ATP and the Core “α-Crystallin” Domain of the Small Heat-shock Protein αB-crystallin*

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Paul J. Muchowski, Lara G. Hays, John R. Yates III, and John I. Clark

From the Department of Biological Structure, Box 357420, the Department of Molecular Biotechnology, Box 356485, and Department of Ophthalmology, Box 357730, University of Washington, Seattle, Washington 98195-7420

Electrospray ionization mass spectrometry (ESI-LC/MS) of tryptic digests of human αB-crystallin in the presence and absence of ATP identified four residues located within the core “α-crystallin” domain, Lys80, Lys102, Arg116, and Arg123, that were shielded from the action of trypsin in the presence of ATP. In control experiments, chymotrypsin was used in place of trypsin. The chymotryptic fragments of human αB-crystallin produced in the presence and absence of ATP were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Seven chymotryptic cleavage sites, Trp64, Phe68, Phe83, Phe94, Phe110, and Tyr122, located near or within the core α-crystallin domain, were shielded from the action of chymotrypsin in the presence of ATP. Chemically similar analogs of ATP were less protective than ATP against proteolysis by trypsin or chymotrypsin. ATP had no effect on the enzymatic activity of trypsin and the Km for trypsin was 0.051 mM in the presence of ATP and 0.029 mM in the absence of ATP. The results demonstrated a function for the conserved core α-crystallin domain (35–37) that was characterized by the presence of a conserved core “α-crystallin” domain that spans 80–100 amino acids in the C-terminal domain (2, 5, 6). sHSPs are expressed ubiquitously in nature (5, 6), are up-regulated in several neurodegenerative diseases that include multiple sclerosis (7), Alzheimer’s (8, 9), and Creutzfeldt-Jacob disease (10), and are mutated in inherited diseases including a desmin-related myopathy and cataract (11, 12). Initially thought to be lens-specific structural proteins, both αA- and αB-crystallin have been identified in a variety of non-lens tissues (13, 14). While α-crystallins and sHSPs function as molecular chaperones both in vitro and in vivo (15–35) a function for the conserved core α-crystallin domain has yet to be demonstrated. In this report trypsin proteolysis identified regions in the core domain that may be involved with the effect of ATP on chaperone activity of sHSPs.

In contrast to the large heat-shock protein families HSP60, HSP70, HSP90, and HSP100, the effect of ATP on the molecular chaperone functions of sHSPs has only been reported recently (31). Several publications have supported a structural and functional relationship between ATP and the sHSPs. Equilibrium binding studies, intrinsic tryptophan fluorescence, and 31P nuclear magnetic resonance spectroscopy demonstrated an interaction between ATP and total bovine α-crystallin (36, 37). Quenching of intrinsic fluorescence in the presence of ATP has been reported for both total α-crystallin and for αB-crystallin, suggesting ATP-induced conformational changes (31, 37). ATP induced a conformational change in total α-crystallin that was accompanied by a concomitant internalization of hydrophobic surfaces previously exposed, as measured by ANS binding (38). Both αA- and αB-crystallin have been reported to possess autophosphorylation activity that depends on magnesium and requires the binding and hydrolysis of ATP (39–41). Functionally, ATP enhanced the refolding of citrate synthase by αB-crystallin 2-fold, enhanced the suppression of citrate synthase thermal aggregation by αB-crystallin 2-fold (31), increased the refolding yield of xylose reductase by total α-crystallin from 1% to nearly 60% (38), and increased the binding of α-crystallin to lens membranes by 35% (36). Although numerous published reports support a functional interaction between ATP and sHSPs, the structural basis for the effects of ATP on the functions of sHSPs remains unknown.

In the current study, ATP protected human αB-crystallin against proteolysis by the serine proteases, trypsin, and chymotrypsin. ESI-LC/MS and LC-MS/MS determined that specific amino acid sequences containing the cleavage sites shielded from proteolysis in the presence of ATP were located near or within the C-terminal core α-crystallin domain, spanning residues Glu67–Ile161 (6). The results in this report indicate that the core α-crystallin domain may have a structural role associated with the effect of ATP on the chaperone function of human αB-crystallin, a sHSP.

