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βB1-Crystallin: Thermodynamic Profiles of Molecular Interactions

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Abstract

Background: β-Crystallins are structural proteins maintaining eye lens transparency and opacification. Previous work demonstrated that dimerization of both βA3 and βB2 crystallins (βA3 and βB2) involves endothermic enthalpy of association (~8 kcal/mol) mediated by hydrophobic interactions.

Methodology/Principal Findings: Thermodynamic profiles of the associations of dimeric βA3 and βB1 and tetrameric βB1/βA3 were measured using sedimentation equilibrium. The homo- and heteromolecular associations of βB1 crystallin are dominated by exothermic enthalpy (−13.3 and −24.5 kcal/mol, respectively).

Conclusions/Significance: Global thermodynamics of βB1 interactions suggest a role in the formation of stable protein complexes in the lens via specific van der Waals contacts, hydrogen bonds and salt bridges whereas those β-crystallins which associate by predominately hydrophobic forces participate in a weaker protein associations.

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Introduction

The transparency and refraction of the mammalian lens are dependent on the molecular associations of the crystalline proteins. These proteins form a βγ-crystallin superfamily those members are similar in structure and contain Greek key motifs. The β-crystallins constitute the major proportion of the lens proteins and seven subtypes have been identified, four of which are acidic (βA1, βA2, βA3, and βA4), and three basic (βB1, βB2, and βB3). Most β-crystallins are monomer-dimer systems [1–3] but some, for example, βA2 and βA4, have low intrinsic solubility and exhibit only weak self-associations [4,5]. The *in vivo* heteromolecular interactions of acidic with basic β-crystallins can circumvent solubility issues [4–6]. Under physiological conditions only β-crystallins are known to associate into dimers, tetramers, and higher-order oligomers [3,7–9]. Although the interactions of the β-crystallins have been well studied [4,10], the detailed molecular mechanisms of most associations remain obscure.

We previously demonstrated that the self-associations of both βA3 and βB2 are mediated by hydrophobic interactions [11]. Here we describe the energetics controlling βB1 dimerization and tetramer formation with βA3. βB1 and βA3 crystallins are major component in the human lens [12,13] and both recently were found in non-lens tissues including the retina [14–16]. We show that the molecular associations of βB1 crystallin are dominated by exothermic enthalpy. This indicates that βB1 plays an important role in the formation of stable protein complexes mediated by specific (stronger) interactions stabilized by van der Waals forces, hydrogen bonds, and salt bridges.

Results

Protein molecular weights and associative behavior

βB1 (monomer, 28 kDa) and βA3 (monomer, 25 kDa) elute during size-exclusion chromatography (SEC) with apparent molecular weights of 35 and 42 kDa, respectively, intermediate between that of monomers and dimers (**Fig. S1**). Sedimentation equilibrium analysis indicated the proteins are reversible monomer-dimer systems [2]. When equimolar amounts of βB1 and βA3 were mixed and incubated for 24 hrs at 20°C, a single symmetrical peak with apparent molecular weight of ~70 kDa was observed (**Fig. S1**). Sedimentation equilibrium analysis of the mixture indicated a weight-average molecular weight of 95 kDa close to that predicted for a weak heterotetramer (M_r 106 kDa). The best–fit model for the equilibrium data was a heterodimer - heterotetramer system with a K_d of 8.78 μM (**Table S1**). This result is very similar to that measured for the analogous interaction with murine β-crystallins [17].

βB1 dimerization energetics

To gain information on the energetics of the βB1 interactions, the temperature dependence of association was determined by sedimentation equilibrium over the range 5–30°C. The equilibrium profiles are shown in **Fig. S2 Panel A**. βB1 is a reversible monomer- dimer system with the equilibrium position shifting towards monomeric protein at higher temperatures as indicated by increased K_d values (**Table S1**). For example, the dimer fraction of βB1 (total 18 μM) decreased from 81% at 10°C to 50% at 30°C.

Table 1. Thermodynamic profiles for the associations of homodimeric βB1 and βA3 and tetrameric βB1/βA3.

