



# A P39R mutation at the N-terminal domain of human $\alpha$ B-crystallin regulates its oligomeric state and chaperone-like activity

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## ABSTRACT

Recent structure analyses of  $\alpha$ B-crystallin have proposed some models of the N-terminal domain and the manner of oligomerization, whereas the effects of the significantly high content of Pro residues at the N-terminal domain remain unclear. We report the properties of a novel P39R mutant of  $\alpha$ B-crystallin. The content of  $\alpha$ -helix was increased, and the molecular size of the P39R mutant was larger than that of wild-type  $\alpha$ B-crystallin. A slight loss of chaperone-like activity was observed using alcohol dehydrogenase (ADH), while a significant increase was detected by insulin assay. The Pro residue at the N-terminal domain of  $\alpha$ B-crystallin is important for oligomerization and function.

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## 1. Introduction

The transparency of eye lens cells is maintained by a highly abundant protein known as  $\alpha$ -crystallin, which is composed of  $\alpha$ A- and  $\alpha$ B-crystallins [1]. Both proteins belong to the small heat shock protein (sHSP) family and function as molecular chaperones to prevent the aggregation of other proteins when exposed to certain types of stress [2]. Structural anomalies such as point mutations of  $\alpha$ -crystallin genes [3] or racemization and isomerization of certain residues by UV irradiation [4,5] are known to cause human cataracts. The expression of  $\alpha$ B-crystallin is widespread in many tissues, including muscle and brain, and has been implicated in myopathies [6], Alexander disease [7], and Alzheimer's disease [8]. For decades, researchers have attempted to describe the high-resolution structure of  $\alpha$ B-crystallin, which is composed of a core structure conserved among all sHSPs that is referred to as the  $\alpha$ -crystallin domain and highly flexible N- and C-terminal domains [9]. The crystal structures of truncated forms containing the  $\alpha$ -crystallin domain and a portion of the C-terminal domain [10,11], together with solid-state NMR measurements of full-length  $\alpha$ B-crystallin [12], have demonstrated that a stable homodimer is formed by the  $\beta$ -sheets of the  $\alpha$ -crystallin domain. However, the flexible N- and C-terminal domains yield diverse oligomeriza-

tion states [13] and prevent the crystallization needed for high-resolution structure determination. Recent studies of a quaternary assembly of  $\alpha$ B-crystallin based on EM, solid-state NMR, and small-angle X-ray scattering (SAXS) analyses proposed homo-oligomer models of 24-meric assembly [14–16]. The models also provide reasonable explanations for the heterogeneity of  $\alpha$ B-crystallin via the dynamic exchange of the dimer subassemblies to form larger oligomerizations such as 26 and 28-mer, and up to 36-mer proteins. On the other hand, these models differ significantly in terms of how contact is achieved between the N- and C-terminal domains and the neighboring subunit. Therefore, further structure analyses will be needed to elucidate the detailed oligomerization mechanism of  $\alpha$ B-crystallin.

Several crystal structures of intact or artificially truncated proteins belonging to the sHSP family have been reported thus far. Wheat HSP16.9 [17] and HSP16.5 from *Methanococcus jannaschii* (MjHSP16.5) [18] consist of 151 and 147 amino acids forming 12 and 24-meric homo-oligomers, respectively. These sHSPs are thought to be highly homologous to 24-meric  $\alpha$ B-crystallin composed of 175 amino acids in the monomeric structure. Only in the crystal structure of wheat HSP16.9, almost the entire N- and C-terminal domain regions of the monomer have been identified. The N-terminal domain of wheat HSP16.9 exhibits an  $\alpha$ -helix-rich structure, which is in good agreement with the proposed model of the N-terminal domain of  $\alpha$ B-crystallin [16]. The amino acid sequence alignment of  $\alpha$ B-crystallin with wheat HSP16.9 and MjHSP16.5 (Fig. 1) revealed that the N-terminal domain of  $\alpha$ B-crystallin contains significantly more Pro residues than do sHSPs. The role played by the high Pro residue content at the N-terminal

Abbreviations: ADH, alcohol dehydrogenase; sHSP, small heat shock protein; CD, circular dichroism; SAXS, small-angle X-ray scattering; DLS, dynamic light scattering.