EXPERIMENTAL PROCEDURES

Materials—Human αB-crystallin was purified as described previously and densitometric analysis of scanned gels containing purified
**Biosystems Mariner ESI-TOF equipped with a microelectrospray ionization source**

The gradient was linear from 2 to 15 minutes of gradient elution consisted of 0.1% acetic acid, 5% acetonitrile, 5% water, and 0.01% Tween 20. ATP, ADP, AMP, ATP-S, or AMP-PNP was added to the reaction mixture at a final concentration of 3.5 mM. At time 0, 5 μl of 0.17 mg/ml trypsin or chymotrypsin was added to each sample and all samples were maintained at 37 °C for the duration of the experiment. A 1.5-μl aliquot was withdrawn immediately after trypsin addition, quenched with 1.5 μl of 100 mM phenylmethylsulfonyl fluoride, and placed on ice. At selected time points, 13.5-μl aliquots were removed and treated identically to the zero time sample. 5 μl of 4 × SDS loading buffer (NoveX, San Diego, CA) was added to 15 μl of each sample and heated for 10 min at 70 °C. Samples were resolved on 4–12% Bis-Tris polyacrylamide gels, run in the presence of MES buffer (NoveX), and stained with Coomassie Blue R-350 (Amersham Pharmacia Biotech).

**Electrospray Ionization Mass Spectrometry (ESI-LC/MS) of αB-crystallin Tryptic Digests**—Samples containing αB-crystallin were proteolysed with trypsin for 30 min in the presence and absence of ATP. The gradient was run in the presence of Tricine buffer (Novex) and stained with Coomassie Blue R-350 (Amersham Pharmacia Biotech).

**Samples containing αB-crystallin were proteolyzed with trypsin for 30 min in the presence and absence of ATP using conditions identical to those described above.** One pmole of digest was loaded onto a microelectrospray column and subjected to analysis by ESI-LC/MS. The microelectrospray columns were constructed from a 360-μm outer diameter × 100-μm inner diameter fused silica capillary with the column tip tapered to a 5–10-μm opening. The columns were packed with PerSeptive Biosystems POROS 10 R2 (Boston, MA) to a length of 10–12 cm. The flow from the high performance liquid chromatography pumps (typically 150 μl/min) was split pre-column to achieve a flow rate of 500–1000 nl/min. The mobile phase used for gradient elution consisted of (A) 0.5% acetic acid, (B) acetonitrile/water 80:20 (v/v) containing 0.5% acetic acid. The gradient was linear from 2 to 80% B in 30 min. Mass spectra were recorded on a PerSeptive Biosystems Mariner ESI-TOF equipped with a microelectrospray ionization source (43). Electrospray was performed at a voltage of 1.9 kV. Mass spectra were acquired by scanning a m/z range of 500–2000 every 2 s for the duration of the high performance liquid chromatography gradient. Deconvolution of the mass spectra was performed to determine the peptide molecular weights (44).

**Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) of αB-crystallin Chymotryptic Fragments**—αB-crystallin was proteolysed by chymotrypsin for 15 min at 37 °C as described above for proteolysis reactions using trypsin. When a sample was collected at each time point for the SDS-PAGE analysis, a second sample was collected for analysis using LC-MS/MS. To separate the largest chymotryptic fragments of αB-crystallin, a 16% Tricine polyacrylamide gel was run in the presence of Tricine buffer (NoveX) and stained with Coomassie Blue R-350 (Amersham Pharmacia Biotech). After separation, the fragments were cut from the gel and subjected to in-gel digestion (45). Soluble fragments were recovered from the in-gel digestion and identified using microcolumn high performance liquid chromatography (46) and automated tandem mass spectrometry. The microelectrospray columns and high performance liquid chromatography set-up were as described for ESI-LC/MS. Mass spectra were recorded on an LCQ ion trap mass spectrometer (Finnigan MAT LCQ, San Jose, CA) equipped with a microelectrospray ionization source (43). Electrospray was performed at a voltage of 1.6 kV. Tandem mass spectra were acquired during the entire gradient run automatically as described previously (47). Protein sequence data bases were searched directly with the tandem mass spectra using the computer program SEQUEST (48). The version 29.5 OWL data base (210232 entries) was obtained as previously (47). Protein sequence data bases were searched directly with the tandem mass spectra using the computer program SEQUEST (48). The version 29.5 OWL data base (210232 entries) was obtained as previously (47). Protein sequence data bases were searched directly with the tandem mass spectra using the computer program SEQUEST (48). The version 29.5 OWL data base (210232 entries) was obtained as previously (47). Protein sequence data bases were searched directly with the tandem mass spectra using the computer program SEQUEST (48). The version 29.5 OWL data base (210232 entries) was obtained as previously (47). Protein sequence data bases were searched directly with the tandem mass spectra using the computer program SEQUEST (48). The version 29.5 OWL data base (210232 entries) was obtained as previously (47). Protein sequence data bases were searched directly with the tandem mass spectra using the computer program SEQUEST (48). The version 29.5 OWL data base (210232 entries) was obtained as previously (47). Protein sequence data bases were searched directly with the tandem mass spectra using the computer program SEQUEST (48). The version 29.5 OWL data base (210232 entries) was obtained as previously (47). Protein sequence data bases were searched directly with the tandem mass spectra using the computer program SEQUEST (48). The version 29.5 OWL data base (210232 entries) was obtained as previously (47). Protein sequence data bases were searched directly with the tandem mass spectra using the computer program SEQUEST (48). The version 29.5 OWL data base (210232 entries) was obtained as previously (47). Protein sequence data bases were searched directly with the tandem mass spectra using the computer program SEQUEST (48).

