separation behavior, are areas of cataract research.

Several x-ray structures of γA-γF crystallins are now known, and they all show very similar two-domain pairing about a hydrophobic interface that contributes toward stability (14–17). γS-crystallin is a major structural protein in the human eye lens (18). Human and bovine γS-crystallins and their isolated domains are very stable and show two-state unfolding, allowing detailed quantitative thermodynamic properties of the proteins to be evaluated (19). Computer simulations of heat-induced unfolding of bovine γS-crystallin also indicate high stability and furthermore suggest that the first stage of unfolding involves the dissociation of the paired domains (20). Conformational changes to aging crystallins can derive from a variety of covalent changes. Oxidation of cysteine and methionine residues have been detected in human crystallins (21). Deamidation of human γ-crystallins is correlated with aging (22, 23) and with increased insolubilization of crystallins, particularly γS (21). Deamidation alters the charge balance, adding a negative charge to a previously neutral area, but it is also thought to mark the nonenzymic formation of isomers such as β-aspartate that would alter the backbone covalent structure (24).

So far for γS-crystallin, only the C-terminal domain of the bovine protein has been solved by x-ray crystallography (25), showing how two domains self-associate to form a dimer in an analogous way to that of the native two-domain γ-crystallins,
although the pairing is less symmetrical. Surprisingly, one of the domains has an altered conformation in its tyrosine corner, a usually highly conserved feature of most β-sandwich proteins (26). In fact, the tyrosine corner has been proposed as a possible folding nucleus in a prokaryote protein with a related βγ-crystallin fold (27), although this has not been universally supported (28). Because it is unclear to what extent the lattice interactions in the crystal structure influenced pairing and conformation, further three-dimensional structures are required. Here we show that the C-terminal domains of human γS-crystallin pair about a slightly distorted 2-fold axis to form a dimer with both tyrosine corners in a nonstandard conformation.

**EXPERIMENTAL PROCEDURES**

**Protein Expression**—The human γS-crystallin C-terminal domain (HGSC)\(^1\) was cloned in the pET3a vector essentially as described for the C-terminal domain of calf γS-crystallin (25). The novel initiation codon was introduced in the human γS sequence (19) at a position that replaced the first glycine in the linker sequence by PCR-mediated mutagenesis using the following primers: GTTCATCTGCCTCATATGGGCCAGTATAAG (forward) and GGATCCATGTCATTACCATGTC (reverse). The HGSC plasmid DNA, coding for residues 91–177 (topologically equivalent to residues 86–172 of γB-crystallin) was transformed into *Escherichia coli* strain BL21(DE3) pLysS competent cells. Colonies were picked to inoculate and grown overnight at 37 °C with shaking in 10 ml of 2YT medium (5 gliter NaCl, 10 gliter yeast extract, 16 gliter peptone 140) with 10 µl of ampicillin (100 µg/ml) and 15 µl of chloramphenicol (50 µg/ml). Large scale growth was performed with an overnight culture of 500 ml of 2YT medium containing 250 µl of ampicillin (50 µg/ml) after inoculation at 100:1 from the 10-ml overnight growths. The flasks were shaken at 37 °C and induced by the addition of 250 µl isopropyl-β-D-thiogalactopyranoside after the culture was grown to an A\(_{600}\) of 0.4–0.6 (3–4 h). Growth was continued overnight, whereupon cells were harvested by centrifugation at 5000 rpm for 15 min at 4 °C. The pellets were resuspended in 10 ml of 25 mM Tris-HCl, pH 8.0, 10 mM EDTA, 50 mM glucose with a protease inhibitor (5 µl of Pefabloc (Merck)) and frozen at −20 °C.