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### 3. Results and discussion

#### 3.1. Circular dichroism spectral studies

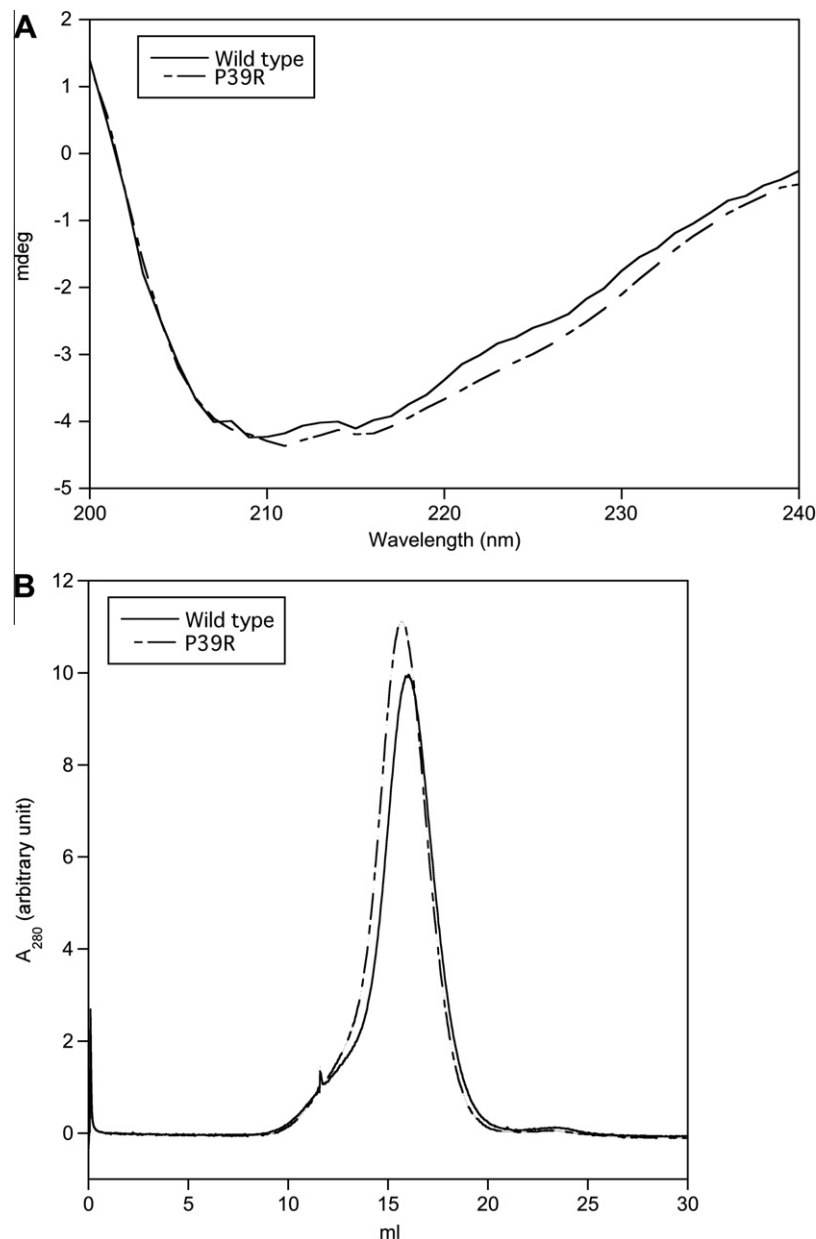
The secondary structures of the wild-type and P39R mutant  $\alpha$ B-crystallins were determined by far-UV CD spectral analysis (Fig. 2A and Table 1). As expected, the mutation of Pro39 to Arg increased the  $\alpha$ -helix content from 19.5% to 22.9%. In contrast, the  $\beta$ -sheet content of the P39R mutant showed a reduction to 30.2%, reduced from 35.5% in the wild-type. Few changes were observed in the  $\beta$ -turn and random coil contents. Because the highly conserved core domain is very stable with a dimer of  $\beta$ -sandwiches [10–12] and the C-terminal domain is thought to be highly flexible, it is likely that the changes in the secondary structure contents observed in the CD spectral analyses were due solely to structural changes at the N-terminal domain of the P39R mutant  $\alpha$ B-crystallin. The predicted secondary structures of the N-terminal domain of wild-type