Crystallin	ΔC_p cal/deg mol	ΔS_a e. u.	ΔH_a kcal/mol	$-T\Delta S_a$ kcal/mol	ΔG_a kcal/mol
βB1	0	−43.2 (±19.3)	−13.3 (±5.6)	12.7 (±5.8)	−0.6
	−2.5 (±0.9)	−69.3 (±15.5)	−21.2 (±4.6)	20.3 (±4.5)	−0.9
βA3	0	29.8 (±10.2)	8.0 (±3.0)	−8.7 (±3.0)	−0.7
	−0.8 (±0.6)	27.8 (±8.5)	7.2 (±2.5)	−8.1 (±2.5)	−0.9
[βB1/βA3]$_4$	0	−81.1 (±21.2)	−24.5 (±6.2)	23.8 (±6.2)	−0.7
	−1.6 (±1.6)	97.9 (±27.4)	−29.6 (±8.1)	28.7 (±8.0)	−0.9

Thermodynamics parameters, enthalpy ΔH_a, and entropy ΔS_a changes were determined using linear ($\Delta C_p = 0$) and nonlinear ($\Delta C_p \neq 0$) fitting functions into van't Hoff plots. The Gibbs free energy changes ΔG_a were calculated using formula $\Delta G_a = \Delta H_a - T\Delta S_a$, where T is temperature in K; e.u. = 1 cal/(deg mol).
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The calculated free energy ΔG_a values for dimerization also decrease with increase in temperature. This is due to the negative contributions from both enthalpy ΔH_a and entropy ΔS_a (**Table 1**) which were derived from plots (non-linear) of In K_d and C_o versus 1/T (**Fig. 1A, B**).

βA3 dimerization energetics

To be consistent with our previous work with murine βA3 crystallin [11], we analyzed the association behavior of the human protein (sequence identity with murine protein 95%). As expected, human βA3 formed tighter dimers (lower K_d's) at higher temperatures (**Table S1**) [11]. Thus, in contrast to βB1, the dimer fraction of βA3 increases with temperature (58% at 5°C and 80% at 30°C). There was a linear dependence of the $\ln K_0/C_0$ on $1000/T$ using the change in heat capacity ΔC_p zero or non zero values. (**Table S1**, and **Fig. 1A, B**). Hence, the self-association of human βA3 is characterized by positive enthalpy ΔH_a and entropy ΔS_a at zero and nonzero ΔC_p (**Table 1**), which is similar to our previously published analysis of the murine protein [2].

βB1/βA3 tetramerization energetics

The βB1/βA3 complex is best modeled as a reversible heterodimer-heterotetramer system over the temperature range studied (5–30°C) with a tendency to form weaker tetramers at