**Protein Isolation**—The highly expressed protein was isolated from the soluble fraction. Whole cell lysate was prepared from the thawed pellet by addition of DNase I and MgCl\(_2\) to the suspension giving final concentrations of 10 µg/ml and 10 mM, respectively, followed by sonication on ice using 10-s pulses with cooling in between. The pellet was spun down at 20,000 rpm for 30 min at 4 °C before dialyzing the supernatant overnight at 4 °C with stirring against buffer A (25 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM dithiothreitol). The solution was then filtered through a 0.4-µm nitrocellulose filter followed by a 0.2-µm nitrocellulose filter before being loaded onto a HiLoad 16/10 Q Sepharose High Performance column (Amersham Biosciences, Inc.). The column was run at 4 ml/min on a Gradientfrac with the following program: 1) 20 ml of 100% buffer A; 2) gradient from 0–70% buffer B (buffer B was buffer A with 1 mM NaCl over 160 ml; 3) 60 ml of 100% buffer B; and 4) 100 ml of 100% buffer A. The HGSC peak eluted at about 15% buffer B, in line with the predicted pl of 6.0. The identity of the protein was confirmed by electrospray mass spectrometry with the measured mass of 10,412 in close agreement with the calculated mass of 10,414 and indicating that the initiating methionine had been cleaved. The HGSC protein fractions were concentrated to 1 mg/ml and equilibrated against 25 mM Bis-Tris-propane HCl, pH 7.5, using an Amicon (Millipore, Watford, Hertfordshire, UK) cell equipped with a YM3 membrane. The concentrated protein was stored at −20 °C.

The size of the protein was estimated using chromatography on a Superose 12HR 10/30, with 25 mM Bis-Tris-propane HCl, 0.2 mM NaCl, pH 6.5 or 8.0, as running buffer. The HGSC eluted at 15.3 ml, over a wide range of protein concentration application (0.2–6.5 mg/ml) at both pH 6.5 and 8.0. For comparison, full-length bovine γS-crystallin at pH 8.0 elutes at 14.4 ml, in agreement with the monomeric nature of the C-terminal domain of human γS in solution, as determined using ultracentrifugation (19).

**Crystallization**—The crystals were grown using the hanging drop vapor diffusion method with conditions for crystal growth optimized from Hampton (Laguna Niguel, CA) Crystal Screen II condition 13, with polyethylene glycol monomethyl ether 2000 as precipitant. 1 µl of protein at ~10 mg/ml 25 mM Bis-Tris-propane HCl, pH 7.5, was added to 1 µl of well solution containing 0.2 mM ammonium sulfate, 0.1 mM sodium acetate, pH 5.0, and 20–28% polyethylene glycol monomethyl ether 2000. The optimum crystals, formed at 24% polyethylene glycol monomethyl ether 2000 after 4 days growth at room temperature, were hexagonal bipyramidal with dimensions of ~0.3 × 0.1 × 0.1 mm\(^2\).

**Data Collection and Processing**—Intensity data to 2.4 Å were collected from a cryo-cooled (100 K) crystal using the Daresbury SRS source at Station 9.6 using an ADSC imaging plate. No cryoprotectant was added. The data were processed using the program MOSFLM (29). Scaling was carried out with the program SCALA (30), and the data were truncated with TRUNCATE (31). The crystals were either space group P6\(_2\)22 or P6\(_3\)22 with two molecules in the asymmetric unit and crystallizing in a solvent of 61% (V\(_c\) = 31.15 Å\(^3\)) of 2.4 Å. The crystal data and statistics from data processing are listed in Table I. Structure Determination—Molecular replacement was undertaken with the program AMoRe (32) with the B chain coordinates from the bovine γS-C domain (25) as a search model. Data from 15–2.4 Å were used in both the rotation and translation function searches with a Patterson cut-off radius of 15 Å and a radius of integration of 0.75% (the maximal distance from the center of mass being 21.5 Å). A successful solution was found indicating two molecules in the asymmetric unit, using the P6\(_2\)2 space group, with a correlation coefficient of 63.7 and an R factor of 42.3%.