**Table 1**

Secondary structure contents (%) of wild-type and P39R mutant  $\alpha$ B-crystallins.

	$\alpha$ Helix	$\beta$ Sheet	$\beta$ Turn	Random coil
Wild-type $\alpha$ B	19.5	35.5	18.8	26.2
P39R	22.9	30.2	19.6	27.2

$\alpha$ B-crystallin are composed of several helices and  $\beta$ -strands [15,16] (Fig. 1). Therefore, it was assumed that the structural changes induced by the mutation at Pro39 to Arg are primarily limited to the N-terminal domain of  $\alpha$ B-crystallin. It has been reported that the conformation of the N-terminal domain is related to its oligomerization states [15,16], and that the deletion of the N-terminal domain results in a larger oligomer than that of the wild-type  $\alpha$ B-crystallin [21]. Since the Pro residue generally acts as a structural disruptor in a regular  $\alpha$ -helix, mutation at Pro39 to Arg



**Fig. 2.** (A) Far-UV CD spectra of wild-type and P39R mutant  $\alpha$ B-crystallins. (B) Elution profiles of size exclusion chromatography for wild-type and P39R mutant  $\alpha$ B-crystallins.

**Table 2**  
Hydrodynamic radius of wild-type and P39R mutant  $\alpha$ B-crystallins.

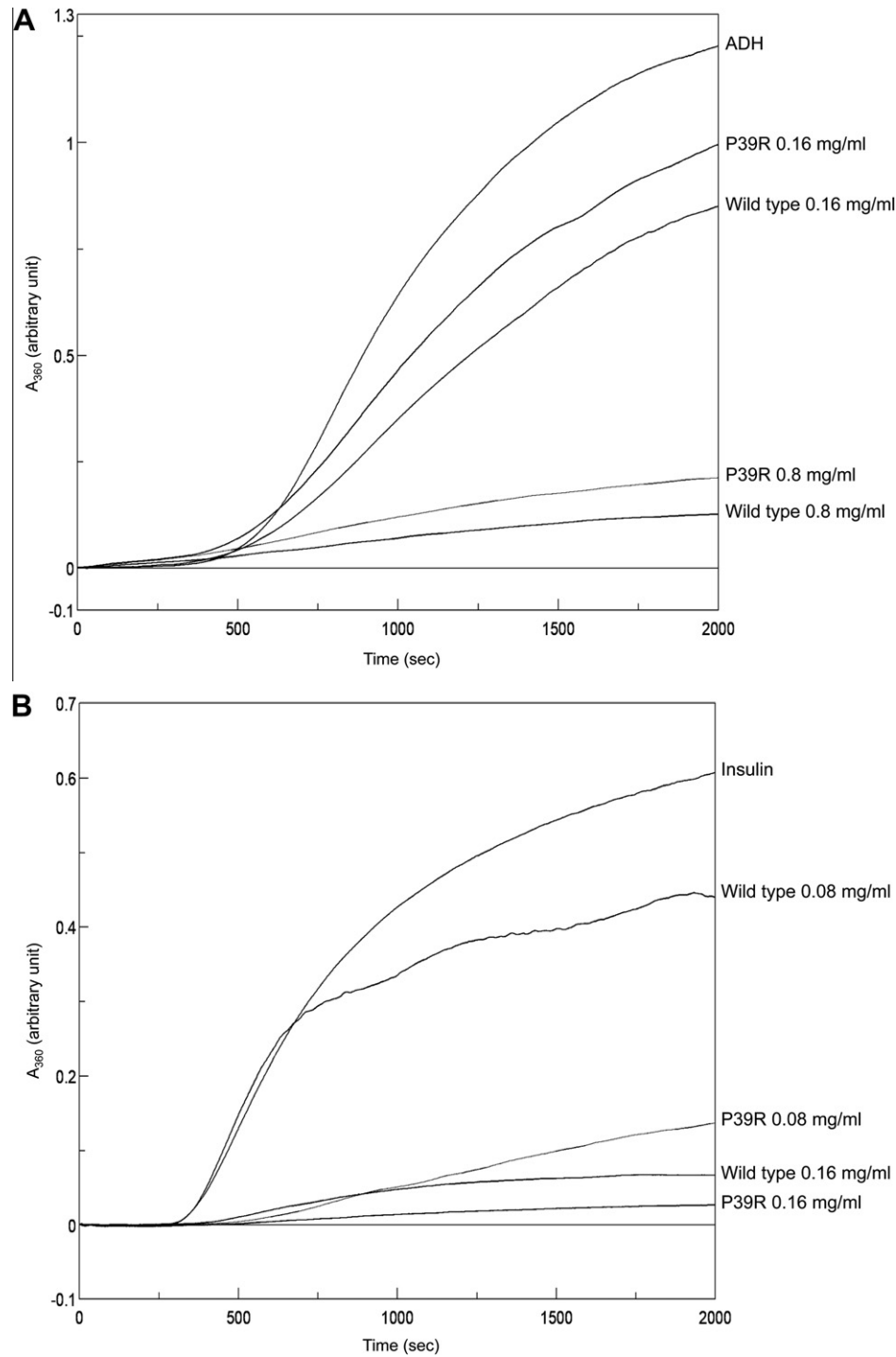
	Rh (nm)	Cp/Rh (%)
Wild-type $\alpha$ B	8.0	14.5
P39R	8.3	18.3

Rh, hydrodynamic radius.  
Cp/Rh, polydispersity index.

contributes to increase  $\alpha$ -helix content of the N-terminal domain of  $\alpha$ B-crystallin.