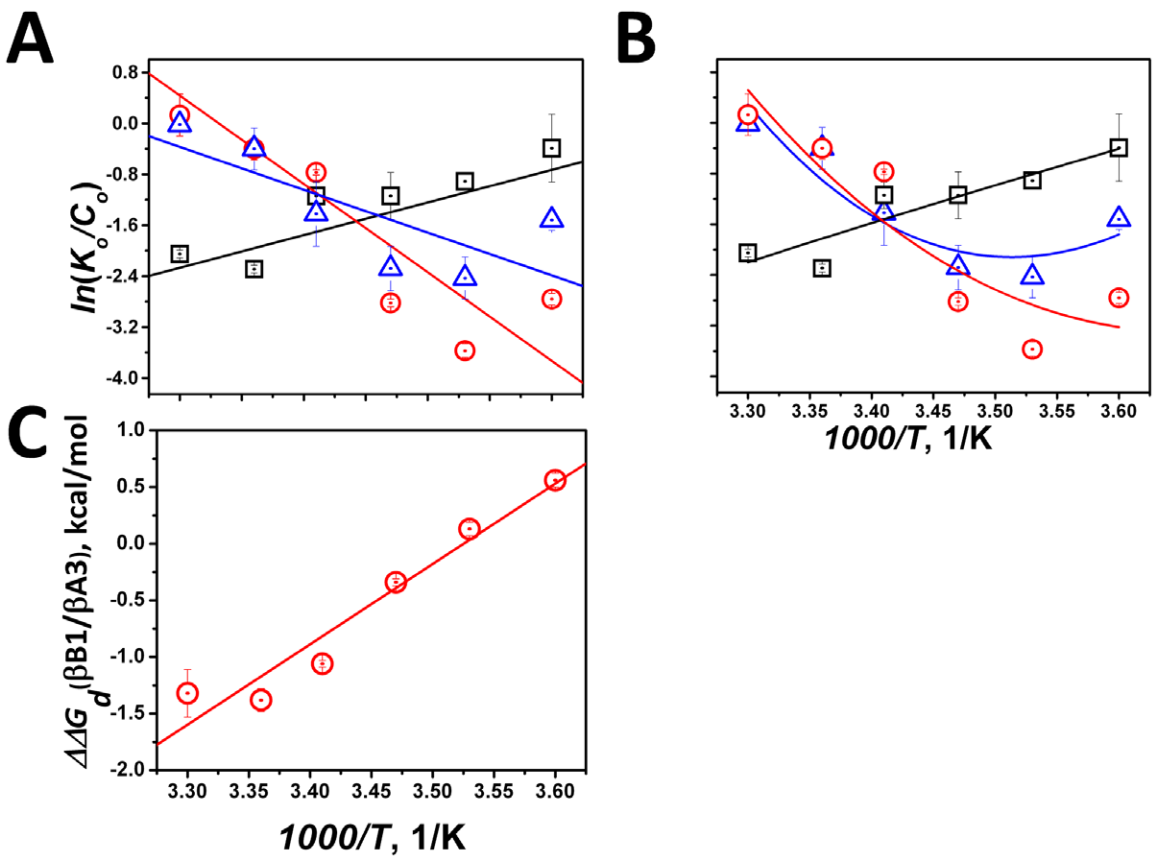

Figure 1. The temperature dependence of changes in the free energies for the dimeric association of βB1 and βA3 and the tetrameric association of βB1/βA3. Asssociation of βB1, βA3 and βB/βA3 are shown by blue open triangles, black open squares and red open circles, respectively. Panels A and B: van't Hoff plots where $ln(K_d/C_o)$ is plotted as function of the reciprocal of absolute temperature ($1000/T$), K_d's are the dissociation constants obtained from analytical ultracentrifugation, and C_o is the μM concentration. Panel A: the difference in heat capacity (ΔC_p) is constrained to be 0, resulting in a linear function; Panel B: ΔC_p is not constrained and has a nonzero value. Panel C: temperature dependence of Gibbs free energy gained in formation of βB1/βA3. $\Delta\Delta G_d$ ($\beta B1/\beta A3$) is defined as a difference between Gibbs free energy changes of tetrameric βB1/βA3 and that of individual components (βB1 and βA3). Concentrations for βB1, βA3, and βB1/βA3 crystallins were each 0.5 mg/ml.
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higher temperature (**Fig. S1, Fig. S2 Panel B,** and **Table S1**). The relationship between the logarithm of the K_d and the reciprocal of the absolute temperature demonstrated by the van't Hoff plot is not linear (**Fig. 1A, B**). When $\Delta\Delta G_a$ is plotted against the reciprocal of the absolute temperature (**Fig. 1C**) $\Delta\Delta G_a$ values negatively increase with increasing temperature. Tetramer formation is, therefore, associated with exothermic enthalpy ΔH_a and entropy ΔS_a (**Table 1**) and, analogous to βB1 dimerization, ΔG_a decreases with increasing temperature (**Fig. 1C**).

Discussion

We have previously shown that both murine βA3 and βB2 are monomer – dimer systems with a tendency to form tighter dimers at higher temperatures [11]. Moreover, the self-association of these crystallins, characterized by positive enthalpy and entropy changes, is entropically driven and mediated by hydrophobic interactions. These endothermic associations ($\Delta H>0$) are dominated by hydrophobic effects entropically driven by water. Here we have shown a similar energetic profile for human βA3 (**Table S1**) indicating that nonpolar regions of the protein, previously accessible to solvent in the isolated subunits, become buried upon dimer formation [18].

The self-association of βB1 energetically differs from that of βA3 and βB2 in that its dimers are destabilized at higher temperatures. The thermodynamic profile (**Table 1**) indicates that both the dimerization of βB1 and formation of the βB1/βA3 complex are exothermic processes ($\Delta H<0$). With tetrameric βB1/βA3 formation, decreasing negative values of ΔG confirm that the complex is less stable at higher temperatures. Large exothermic enthalpy change $\Delta H_a = -29.6\pm8.1$ kcal/mol and negative entropy $\Delta S_a = -97.9\pm27.4$ e.u. are accompanied with a negative heat capacity change $\Delta C_p = -1.6\pm1$ cal/deg mol. Thus, the profile suggests that tetramer formation is controlled by enthalpy and interactions between the subunits are mediated by van der Waals interactions, hydrogen bonds, and salt bridges [18].