**Structure Refinement**—Refinement of the structure was undertaken using CNSsolve version 0.9 (33). The reflections were divided, at random, into working and test (7.5% of the data) sets, to allow both the graphical and free R factors to be followed. The test set of reflections was excluded from the map calculations. Early in refinement, the noncrystallographic symmetry at the dimer interface was maintained by use of restraints (initial noncrystallographic symmetry restraints were 20 kcal/mol). Both simulated annealing and minimization methods were tried for refinement, with a maximum likelihood target using amplitudes. The refinement method giving the best reduction in the R factor for a cycle was chosen, and individual isotropic B-factor refinement was then undertaken. In each cycle, both 2F\(_{o}\) − F\(_{c}\) and F\(_{c}\) − F\(_{o}\) electron density maps were calculated, and manual rebuilding was undertaken using the program O (34). Water molecules were added using the CCP4 (35) programs PEAKMAX and WATERPEAK to select potential sites. Some later rounds of refinement were undertaken using the CCP4 programs REFMAC (36) and ARP_WARP (37) interspersed with CNSsolve refinement. Noncrystallographic symmetry restraints

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\(^1\) The abbreviations used are: HGSC, human γS-crystallin C-terminal domain; BGBC, bovine γB-crystallin C-terminal domain; BGSC, bovine γS-crystallin C-domain.
were lowered to 5 kcal/mol and finally removed. The final values for a crystallographic $R$ factor of 21.6% and for a free $R$ factor of 26.4% were obtained after 35 cycles of refinement.

The final solution contains two molecules of human $\gamma$S-crystallin C-domain (A and B) in the asymmetric unit, together with 90 water molecules. The statistics after refinement are given in Table II. Electron density for all the residues is visible, with just some side chain density remaining unclear. The Ramachandran plot from PROCHECK (38) shows 86.2% of the residues in the most favored regions and 13.8% in the additional allowed regions. The coordinates have been deposited in the RCSB protein data bank, under code number 1ha4.

**Solvent Accessibility**—The program NACCESS (39) was used to calculate solvent accessible surface areas using the default probe radius of 1.4Å.

**Figures**—The figures were produced using the programs MOLMOL (40) and POVRAY version 3.1.

## RESULTS

**Human $\gamma$S-crystallin C-terminal Domain Forms a "Dimer" in the Crystal Lattice**—The refined electron density shows that the two molecules of the HGSC in the asymmetric unit have a very similar structure (backbone root mean square deviation of 0.29 Å), and they are very similar to bovine $\gamma$B-crystallin C-terminal domain (BGBC) (backbone root mean square deviation of 0.8 Å). The two HGSC domains form a dimer with a rotation of 176.5° between the two chains (red in Fig. 1A). This 2-fold pairing of two C-terminal domains is similar to the N- and C-terminal domain pairing in other polypeptides of the $\beta\gamma$-crystallin superfamily (HGSC, BGSC, BGC, and the C-terminal domain of bovine $\beta$B2-crystallin) are compared, HGSC has a backbone conformation that follows that of the BGSC A chain rather than the more common conformation seen in BGBG and the C-terminal domain of bovine $\beta$B2-crystallin. Fig. 3A shows a comparison of the HGSC tyrosine corner with that from BGBC. The unusual conformation is centered near residue 148 where $\gamma$B has proline and $\gamma$S has lysine. In the “standard” conformation, the tyrosine (151) hydroxyl oxygen hydrogen bonds with the main chain N-H of Asp147 (bond length, 2.4 Å). In HGSC, the tyrosine hydrogen bonds with the main chain N-H of Lys149 (bond length, 1.96 Å). The new corner conformation results in different positions for the exposed positive charges of lysines 148 and 149 (Fig. 3A).

**Dimer-Dimer Interactions in Human $\gamma$S-C**—There are three different interdimer interfaces present in the crystal lattice (Fig. 4). The solvent accessible surface areas have been calculated for the single HGSC chains, for the AB dimer on its own (Chains A and B together), and for the AB dimer with its symmetrically related partners. These data are shown in Table III. When the amounts of buried surface area in the three lattice interfaces are compared with the area buried within the dimer, it can be seen that the interface between the chains in the dimer buries 10.9% of the monomer surface, whereas lattice interfaces 1, 2, and 3 bury 9.3, 6.6, and 2.8%, respectively. Interface 1 is thus nearly as extensive as the dimer interface and is similar to one of the four lattice interfaces found in the bovine lattice (data not shown).