3.2. Molecular size analyses

The molecular sizes of the oligomeric complexes of wild-type and P39R mutant  $\alpha$ B-crystallins were estimated by size exclusion chromatography and DLS analyses. Size exclusion chromatography analysis revealed slightly faster elution for the P39R mutant (calculated as ~620 kDa) than that of the wild-type  $\alpha$ B-crystallin (calculated as ~510 kDa, Fig. 2B). These results were confirmed by the DLS data. The hydrodynamic radius of the wild-type and P39R mutant  $\alpha$ B-crystallin was 8.0 and 8.3 nm, respectively (Table 2). These findings indicate that the conformational change at the N-terminal



**Fig. 3.** Chaperone-like activities of wild-type and P39R mutant  $\alpha$ B-crystallins with (A) ADH (0.8 mg/ml) and (B) insulin (0.33 mg/ml) as substrate proteins at 37 °C.

domain would lead to the formation of a larger assembly than that of the 24-meric wild-type  $\alpha$ B-crystallin. This hypothesis does not contradict the assembly architecture proposed by Jehle et al., in which the N-terminal domain in the 24-mer model is located around the binding area for another molecule to form even larger oligomers, such as 26 or 28-mer oligomers [16]. The conformation of the N-terminal domain of P39R mutant  $\alpha$ B-crystallin would be preferred to form a larger assembly than that of wild-type  $\alpha$ B-crystallin.

