Crystal structure of truncated human β B1-crystallin

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Abstract

Crystallins are long-lived proteins packed inside eye lens fiber cells that are essential in maintaining the transparency and refractive power of the eye lens. Members of the two-domain $\beta\gamma$ -crystallin family assemble into an array of oligomer sizes, forming intricate higher-order networks in the lens cell. Here we describe the 1.4 Å resolution crystal structure of a truncated version of human β B1 that resembles an in vivo age-related truncation. The structure shows that unlike its close homolog, β B2-crystallin, the homodimer is not domain swapped, but its domains are paired intramolecularly, as in more distantly related monomeric γ -crystallins. However, the four-domain dimer resembles one half of the crystallographic bovine β B2 tetramer and is similar to the engineered circular permuted rat β B2. The crystal structure shows that the truncated β B1 dimer is extremely well suited to form higher-order lattice interactions using its hydrophobic surface patches, linker regions, and sequence extensions.

Keywords: β B1; cataract; crystallin; domain swapping; eye lens; oligomer assembly; protein modification

The transparency and refractive power of the eye lens is dependent on an even distribution of the proteins in the lens cells on the scale of the wavelength of visible light (Delaye and Tardieu 1983). Because a lens cell lasts a lifetime with virtually no protein turnover, its contents must be extremely stable (Jaenicke and Slingsby 2001). With age, however, lens proteins lose solubility and stability, which can result in light scattering that causes cataract (Horwitz 2000). The majority of vertebrate eye lens proteins can be grouped in two families, α -crystallins and $\beta\gamma$ -crystallins, which in turn consist of the monomeric γ -crystallin and the oligomeric β -crystallin branches. The β -crystallin family comprises four acidic (β A1, β A2, β A3, β A4) and three basic (β B1, β B2, β B3) polypeptides (Wistow and Piatigorsky 1988), which associate to form homo- and hetero-oligomers in a range of sizes (Slingsby and Bateman 1990).

The tertiary structures of the β - and γ -crystallins are very similar. Both β - and γ -crystallin monomers consist of two similar domains connected by a short linker. Each domain is composed of two similar Greek key motifs of ~40 amino acids folded into a wedge-shaped β -sheet sandwich with approximate twofold symmetry. Based on the crystal structures of β - and γ -crystallins, the main structural difference between the two families was thought to be the intramolecular domain pairing of γ -crystallins versus the domain swapped and intermolecular domain pairing of β -crystallins (Bax et al. 1990). Long N-terminal sequence extensions further distinguish the β -crystallin family from the γ -crystallins, with the basic β -crystallins also possessing C-terminal extensions (Lubsen et al. 1988). Recent evidence of the involvement of β B2 extensions in stabilization of higher-order hetero-oligomeric interactions with β A3 (Werten et al. 1999) indicate that they might play a crucial role in higher oligomer assembly in ways that echo the function of N- and C-terminal extensions in higher oligomer assembly of the small heat shock protein family of which α -crystallin is a member (van Montfort et al. 2001).

The different β -crystallin polypeptides in adult human and bovine lenses can be separated by gel filtration chromatography in three size fractions: β H-, β L1-, and β L2-

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crystallins (Zigler Jr. et al. 1980; Bindels et al. 1981). Formation of the 200-kD β H-crystallin is thought to involve β B1, which has an N-terminal extension of >50 amino acids (Berbers et al. 1982). The presence of various N-terminally truncated forms of β B1 in the lower-molecular-weight fractions together with the extensive degradation (15–41 residues) of the N-terminal extension of human β B1 with age as shown by mass spectroscopy (David et al. 1996; Lampi et al. 1998) strengthens the idea that also the N-terminal extension of β B1 is involved in higher-order assembly (Ajaz et al. 1997).

Because of the proposed function of β B1 in controlling higher assembly of β -crystallins and the potential role of truncated versions of the protein in cataract formation (Hanson et al. 2000), we set out to solve the structure of human β B1 (h β B1) and use it as a first step to gain further insight in the intermolecular relationships of the β -crystallins and

their higher oligomer assemblies. Despite extensive crystallization trials, crystallization of full-length human β B1 was unsuccessful. Therefore, crystallization experiments were carried out with various truncated forms of the protein. Here we describe the 1.4 Å crystal structure of a truncated human β B1 (trh β B1) that lacks more than the first 41 N-terminal residues (Bateman et al. 2001) and resembles truncations occurring in the aging human eye lens (Ma et al. 1998).