A summary overview of the homo- and hetero-associations of β-crystallins is presented in **Fig. 2**. βB1 mediates protein interactions using van der Waals contacts and hydrogen bonds which suggest that contact involve complementary shapes of protein surfaces with a higher biological specificity [19]. In contrast the formation of dimeric βA3 and βB2, are driven by hydrophobic forces which are usually less specific. In these associations, hydrophobic residues at the surface interfaces become excluded from direct contact with surrounding water molecules.

Currently we cannot completely rule out the possibility that heterotetramers are formed by the association of homodimers rather than heterodimers but in either case it does not affect our analyses and conclusions. However; the precise mechanism of βB1/βA3 association appears to involve the association of heterodimers [2]. The kinetics, equilibrium position and balance of so-called "close" and "open" conformational isomers could be affected by interactions of core domains or with the N-terminal extensions of βA3 and βB1 [1,17]. The dimerization of βB1 may 'induce' a conformational shift which favors interaction with βA3. Such a model would explain why both, homo- and hetero-association of βB1 are driven by enthalpy.

Previous dynamic light scattering analysis demonstrated that in fetal calf lenses soluble crystallins form a broad distribution of protein complexes with sizes of 8–14 nm with β- and γ-crystallins at the leading edge of this distribution [20]. Proteomic analysis has demonstrated that large molecular complexes are often built around a stable core of proteins, which are expanded thorough the attachment of weakly bound exchangeable peripheral proteins often stabilized by dynamic transient interactions [21,22]. These exchangeable components could be for example, so-called 'weak' dimers which have relatively high K_d's [23]. The protein interfaces in 'weak' dimers are loosely packed and more hydrophobic than in average protein transient complexes.

βB2 and βA3 crystallins could have a propensity to be components of a peripheral protein network. The less specific and more transient nature of their interactions would give these crystallins more flexibility for binding. On the other hand, the more specific and stronger exothermic interactions involving βB1 make this crystallin more suitable for formation of the stable core of the lens proteins.

Although we have described the interactions of the crystallins as being mediated by either the weaker hydrophobic or the more specific van der Waals interactions, both may occur but on average one dominates energetically. It is known, for example, from antibody – antigen interactions, that initial contacts may involve hydrophobic interactions via interface aromatic residues followed by more specific and tighter H- bonding and salt bridges [18,24].

In conclusion, the global thermodynamics of βB1 interactions indicate that they contribute in more stable protein complexes in the lens via specific van der Waals contacts, hydrogen bonds and salt bridges whereas those β-crystallins which associate by predominately hydrophobic forces are more likely to participate in a weaker protein associations.

Materials and Methods

Expression, purification and association of βB1- and βA3-crystallins

Wild type recombinant murine βB1 and human βA3 were expressed as soluble proteins in *E.coli* and purified as previously described by ion-exchange and size-exclusion chromatographies [2,17]. Murine βB1 was used which has a 95% sequence similarity (>80% sequence identity) with the human protein and is, therefore, a reasonable surrogate. The purified proteins were dialyzed overnight against Buffer A (50 mM Tris-HCl, 1 mM EDTA, 0.15 M NaCl, 1 mM TCEP, at pH 7.5) at 4 °C. Protein concentrations were estimated from $A_{280/260}$ (Beckman Coulter DU650, CA) and adjusted to 0.5 mg/ml. For the formation of complexes between βB1 and βA3, an equimolar mixture (~20 μM each) was incubated at room temperature for 24 h. Aliquots (250 μl) were loaded on an analytical grade Superdex 75 HR10/30 column, precalibrated with standards (bovine serum albumin, 67 kDa, ovalbumin, 43 kDa, chymotrypsinogen, 25 kDa, and ribonuclease A, 13.7 kDa; Sigma, MO). Samples were eluted at a flow rate of 0.5 ml/min and 0.5 ml fractions were collected.