### 3.3. Chaperone-like activity

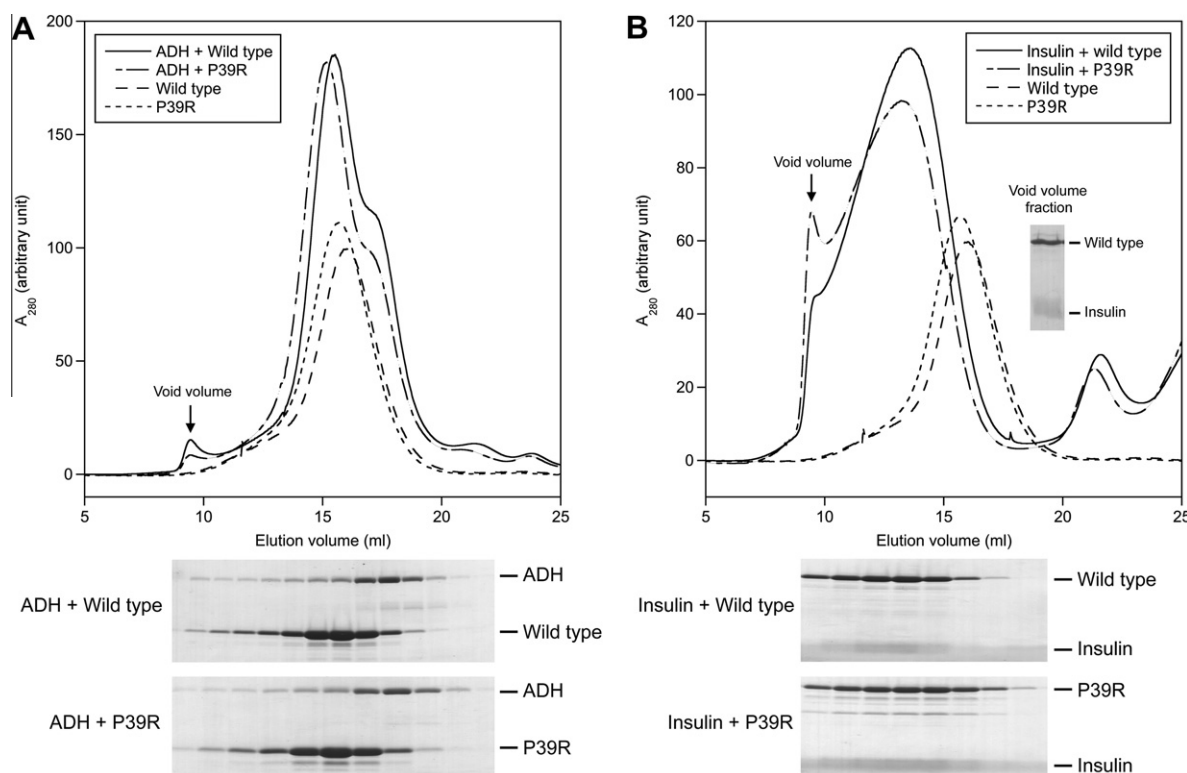
The suppression of aggregation was analyzed for denatured ADH and insulin (Fig. 3). In the ADH assay, the addition of 0.8 mg/ml of wild-type or P39R mutant  $\alpha$ B-crystallin reduced aggregation to 10.2% and 17.2% of the control, respectively, at 2000 min. Fewer  $\alpha$ B-crystallins caused a weak suppression of aggregation; the addition of 0.16 mg/ml of wild-type or P39R mutant  $\alpha$ B-crystallin reduced aggregation to 69.0% and 80.8% of the control level, respectively. Thus, the P39R mutant displayed a slight loss of chaperone-like activity with respect to that of the wild-type  $\alpha$ B-crystallin.