Overall structure

The two independent monomers in the crystal form a dimer related by an approximate twofold axis (Fig. 1c). The trh β B1 monomer, comprising residues 54 to 236 (corresponding to –4 to 174 of the topological numbering based on the γ B sequence, see Fig. 2), is composed of the two characteristic β γ -crystallin domains, which are connected by a short linker. With the crystal structure of bovine β B2

Figure 1. Ribbon diagrams showing the assembly characteristics of the domains in monomeric bovine γ B-crystallin (a; PDB ID 1amm). Domain pairing is intramolecular and buries an interface defined as PQ by analogy with the interface defined in the β B2 tetramer. (b) Rat circularly permuted β B2 dimer (PDB ID 1bd7). Domain pairing buries the PQ interface and is intramolecular, whereas the QR interface, by analogy with the interface defined in the β B2 tetramer, is between monomers. (c) Human truncated β B1 dimer. Domain pairing buries the PQ interface and is intramolecular, whereas the QR interface is between monomers. (d) Bovine β B2 dimer (PDB ID 2bb2). The crystallographic dyad P is perpendicular to the page; domain pairing buries the PQ interface and is intermolecular. (e) Bovine β B2 lattice tetramer. The P-axis is perpendicular to the page, Q is vertical, and R is horizontal. The PQ interface is intermolecular, and the QR interface is between dimers. All figures were made by using MOLSCRIPT/Raster3D (Kraulis 1991; Merritt and Bacon 1997).

Figure 2. Sequence alignment of human β B1 (hbetaB1, P53674), bovine β B2 (bbetaB2, P02522), circularly permuted rat β B2 (rcpbetaB2, based on P26775 modified according to Wright et al. 1998), and bovine γ B (bgammaB, P02526) with the different domains and extensions indicated. The residue numbering and secondary structure shown at the top of the alignment are of human β B1, the numbering below is of γ B. Conserved glycine and serine motifs are shown in blue. Asp107, Asp108, Arg169, and Arg171 in β B2 are shown in red. Interaction sites A–D making up dimer–dimer interfaces 1 and 2 are shown in purple (residues 54–57 and 235–236), pink, dark green, and light green, respectively.

in mind and the observation that both β B2 and truncated h β B1 form dimers in solution (Bateman et al. 2001), the arrangement of the domains in trh β B1 was expected to be similar to the domain-swapped bovine β B2 (Fig. 1d). Surprisingly, the N- and C-terminal domains of trh β B1 pair in an intramolecular fashion as in γ -crystallin and the respective linkers have a similar conformation (Fig. 1a,c). The structural similarity between the two proteins is further underlined as the 1.2 Å structure of γ B and the 1.4 Å trh β B1 structure could be superimposed with a root mean square deviation (rmsd) of 1.41 Å using 171 C α -coordinates. In addition to the similarity in domain pairing with the γ -crystallins, the trh β B1 dimer closely resembles the top half of the crystallographic tetramer of bovine β B2 (rmsd 1.20 Å using 346 C α -coordinates; Fig. 1e). The domain arrangement is also similar to that of the engineered circularly permuted rat β B2 (cp β B2) dimer (rmsd 1.19 Å using 340 C α -coordinates; Fig. 1b; Wright et al. 1998). The intramolecular domain pairing in the trh β B1 dimer thus gives rise to an interface analogous to the PQ interface (see Fig. 1 for definition) in bovine β B2, whereas the intermolecular dimer interface in trh β B1 is analogous to the QR interface in β B2

(Lapatto et al. 1991). In both trh β B1 monomers, Cys79 was oxidized to a sulfinic acid, which most likely is a result of the slow crystallization of trh β B1.

Domain interactions

The crystal structures of trh β B1, full-length domain-swapped bovine β B2, bovine γ -crystallins, and circularly permuted “swapped back” cp β B2 show the versatility of the $\beta\gamma$ system for domain assembly (Fig. 1). However, identification of the determinants of domain swapping and elucidation of the mechanisms governing the different oligomerization states in these proteins is a daunting task. Intuitively, one would expect the linker connecting the two domains in a $\beta\gamma$ -crystallin monomer to play a crucial role. Yet, neither its length nor its sequence appears to be important, as the γ B and β B2 linkers have the same length (Fig. 2) and γ B remained monomeric after exchange of its linker with that of β B2 (Mayr et al. 1994).