Analytical Ultracentrifugation

A Beckman Optima XL-I analytical ultracentrifuge with absorption optics, an An-60 Ti rotor, and standard double-sector centerpiece cells were used for sedimentation equilibrium experiments. All analyses were performed using duplicate protein samples. Data were collected after 16 hours at 18,500 rpm at 20°C. The baselines were established by overspeeding at 45,000 rpm for another 4 hours. Equilibrium profiles were analyzed by standard Optima XL-I Origin-based data analysis software. Solvent density was estimated as previously described [25]. Monomeric molecular weights M_r and molar extinction coefficients were used for calculation of dissociation constants K_d. The M_r and K_d were measured in duplicate and averaged. Equilibrium data was collected with 5 °C temperature increments for the ranges: 5–25°C and 15–30°C.

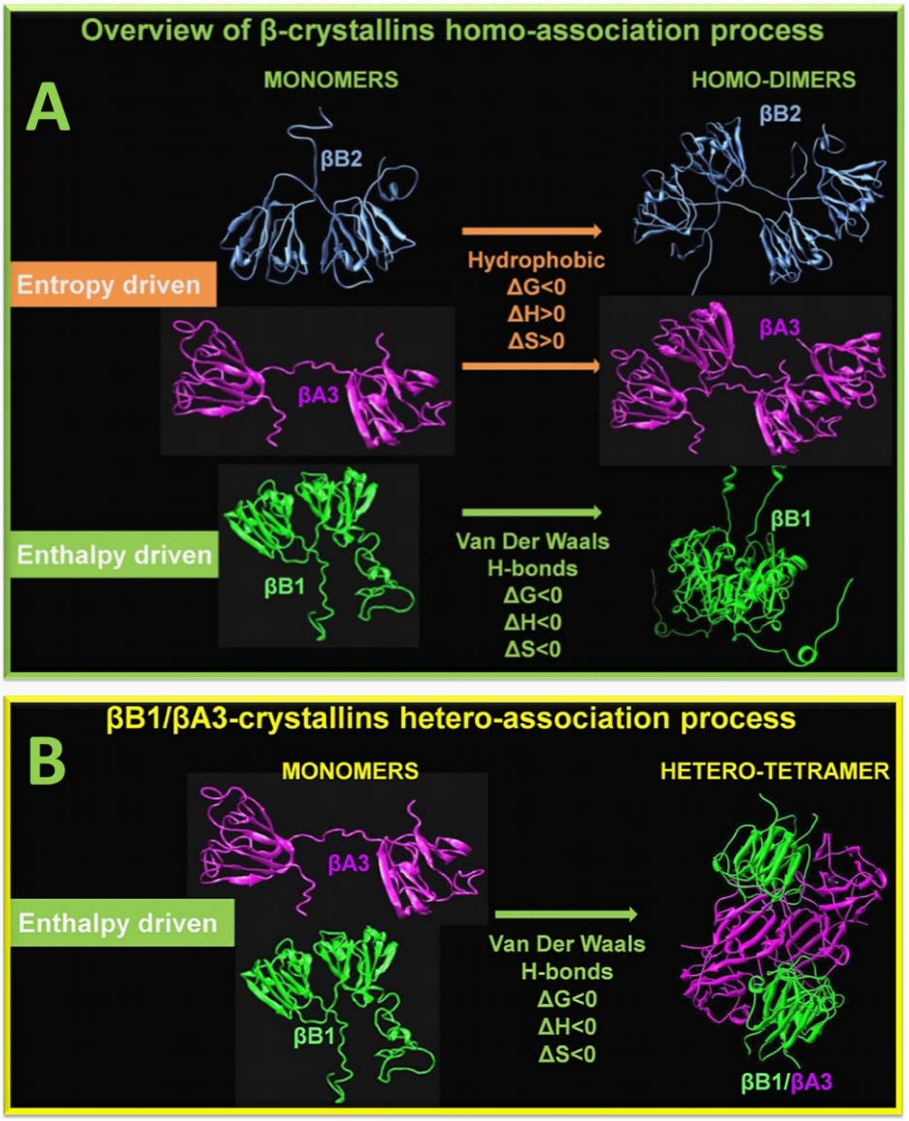

Figure 2. Overview of the homo- and heteromolecular associations of β-crystallins. Top panel: βA3, βB1, and βB2 self-associate in a reversible manner to form dimers. The homo-associations of βA3 and βB2 exhibit endothermic enthalpy and are driven by entropy as a result of hydrophobic interactions between protein molecules. In contrast, the self-association of βB1 is driven by exothermic enthalpy due to van der Waals interactions and hydrogen bonds at the dimer interface. Bottom panel: The βB1/βA3 complex is likely formed by the association of hetero-dimers but we cannot rule out that it is formed from homodimers. Similar to that of βB1 alone, the formation of the tetramer is driven by exothermic enthalpy. Structures of βB1 and βB2 were obtained from the protein database RCSB (files: 1 oki and 1 blb, respectively). Closed and open structures of βA3 were modeled as described earlier [1]. From our results we cannot say which monomer conformation exists within the hetero-tetramer. However, the majority of known crystal structures of β-crystallins (3 of 4) are of the closed monomer type suggesting this is the most stable conformation. Therefore, the structure of the hypothetical tetrameric βB1/βA3 complex was generated using the crystal packing of βB1 crystallin as a template (PDB file: 1 oki).
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Energetics of monomer-dimer and dimer-tetramer equilibrium

The temperature dependence of association was examined for homodimer and hetero-tetramer associations between 5–30 °C, using a previously described [11] equation:

$$ln(K_d/C_o) = (1/R)\left[\Delta C_p(293.15/T - ln(293.15/T) - 1) - \Delta H^\circ/T + \Delta S^\circ\right] \quad (1)$$

where K_d is the dissociation constant, measured by AUC; C_o is the molar concentration of protein (μM); R is universal gas constant; T is temperature (K); and ΔC_p, ΔH°, and ΔS° are changes in protein heat capacity, enthalpy and entropy, respectively. The experimental data were fitted in two ways: first; where ΔC_p was constrained to be zero and second; where ΔC_p was nonzero. The effect of protein concentration was excluded from the analysis by normalization to the protein molar concentration C_o (See formula 1).

Supporting Information

Figure S1 Size-exclusion chromatography profiles obtained for individual proteins and the βB1/βA3 complex.

The chromatographic profile obtained immediately after mixing of equimolar amounts of βB1 and βA3 is shown in green and following 24 hours of incubation, by the red line. The elution positions of molecular weight standards are shown at the top of the figure.
(TIF)

Figure S2 Sedimentation equilibrium profiles of βB1 and βB1/βA3 complex at various temperatures. In the main panels, open circles show the protein concentration profile represented by the UV absorbance gradients in the centrifuge cell at 280 nm. The solid lines indicate the calculated fits for the βB1monomer-dimer (Panel A) or heterodimer – heterotetramer βB1/βA3 complex (Panel B) associations. Residuals in the smaller upper panels show the difference in the fitted and experimental values as a function of radial position. In many of the profiles, the residuals at the bottoms of the cells reveal systematic patterns indicative of aggregating protein; this data was not included in the analyses.
(TIF)

Table S1 Dissociation constants (K_d) and changes in Gibbs free energy of association obtained at different temperatures for the formations of dimeric βB1, and βA3, and tetrameric βB1/βA3 Association free energy change ΔG_a estimated as $\Delta G_a = -\Delta G_d$, where the dissociation free energy change is $\Delta G_d = -RT\ ln(K_d/C_o)$; K_d is the dissociation constant in μM and C_o is the protein sample concentration in μM; standard errors were calculated from 2–3 times repeated data and are shown in parentheses.
(DOCX)

Author Contributions

Conceived and designed the experiments: YS PW. Performed the experiments: MD YS PW. Analyzed the data: MD YS PW. Contributed reagents/materials/analysis tools: MD YS PW. Wrote the paper: MD YS PW.