In contrast, the P39R mutant showed a significant increase in chaperone-like activity in the insulin assay. The addition of 0.16 mg/ml of wild-type or P39R mutant  $\alpha$ B-crystallin reduced aggregation to 10.1% and 4.0% of the control level, respectively, at 2000 min. Dilution by half of the wild-type  $\alpha$ B-crystallin (0.08 mg/ml) resulted in a much weaker suppression of aggregation, i.e., of 66.5% of the control, whereas dilution by half of the P39R mutant  $\alpha$ B-crystallin retained to some extent of chaperone-like activity, i.e., of 20.7% of the control.

To elucidate how complexes are formed in chaperone-like activity assays, size exclusion chromatography was performed under

conditions similar to those used for the chaperone-like activity assays. Protein concentrations were set to approximately ten times those used in the chaperone-like activity assays, in order to achieve clearer signals in the chromatograms and for subsequent PAGE analyses. For the assays of ADH and wild-type  $\alpha$ B-crystallin, very large aggregates were eluted at the void volume, but a large portion of the  $\alpha$ B-crystallin was eluted at 15.4 ml, just prior to the elution volume (16.0 ml) of the control of wild-type  $\alpha$ B-crystallin (Fig. 4A). The SDS-PAGE analysis of the void volume fraction demonstrated that the very large aggregates are derived from complexes of ADH and wild-type  $\alpha$ B-crystallin, whereas the fraction at the highest peak contains primarily  $\alpha$ B-crystallin and the fraction occurring just after the highest peak (shoulder peak at 17.0 ml) contains primarily ADH. These observations indicate that a few very large complexes (>10 MDa) of ADH and wild-type  $\alpha$ B-crystallin are formed, but that a large portion of the  $\alpha$ B-crystallin binds to no substrates or to only a small amount of substrate. Similar results were obtained in the assay of the ADH and P39R mutant  $\alpha$ B-crystallin, except that the ratio of the peak of very large aggregates at the void volume to the main peak was reduced. For the assay of insulin and wild-type  $\alpha$ B-crystallin, very large aggregates were also eluted at the void volume, but the highest peak (13.6 ml) of elution volume was observed significantly before that of the control of wild-type  $\alpha$ B-crystallin (Fig. 4B). The SDS-PAGE analysis demonstrated that both peaks are derived from complexes of insulin and wild-type  $\alpha$ B-crystallin. Similar results were obtained for the assay of insulin and the P39R mutant  $\alpha$ B-crystallin, except that the ratio of the peak of very large aggregates at the void volume to the main peak was significantly increased.

The generation of the very large aggregates at the void volume corresponded to the observed chaperone-like activities. Therefore, the chaperone-like activity of  $\alpha$ B-crystallin would depend on how the very large aggregates, the molecular size of which was



**Fig. 4.** Elution profiles and SDS-PAGE analyses of fractions of size exclusion chromatography for the complexes of (A) denatured ADH and (B) denatured insulin with wild-type and P39R mutant  $\alpha$ B-crystallins. The elution profiles of the control of wild-type and P39R mutant  $\alpha$ B-crystallins are overlaid. SDS-PAGE for the fraction of the void volume concentrated tenfold for the insulin and wild-type  $\alpha$ B-crystallin assay is shown in the inset of elution profiles.



estimated at more than 10 MDa, were efficiently generated. It has been previously reported that residues 57–69 and 93–107 are involved in the binding with denatured ADH [22]. Residues 57–69 are located at the end of the N-terminal domain. The conformational change at the N-terminal domain of the P39R mutant  $\alpha$ B-crystallin would prevent residues 57–69 from interacting with denatured ADH, which would in turn suppress the formation of very large aggregates.

There is currently no information about the binding site for denatured insulin, but the mechanism of interaction clearly differs between the  $\alpha$ B-crystallin-ADH and  $\alpha$ B-crystallin-insulin complexes. In the insulin assay, most wild-type and P39R mutant  $\alpha$ B-crystallins formed complexes of approximately 2200–2800 kDa, whereas these complexes were not observed in the ADH assay. A major difference between the conditions used for the insulin and ADH assays was the molar ratio of the substrate proteins to  $\alpha$ B-crystallins. For the insulin assay, the molar ratios of the substrate protein to  $\alpha$ B-crystallins were about 60 times higher than those of the ADH assay. Thus, complexes of approximately 2200–2800 kDa would have been responsible for the chaperone-like activity, in addition to the generation of very large aggregates at the void volume.