HIs$^{117}$, a residue in the long loop region between strands c and d of the first Greek key motif, participates to some extent in all three interfaces (Fig. 4).

It is residues in the smaller HGSC interface 3 that show the major conformational differences when compared with other $\gamma$-crystallin C-terminal domains (Fig. 4C). Only one of the residues that differs between the human and the bovine sequences is involved in lattice interactions for the human form. This is tyrosine 103 that interacts with conserved glutamine 101 in interface 3, whereas this residue is a histidine in the bovine protein.

**The Cysteine and Amidie Sites**—The solvent accessibilities of Cys$^{109}$ (Cys$^{114}$ in $\gamma$S numbering) 3.7 Å$^2$ and Cys$^{124}$ (Cys$^{129}$ in $\gamma$S numbering) 0.0 Å$^2$ are low because they are buried in the domain core with their 5G atoms 13 Å apart. There is no indication of oxidation. The exposure of the amide containing amino acids, calculated for the A chain from an AB dimer, are ranked in order of accessibility: Glu$^{70/92}$ (30.0 Å$^2$), Glu$^{115/120}$ (105.1 Å$^2$), Glu$^{101/106}$ (100.1 Å$^2$), Asn$^{138/143}$ (97.2 Å$^2$), Glu$^{65/70}$ (50.9 Å$^2$), Glu$^{91/96}$ (30.6 Å$^2$), and Glu$^{143/148}$ (18.4 Å$^2$ (Fig. 5). The least accessible, Glu$^{143/148}$, is buried in the dimer interface and would likely be packed against the N-terminal domain in the intact two-domain $\gamma$S molecule. The side chain of Asn$^{138/143}$, a residue shown to be deamidated in human cataract (44), is not form (see Fig. 4 in Ref. 25). A cluster of interactions is close to the 2-fold axis; each Gln$^{142}$ and Glu$^{172}$ side chain interacts with backbone polar atoms of its symmetrically related partner, and each Arg$^{142}$ interacts with its symmetrically related partner side chain. The only sequence difference between the two species at the interface is Val$^{130}$ in human that replaces alanine in the bovine and is likely to contribute to the differing symmetries.

**Tyrosine Corner Structure**—It is apparent from a superposition of these residues of $\gamma$-crystallin C-termimol domers that there are two regions, calculated using difference distance plots (43), where the domain conformation differs: the tyrosine corners and a dimer-dimer interface region (Fig. 1A). When tyrosine corners from four C-terminal domains of members of the $\beta\gamma$-crystallin superfamily (HGSC, BGSC, BGC, and the C-terminal domain of bovine $\beta$B2-crystallin) are compared, HGSC has a backbone conformation that follows that of the BGSC A chain rather than the more common conformation seen in BGBG and the C-terminal domain of bovine $\beta$B2-crystallin. Fig. 3A shows a comparison of the HGSC tyrosine corner with that from BGBC. The unusual conformation is centered near residue 148 where $\gamma$B has proline and $\gamma$S has lysine. In the “standard” conformation, the tyrosine (151) hydroxyl oxygen hydrogen bonds with the main chain N-H of Asp147 (bond length, 2.4 Å). In HGSC, the tyrosine hydrogen bonds with the main chain N-H of Lys149 (bond length, 1.96 Å). The new corner conformation results in different positions for the exposed positive charges of lysines 148 and 149 (Fig. 3A).