In addition, a structural comparison of trh β B1, cp β B2, bovine β B2, and γ B showed that the differences in their inter- and intramolecular interfaces are small. Compared

with bovine β B2, the only extra interaction in the intermolecular QR interface in circularly permuted β B2 (Fig. 3B,C) is an intermolecular interaction between Arg171 and Asp107 (Wright et al. 1998). A similar interaction is present between the corresponding Arg232 and Asp168 in trh β B1 (Fig. 3A). In the monomeric γ -crystallins, the equivalent of Arg171/Arg232 is always hydrophobic, and thus, the formation of an intermolecular salt-bridge is impossible. This indicates that the salt-bridge may play a role in the dimer formation of intramolecularly domain-paired crystallins, but its absence in the bovine β B2 tetramer shows that it is not essential in the domain-swapped crystallin. Instead, Arg171 in β B2 forms an intramolecular salt-bridge with Asp108. In trh β B1, cp β B2, and γ B, this aspartic acid (Asp169 in trh β B1 and Asp108 in cp β B2 and γ B) interacts with a strictly conserved arginine residue (Arg230 in trh β B1 and Arg169 in cp β B2 and γ B). In the structures of these three proteins, the arginine side chain adopts a similar conformation, but in β B2, it reaches out across the PQ interface. The conformation of this arginine coupled with its interaction

with Asp108/Asp169 thus appears to correlate with intramolecular domain pairing in the β γ -crystallin family.

Results and Discussion

Higher assembly

The presence of β B1 in the high-molecular-weight β H-crystallin fraction in gel filtration experiments indicated its involvement in higher-order assembly in the vertebrate eye lens. Indeed, the crystal packing of trh β B1 shows that even a truncated form of human β B1 can contribute to assembly. In our crystals, one trh β B1 dimer interacts with no less than eight other dimers in the crystal lattice by using two different dimer-dimer interfaces (Figs. 2,4; Table 1). Interface 1 is composed of an interaction site A from one dimer and an interaction site B from a dimer related by crystallographic symmetry (Fig. 4A). Interaction site A comprises part of the N-terminal arm, Ala145 in the linker region between the two h β B1 domains and Gln235 and Trp236 in the C-termi-

Figure 3. Zoomed out views down the Q dyad of respective β B1 (A), cp β B2 dimers (B), and the top half of the β B2 tetramer (C). Asp168, Asp169, Arg230, and Arg232 in trh β B1 and the equivalent Asp107, Asp108, Arg169, and Arg171 in cp β B2 and β B2 are labeled. The monomers in the β B1 dimer are shown in gray and light gray; in cp β B2, in magenta and pink, and the domains belonging to different monomers in the top half of β B2 are shown in gray, light gray, and green, and light green, respectively.

Figure 4. (A) Close-up of interface 1. The interacting dimers are shown in yellow and blue ribbon representation. Residues from interaction site A are on the yellow dimer and are shown in dark pink. Residues from interaction site B on the blue dimer are shown in pale pink. Interaction site B is also shown in pink mapped to the semi-transparent surface of the second dimer. (B) Close up of interface 2. The interacting dimers are shown in magenta and blue ribbons, respectively. Residues from interaction site C are shown in green. Residues from interaction site D are shown in light green. Interaction site D is also shown in light green mapped to the semitransparent surface of the second dimer. Figure 4 was generated by using Aesop (M. Noble, unpubl.).

nal extension. Interaction site B on the interacting dimer is mainly composed of residues from the N-terminal domain from one of its monomers. However, the largest hydrophobic contribution is made by Trp174 located in the C-terminal domain of the other monomer in this dimer. Likewise,

interface 2 consists of interaction sites C and D on respective interacting dimers (Fig. 4B). Interaction site C is composed of residues from both the N- and C-terminal domains of one of the monomers in the dimer, whereas interaction site D comprises two linker residues and residues from the

Table 1. Intermolecular interactions that stabilize the h β B1 dimer and higher-order interactions between h β B1 dimers.