In conclusion, the conformational changes in the N-terminal domain of  $\alpha$ B-crystallin induced by the mutation of Pro39 to Arg greatly affect the oligomerization and chaperone-like activity of  $\alpha$ B-crystallin. It has been reported that the N-terminal domain of  $\alpha$ B-crystallin is primarily important for both its oligomerization and chaperone-like activity [21], and the hydrophobic region (residues 41–58) of the N-terminal domain is involved in the recognition of denatured proteins [23]. Our study demonstrated that the Pro39 is also a regulatory residue for the oligomerization and chaperone-like activity of  $\alpha$ B-crystallin. The Pro-rich environment at the N-terminal domain of  $\alpha$ B-crystallin would allow for the diversity of conformations of the N-terminal domain and is expected to be responsible for both polydisperse oligomerization and interactions with various denatured proteins.

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## References

- [1] J. Horwitz, Alpha-crystallin, *Exp. Eye. Res.* 76 (2003) 145–153.
- [2] J. Horwitz, Alpha-crystallin can function as a molecular chaperone, *Proc. Natl. Acad. Sci. USA* 89 (1992) 10449–10453.
- [3] S.G. Bhagyalaxmi, P. Srinivas, K.A. Barton, K.R. Kumar, M. Vidyavathi, J.M. Petrash, G. Bhanuprakash Reddy, T. Padma, A novel mutation (F71L) in alphaA-crystallin with defective chaperone-like function associated with age-related cataract, *Biochim. Biophys. Acta* 1792 (2009) 974–981.
- [4] N. Fujii, Y. Ishibashi, K. Satoh, M. Fujino, K. Harada, Simultaneous racemization and isomerization at specific aspartic acid residues in alpha B-crystallin from the aged human lens, *Biochim. Biophys. Acta* 1204 (1994) 157–163.
- [5] N. Fujii, K. Satoh, K. Harada, Y. Ishibashi, Simultaneous stereoinversion and isomerization at specific aspartic acid residues in alpha A-crystallin from human lens, *J. Biochem.* 116 (1994) 663–669.
- [6] P. Vicart, A. Caron, P. Guicheney, Z. Li, M.C. Prevost, A. Faure, D. Chateau, F. Chapon, F. Tome, J.M. Dupret, D. Paulin, M. Fardeau, A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy, *Nat. Genet.* 20 (1998) 92–95.
- [7] K. Kato, Y. Inaguma, H. Ito, K. Iida, I. Iwamoto, K. Kamei, N. Ochi, H. Ohta, M. Kishikawa, Ser-59 is the major phosphorylation site in alphaB-crystallin accumulated in the brains of patients with Alexander's disease, *J. Neurochem.* 76 (2001) 730–736.
- [8] L.E. Goldstein, J.A. Muffat, R.A. Cherny, R.D. Moir, M.H. Ericsson, X. Huang, C. Mavros, J.A. Coccia, K.Y. Faget, K.A. Fitch, C.L. Masters, R.E. Tanzi, L.T. Chylack Jr., A.I. Bush, Cytosolic beta-amyloid deposition and supranuclear cataracts in lenses from people with Alzheimer's disease, *Lancet* 361 (2003) 1258–1265.
- [9] R.C. Augusteyn, Alpha-crystallin: a review of its structure and function, *Clin. Exp. Optom.* 87 (2004) 356–366.
- [10] C. Bagneris, O.A. Bateman, C.E. Naylor, N. Cronin, W.C. Boelens, N.H. Keep, C. Slingsby, Crystal structures of alpha-crystallin domain dimers of alphaB-crystallin and Hsp20, *J. Mol. Biol.* 392 (2009) 1242–1252.