## Table II

| Refinement statistics | Value |
|-----------------------|-------|
| Final $R_{free}$      | 26.4% |
| Final $R$             | 21.6% |
| Number of Reflections | 10121 (working)/8444 (test) |
| Average $B_{iso}$ of protein atoms: main chain | 28.7 Å$^2$ |
| Average $B_{iso}$ of protein atoms: side chain | 32.0 Å$^2$ |
| Number of protein atoms | 1468 |
| Number of water molecules | 90 |
| Root mean square deviations in bond length | 0.0093 Å |
| Root mean square deviations in bond angles | 1.388° |
| Bulk solvent parameters | Density = 0.409, e/Å$^3$, $B = 51.16$ Å$^2$ |
| Number of molecules in asymmetric unit | 2 |
| Solvent content | 61% |

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The tyrosine corner in human γS-crystallin. A, the two chains of the human γS C-terminal domains and the two chains of bovine βγ C-terminal domains have been superposed to show the different hydrogen bonding patterns in the two types of tyrosine corner. The yellow hydrogen bond occurs in the standard conformation from BGBC (in red), where the side chain oxygen of Tyr141 hydrogen bonds to the main chain N-H of Arg147, this conformation being the same as that found in the B chain of BGSC. The turquoise hydrogen bonds occur in the unusual conformation from HGSC (in blue), where Tyr141 hydrogen bonds to Lys146 main chain nitrogen. This conformation is the same as that found in the A chain of BGSC. B, an alignment of some human βγ-crystallin C-terminal domain tyrosine corner sequences shows the conservation of at least one of Pro146 or Gly146 (shown in red) in all sequences except for γS.

involved in hydrogen bonding to either backbone or side chain atoms of neighboring residues; therefore deamidation will not destabilize the molecule by disruption of hydrogen bonds. At neutral pH, the C-terminal domain of γS-crystallin has a charge of −1, and the addition of another negative charge by deamidation of Asn138 is likely to be that of Asn138 with Glu114 because the closest atoms of these two residues are the termini of the side chains (7.2 Å apart).

The two-domain human γS-crystallin was modeled using the complete bovine β-crystallin as a template, with the C-terminal domain replaced by the human γS coordinates and the residues in the N-domain mutated to match the human γS sequence. In this model, the only residue on the N-terminal domain that is within 10 Å of Asn138 is Met102, with the main chain carbonyl of Asn138 being 8.9 Å from the side chain of Met102. This interaction with methionine is also seen in the x-ray structures of bovine γD-, γE-, and γF-crystallins, but at a distance of 6.3–6.5 Å, and it is the only residue from the N-terminal domain within 10 Å of Asn138. Deamidation of Asn138 is thus unlikely to perturb electrostatic interactions in the N-terminal domain.

Two other amide containing residues are involved in lattice contacts: Gln101 and Gln106. Gln115 hydrogen bonds to Thr105 and Thr106 and is close to Gln101 in interface 1 (Fig. 4A). This residue is also involved in a similar interaction in the lattice of the bovine γS-crystallin C-terminal domain dimer. In HGSC interface 2, Gln101 hydrogen bonds from chain A interacts with the main chain of two residues from chain B: Arg119 and Glu120 (Fig. 4B) and in interface 3 (Fig. 4C), it interacts with its symmetrically related partner as well as with Met102 and Tyr103.

DISCUSSION

The human γS-crystallin C-terminal domain forms dimers in the crystal lattice (although not in solution) using a similar interface to those observed between N- and C-terminal domains in other βγ-crystallins and is likely to form a similar intramolecular interface with its own N-terminal domain. The dyad is more exact than in the corresponding bovine γS construct. The recreation of these 2-fold interactions between single domains underscores the idea that domain pairing is an ancestral dimer trait. However, without the covalent linker, the local concentration of a single domain is insufficient to form a dimer in solution (19). The weakness of the interface interaction renders it susceptible to deformation, and it is the first likely hydrophobic surface to become water exposed during denaturation, in line with computer simulated unfolding studies of β-crystallin (20).