Monomer–monomer interactions

P: Ile114(O)-Arg201(NH₂); Lys117(O)-Ser172(O γ); Glu119(N)-Gln226(O ϵ 1); Glu119(O)-Gln226(N ϵ 2); Arg122(NH₂)-Tyr197(OH); Trp126(O)-Arg201(N ϵ); Arg201(NH₂); Tyr130(OH)-Ser127(O γ)^a; Ser172(O γ)-Lys117(O); Tyr197(OH)-Arg122(NH₂); Arg201(N ϵ)-Trp126(O); Arg201(NH₂)-Ile114(O); Trp126(O); Gln226(O ϵ 1)-Glu119(N); Gln226(N ϵ 2)-Gln119(O); Arg232(NH₂)-Asp168(O δ 2)

H: Glu116-Pro171; Lys117-Trp174, Val175; Gly118-Trp174, Pro198; Tyr120-Pro198; Pro121-Tyr197; Thr125-Tyr197, Arg201; Trp126-Arg201; Tyr130-Asn124^b, Ser128^a; Pro171-Glu116; Tyr174-Lys117, Gly118; Val175-Lys117; Lys234; Tyr176-Lys234; Tyr197-Thr125, Pro121; Pro198-Gly118, Tyr120; Tyr200-Arg229; Arg201-Thr125, Trp126; Arg229-Tyr200; Lys234-Val175, Tyr176

Dimer–dimer interactions

Interface 1

P: Asn57A(N δ 2)-Asn67B(O), Gln69B(O ϵ 1); Ala145A(N)-Glu74B(O ϵ 2); Gln235A(O)-Arg72B(N)
H: Pro54A-Trp174A, Ile94B, Ser96B, Lys117B, Glu119B; Pro55A-Trp174A; Gly56A-Phe68B, Ile94B; Asn57A-Phe68B, Gln69B; Ala145A-Glu74B; Gln235A-Gly70B, Arg72B; Trp236A-Arg72B, Ala73B, Glu74B, Val61B

Interface 2

P: Gln105A(N ϵ 2)-Glu164B(O ϵ 1); Gln222A(N ϵ 2)-Gln146B(O ϵ 1); Arg131A(NH1)-Asp211B(O δ 2); Arg131A(NH2)-Asp211B(O δ 1); Arg131A(O)-Lys186B(N ζ); Arg134A(NH1)-Gly160B(O); Arg134A(NH2)-Phe158B(O), Gly160B(O)
H: Arg134A-Thr162; Phe221A-Glu147B, His148B, Lys149B, Ser189B

The monomer–monomer interactions are topologically equivalent to the interface in β B2 previously defined as QR (Lapatto et al. 1991). P and H denote hydrogen bonds/ionic interactions and hydrophobic interactions, respectively.

^a Interactions only from molecule A to B.

^b Interactions only from molecule B to A. All contacts in Table 1 and Table 2 were calculated by using the program CONTACT of the CCP4 suite and manually evaluated by using the graphics programs O (Jones et al. 1991) and Quanta (Accelrys Inc.). Trh β B1 dimers interact with each other using two different interfaces. Interface 1 is composed of interaction sites A and B; interface 2, of interaction sites C and D. Residues before the hyphen are from interaction sites A or C; after the hyphen from interaction, sites B or D. Using these two different interfaces, each trh β B1 dimer interacts with eight other dimers in the crystal.

Table 2. Statistics of data collection

Data set	Low resolution	High resolution
Cell dimensions (Å)	$a = b = 45.16, c = 171.00$	$a = b = 45.04, c = 171.11$
Resolution (Å)	2.7	1.4
Space group	$P4_32_12$	$P4_3$
No. reflections	59,181	315,295
Unique reflections	5244	60,531
Completeness (%)	97.4	85.6
R_{merge}^a	4.5	4.3
$I/\sigma(I)$	11.8	8.9
Refinement		
$R_{\text{cryst}}/R_{\text{free}}^b$		0.186/0.211
RMSD bonds (Å)		0.023
RMSD angles (°)		1.87

^a $R_{\text{merge}} = \sum_i \sum_h |I(h,i) - \langle I(h) \rangle| / \sum_h \sum_i \langle I(h) \rangle$; $I(h,i)$ is the scaled intensity of the i th observation of reflection h , and $\langle I(h) \rangle$ is the mean value. Summation is over all measurements.

^b $R_{\text{cryst}} = \sum_{hkl, \text{work}} |F_{\text{obs}} - kF_{\text{calc}}| / \sum_{hkl} F_{\text{obs}}$, where F_{obs} and F_{calc} are the observed and calculated structure factors, k is a weighting factor, and work denotes the working set of 95% of the reflections used in the refinement. $R_{\text{free}} = \sum_{hkl, \text{test}} |F_{\text{obs}} - kF_{\text{calc}}| / \sum_{hkl} F_{\text{obs}}$, where F_{obs} and F_{calc} are the observed and calculated structure factors, k is a weighting factor, and test denotes the test set of 5% of the reflections used in cross validation of the refinement.