- [11] A. Laganowsky, J.L. Benesch, M. Landau, L. Ding, M.R. Sawaya, D. Cascio, Q. Huang, C.V. Robinson, J. Horwitz, D. Eisenberg, Crystal structures of truncated alphaA and alphaB crystallins reveal structural mechanisms of polydispersity important for eye lens function, *Protein Sci.* 19 (2010) 1031–1043.
- [12] S. Jehle, P. Rajagopal, B. Bardiaux, S. Markovic, R. Kuhne, J.R. Stout, V.A. Higman, R.E. Klevit, B.J. van Rossum, H. Oschkinat, Solid-state NMR and SAXS studies provide a structural basis for the activation of alphaB-crystallin oligomers, *Nat. Struct. Mol. Biol.* 17 (2010) 1037–1042.
- [13] J. Horwitz, Alpha crystallin: the quest for a homogeneous quaternary structure, *Exp. Eye Res.* 88 (2009) 190–194.
- [14] A.J. Baldwin, H. Lioe, G.R. Hilton, L.A. Baker, J.L. Rubinstein, L.E. Kay, J.L. Benesch, The polydispersity of alphaB-crystallin is rationalized by an interconverting polyhedral architecture, *Structure* 19 (2011) 1855–1863.
- [15] N. Braun, M. Zacharias, J. Peschek, A. Kastenmuller, J. Zou, M. Hanzlik, M. Haslbeck, J. Rappsilber, J. Buchner, S. Weinkauff, Multiple molecular architectures of the eye lens chaperone alphaB-crystallin elucidated by a triple hybrid approach, *Proc. Natl. Acad. Sci. USA* 108 (2011) 20491–20496.
- [16] S. Jehle, B.S. Vollmar, B. Bardiaux, K.K. Dove, P. Rajagopal, T. Gonen, H. Oschkinat, R.E. Klevit, N-terminal domain of alphaB-crystallin provides a conformational switch for multimerization and structural heterogeneity, *Proc. Natl. Acad. Sci. USA* 108 (2011) 6409–6414.
- [17] R.L. van Montfort, E. Basha, K.L. Friedrich, C. Slingsby, E. Vierling, Crystal structure and assembly of a eukaryotic small heat shock protein, *Nat. Struct. Biol.* 8 (2001) 1025–1030.
- [18] K.K. Kim, R. Kim, S.H. Kim, Crystal structure of a small heat-shock protein, *Nature* 394 (1998) 595–599.
- [19] S. Rajan, C. Horn, E.C. Abraham, Effect of oxidation of alphaA- and alphaB-crystallins on their structure, oligomerization and chaperone function, *Mol. Cell. Biochem.* 288 (2006) 125–134.
- [20] N.P. Shroff, S. Bera, M. Cherian-Shaw, E.C. Abraham, Substituted hydrophobic and hydrophilic residues at methionine-68 influence the chaperone-like function of alphaB-crystallin, *Mol. Cell. Biochem.* 220 (2001) 127–133.
- [21] C.O. Asomugha, R. Gupta, O.P. Srivastava, Structural and functional roles of deamidation of N146 and/or truncation of NH<sub>2</sub>- or COOH-termini in human alphaB-crystallin, *Mol. Vis.* 17 (2011) 2407–2420.
- [22] K.K. Sharma, H. Kaur, K. Kester, Functional elements in molecular chaperone alpha-crystallin: identification of binding sites in alpha B-crystallin, *Biochem. Biophys. Res. Commun.* 239 (1997) 217–222.
- [23] J.G. Ghosh, A.K. Shenoy Jr., J.I. Clark, N- and C-Terminal motifs in human alphaB crystallin play an important role in the recognition, selection, and solubilization of substrates, *Biochemistry* 45 (2006) 13847–13854.