References

1. Sergeev YV, Wingfield PT, Hejtmancik JF (2000) Monomer-dimer equilibrium of normal and modified beta A3-crystallins: experimental determination and molecular modeling. Biochemistry 39: 15799–15806.
2. Chan MP, Dolinska M, Sergeev YV, Wingfield PT, Hejtmancik JF (2008) Association properties of betaB1- and betaA3-crystallins: ability to form heterotetramers. Biochemistry 47: 11062–11069.
3. Lampi KJ, Oxford JT, Bachinger HP, Shearer TR, David LL, et al. (2001) Deamidation of human beta B1 alters the elongated structure of the dimer. Exp Eye Res 72: 279–288.
4. Liu BF, Liang JJ (2007) Protein-protein interactions among human lens acidic and basic beta-crystallins. FEBS Lett 581: 3936–3942.
5. Marin-Vinader L, Onnekink C, Van Genesen ST, Slingsby C, Lubsen NH (2006) In vivo heteromer formation. Expression of soluble betaA4-crystallin requires coexpression of a heteromeric partner. FEBS J 273: 3172–3182.
6. Bateman OA, Sarra R, Van Genesen ST, Kappe G, Lubsen NH, et al. (2003) The stability of human acidic beta-crystallin oligomers and hetero-oligomers. Exp Eye Res 77: 409–422.
7. Slingsby C, Bateman OA (1990) Qarternary interactions in eye lens beta-crystallins: basic and acidic subunits of beta-crystallins favor heterologous association. Biochemistry 29: 6592–6599.
8. Werten PJ, Lindner RA, Carver JA, de Jong WW (1999) Formation of betaA3/betaB2-crystallin mixed complexes: involvement of N- and C-terminal extensions. Biochim Biophys Acta 1432: 286–292.
9. Takata T, Woodbury LG, Lampi KJ (2009) Deamidation alters interactions of beta-crystallins in hetero-oligomers. Mol Vis 15: 241–249.
10. Hejtmancik JF, Wingfield PT, Sergeev YV (2004) beta-Crystallin association. Exp Eye Res 79: 377–383.
11. Sergeev YV, Hejtmancik JF, Wingfield PT (2004) Energetics of Domain-Domain Interactions and Entropy Driven Association of beta-Crystallins. Biochemistry 43: 415–424.
12. David LL, Lampi KJ, Lund AL, Smith JB (1996) The sequence of human betaB1-crystallin cDNA allows mass spectrometric detection of betaB1 protein missing portions of its N-terminal extension. J Biol Chem 271: 4273–4279.
13. Lampi KJ, Ma Z, Shih M, Shearer TR, Smith JB, et al. (1997) Sequence analysis of betaA3, betaB3, and betaA4 crystallins completes the identification of the major proteins in young human lens. J Biol Chem 272: 2268–2275.
14. Yuan X, Gu X, Crabb JS, Yue X, Shadrach K, et al. (2010) Quantitative Proteomic Comparison of the Macular Bruch's Membrane/Choroid Complex from Age-related Macular Degeneration and Normal Eyes. Mol Cell Proteomics 9: 1031–1046.
15. Sinha D, Klise A, Sergeev Y, Hose S, Bhutto IA, et al. (2008) betaA3/A1-crystallin in astroglial cells regulates retinal vascular remodeling during development. Mol Cell Neurosci 37: 85–95.
16. Zigler JS, Jr., Zhang C, Grebe R, Sehrawat G, Hackler L, Jr., et al. (2011) Mutation in the betaA3/A1-crystallin gene impairs phagosome degradation in the retinal pigmented epithelium of the rat. J Cell Sci 124: 523–531.
17. Dolinska MB, Sergeev YV, Chan MP, Palmer I, Wingfield PT (2009) N-terminal extension of beta B1-crystallin: identification of a critical region that modulates protein interaction with beta A3-crystallin. Biochemistry 48: 9684–9695.
18. Ross PDSS (1981) Thermodynamics of protein association reactions: forces contributing to stability. Biochemistry 20: 3096–3102.
19. Roth CM, Neal BL, Lenhoff AM (1996) Van der Waals interactions involving proteins. Biophys J 70: 977–987.
20. Datiles MB, III, Ansari RR, Suh KI, Vitale S, Reed GF, et al. (2008) Clinical detection of precataractous lens protein changes using dynamic light scattering. Arch Ophthalmol 126: 1687–1693.
21. Gavin AC, Aloy P, Grandi P, Krause R, Boesche M, et al. (2006) Proteome survey reveals modularity of the yeast cell machinery. Nature 440: 631–636.
22. Stein A, Pache RA, Bernado P, Pons M, Aloy P (2009) Dynamic interactions of proteins in complex networks: a more structured view. FEBS J 276: 5390–5405.
23. Dey S, Pal A, Chakrabarti P, Janin J (2010) The subunit interfaces of weakly associated homodimeric proteins. J Mol Biol 398: 146–160.
24. Chothia C, Janin J (1975) Principles of protein-protein recognition. Nature 256: 705–708.
25. Laue TM, Shah BD, Ridgeway TM, Pelletier SL (1992) Computer-aided interpretation of analytical sedimentation data for proteins. In: Harding SE, Rowe AJ, Horton JC, eds. Analytical Ultracentrifugation in Biochemistry and Polymer Science. Cambridge, United Kingdom: Royal Society for Chemistry. pp 90–125.