C-terminal domain of one of the monomers in the interacting dimer. Both interfaces are formed by a mixture of direct hydrophobic and hydrophilic contacts and are strengthened by mediating water molecules.

The trh β B1 structure shows Pro54 stacking against the side chain of Trp174 of a crystallographically related dimer (Fig. 4; Table 1), consistent with a role of the N-terminal sequence extension in higher assembly. Intriguingly, circular dichroism and fluorescence spectroscopies show that homodimer surface tryptophans become buried in β A3/ β A1- β B1-crystallin heteromers (Bateman et al. 2003). Therefore dimer-dimer interactions of the N-terminal sequence extension in the trh β B1 crystal structure may be typical of more complex higher-order molecular-weight interactions between basic and acidic β -crystallins in the lens.

Because the β -crystallin proteins are extremely long-lived as the mature eye lens fiber cell does not have the machinery for macromolecular synthesis or degradation, age-related truncation of β -crystallins could affect the higher-order assembly as well as the solubility and stability of the different β -crystallins, which may ultimately result in cataract formation. Indeed, truncation of h β B1 is correlated with its presence in the lower-molecular-weight β L-crystallins (Ajaz et al. 1997) and extensive age-related β B1 truncations of up to 40 N-terminal residues have been observed in the soluble lens crystallin fraction of normal human lenses (David et al. 1996; Ma et al. 1998). Even more extensive truncations of up to 73 N-terminal residues were found in the insoluble lens crystallin fraction of 50- to 65-year-old human lenses (Hanson et al. 2000). Nevertheless, truncation of up to 47 residues from the N terminus and five residues from the C terminus did not affect in vitro stability (Kim et al. 2002). This is consistent with the trh β B1 struc-

ture as the bulk of the N-terminal extension is clearly not necessary to stabilize the dimeric form. However, truncations reaching into the N-terminal domain would be predicted to cause unfolding and loss of solubility.

Unfortunately, the structure of full-length h β B1 in the context of higher assemblies of β H-crystallin is unknown, although NMR-experiments on h β B1 self-assemblies indicate that most of its N-terminal extension is flexible and has no ordered structure (Lampi et al. 2002). However, in addition to an unexpected domain-pairing, the crystal structure of trh β B1 shows that N-terminal residues close to the globular domains are well suited to form an intricate branched-out hetero-oligomeric network.

Materials and methods

Crystals of truncated human β B1 were grown as described earlier (Bateman et al. 2001). Two native data sets, one to 2.7 Å and one to 1.4 Å resolution, have been collected at the ESRF on beam-line ID14-3 by using a MARCCD detector. The high-resolution data set was collected in a low- and a high-resolution pass (Table 2). Both data sets were processed, scaled, and merged by using MOSFLM and SCALA (Collaborative Computational Project, No. 4, 1994). The 2.7 Å data set was indexed in space group $P4_32_12$, indicating one molecule in the asymmetric unit. Initial phases were obtained by molecular replacement with AMORE (Collaborative Computational Project, No. 4, 1994) using data between 15 and 3.0 Å from the 2.7 Å data set. The N-terminal domain of bovine β B2 comprising residues 1–81 with nonconserved residues truncated to alanine residues was used as a search model. Phase improvement combined with automatic model building with ARP/wARP (Perakis et al. 1999) was carried out by using the high-resolution data set. About 93% of the main chain of the current model was built automatically, the rest of the main chain and all side chains were manually built-in using O (Jones et al. 1991). During refinement of the model with Refmac5 (Collaborative Computational Project,

No. 4, 1994), severe clashes were observed between two tyrosine residues (Tyr130) belonging to molecules related by the crystallographic twofold axis. To account for this breakdown of the crystallographic symmetry, the high-resolution data set, which was initially indexed in $P4_32_1$ assuming one molecule in the asymmetric unit, was reprocessed in $P4_3$ with two molecules in the asymmetric unit related by a noncrystallographic twofold axis and the side chain of Tyr130 occupying different rotamer positions in the two monomers. The final model consists of the two NCS-related monomers, each comprising residues 53–236 and 330 water molecules.

Protein Data Bank accession codes

Coordinates and structure factors have been deposited in the Protein Data Bank, accession codes 1oki and r1okisf.

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