The tyrosine corner is an extremely conserved structural feature of the βγ-crystallin fold. In common with other β-sandwich domains, it occurs only once in the domain, even though the βγ-crystallin domain is made from two similar Greek key motifs (Fig. 1B). Here it is shown that in the human γS-
crystallin C-terminal domain, the tyrosine corner conformation in both partners of the dimer is nonstandard. In the corresponding bovine γS-crystallin structure, where the two domains in the asymmetric unit (chains A and B) pair about a distorted 2-fold axis, the major conformational difference between the chains is in the tyrosine corner, with chain A having an unusual conformation. However, it was not possible to ascertain whether this was due to the distorted 2-fold pairing and/or was a consequence of crystal lattice interactions (25). We hypothesize that the tyrosine corner structure seen in both chains of the human γS-crystallin C-terminal domain, as well as the A chain of the bovine γS-crystallin C-terminal domain, is the favored conformation for γS-crystallins. The consensus sequence for the tyrosine corner is LXPGXY, whereas in γS-crystallin C-terminal domain it is LDKKEY with the lysine pair that replaces proline-glycine increasing the energy of the standard tyrosine corner polyproline II conformation. The more usual γ-crystallin conformation found in the crystal form of the B chain of the C-terminal domain from bovine γS-crystallin is probably being stabilized by the side chain of Lys₁⁴⁸⁻¹⁵³. This forms a salt bridge with the C-terminal carboxylate of chain A, giving a compensation for the higher energy conformation of the backbone. Now that the new conformation has been seen in a γS-crystallin in a different lattice, it is likely to be independent of a secondary lattice effect. It will be interesting to ascertain whether this new conformation contributes to the lower stability of γS-crystallin toward denaturants compared with γB-crystallin (19) and/or affects the folding.

The human γS C-terminal domain sequence is very similar to the bovine (93% identical). Although the two species of crystals are grown under very similar conditions, they have different space groups (human, P6₁22; bovine, P6₁22) and form two kinds of dimer, one almost perfect and one distorted. Only one of the residues that differs between the human and the bovine sequences is involved in lattice interactions, this being Tyr₁⁰³⁻¹⁰⁸ in the human form. This bulky residue occupies a

![FIG. 4. Residues that dominate lattice interactions. The dimer in the asymmetric unit makes three lattice interactions. The green residue His₁¹⁷⁻¹²² is involved in all three, with involvement of the purple residues Gln¹¹⁵⁻¹²⁰ in interface 1 (A) and Gln¹¹²⁻¹¹⁰ in both interfaces 2 (B) and 3 (C). These residues are likely to contribute toward the short range interactions in the normal human lens.](image)

| Interface type | First chain | Second chain | Surface area buried |
|---------------|-------------|--------------|---------------------|
| Single domain | A           | B            |                     |
| Dimer         | A           |              |                     |
| 1             | A           | B            |                     |
| 2             | A           | B            |                     |
| 3             | B           |              |                     |

**TABLE III**

**Solvent-accessible surface areas**

| Interface type | Chain type | First chain SA | Buried | Second chain | Chain type | SA        | Buried | Surface area buried |
|---------------|------------|----------------|--------|--------------|------------|-----------|--------|---------------------|
| Single domain | A          | 5112.3         | N/A    | B            |            | 5152.6    | N/A    |                     |
| Dimer         | A          | 4555.9         | 556.4  | B            | 4582.7     | 569.9     | 1126.3 |                     |
| 1             | A          | 4635.4         | 476.9  | B            | 4676.4     | 476.2     | 953.1  |                     |
| 2             | A          | 4773.5         | 338.8  | B            | 4818.4     | 334.2     | 673.0  |                     |
| 3             | B          | 5008.3         | 144.3  | B            | 5008.3     | 144.3     | 288.6  |                     |
of extra carbons to the polypeptide backbone and tends to be correlated with flexibility of the protein backbone chain (24). It is significant that Asn138(143) is in the highly ordered folded β-hairpin structure that is involved in maintaining the tertiary βγ-crystallin fold (4). If deamidation were to occur to the native protein at this site leading to an altered covalent backbone structure, it would likely destabilize the γS-crystallin domain. Because this residue is resistant to deamidation in the normal aged human γS-crystallin (46), it is unknown whether the molecule has first to be unfolded prior to deamidation or whether other cataractogenic factors are involved that favor deamidation, which then leads to unfolding.

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