**Effect of ATP on Enzymatic Activity of Trypsin**—The control for the effect of ATP on the enzymatic activity of trypsin was done using the BANA assay (Fig. 5) (49). The assay is based on the release of a chromogenic naphthylamidine product, having maximum absorbance at approximately λ = 310 nm, resulting from trypsin cleavage of the synthetic arginine substrate, BANA. Because of a large background absorbance below λ = 310 nm, the progress of the reaction was monitored at λ = 350 nm, the shoulder of the maximum absorbance. The reaction is very sensitive to pH and temperature, which were carefully controlled at 7.4 and 37 °C, respectively. Trypsin was dissolved in the buffer solution used for proteolysis of human αB-crystallin and selected concentrations of the BANA substrate were added. Progress of the reaction was monitored at λ = 350 nm using an Ultrospec 3000, UV/Visible Spectrophotometer (Amersham Pharmacia Biotech). ATP was added to a final concentration of 3.5 mM to evaluate the effect of ATP on the enzymatic activity of trypsin. Kₐ values were calculated using the Lineaweaver-Burk analysis of product formation.

**RESULTS**

**ATP Protects αB-crystallin against Proteolysis by Trypsin**—SDS-PAGE was used to separate the tryptic cleavage products of αB-crystallin generated in the absence and presence of several adenine nucleotides at 37 °C over a 30-min time course (Fig. 1). In the absence of ATP, intact αB-crystallin was rapidly digested by trypsin, a serine protease that cleaves after the basic amino acids Lys and Arg (Fig. 1A) (50). After 30 min of trypsin proteolysis nearly all intact αB-crystallin (molecular mass ~20 kDa) was digested and appeared in the Coomassie Blue-stained gels as relatively small proteolytic fragments (Fig. 1A). In contrast, ATP significantly protected αB-crystallin against proteolysis by trypsin throughout the entire 30-min time course (Fig. 1B). The prominent bands observed in gels after 30 min of trypsin digestion in the presence of ATP were identified as intact αB-crystallin (molecular mass ~20 kDa), a ~18-kDa fragment, a ~12-kDa fragment, and a ~5-kDa fragment. When chemically similar ATP analogs were used in the trypsin digestion, large proteins were retained in the presence of ADP (C) and were barely visible in gels containing AMP (D) and the non-hydrolyzable ATP analogs ATP-S (E) and AMP-PNP (F). The results are consistent with the concept that the core α-crystallin domain contained cleavage sites for trypsin that were inaccessible to proteolysis in the presence of ATP.
ATP Protects aB-crystallin against Proteolysis

**TABLE I**

| Peptide masses observed by ESI-MS | Predicted masses | Peptide sequences |
|----------------------------------|-----------------|-------------------|
| ATP                              | 18,224 ± 4      | 18,227 1–157      |
| AMP                              | 16,855 ± 5.9    | 16,857 12–157     |
| ADP                              | 15,498 ± 6.2    | 15,506 23–157     |
| AMP                              | 11,510 ± 3.9    | 11,512 57–157     |
| AMP                              | 9,583 ± 3       | 9,581 12–92       |
| AMP                              | 8,225 ± 1       | 8,225 23–92       |
| AMP                              | 8,213 ± 2       | 8,212 1–69        |
| AMP                              | 8,029 ± 4       | 8,024 57–123      |
| AMP                              | 7,004 ± 1       | 7,006 57–116      |
| AMP                              | 6,731 ± 2       | 6,733 1–56        |
| AMP                              | 4,004 ± 0       | 4,006 23–56       |
| AMP                              | 3,981 ± 1       | 3,983 83–116      |
| AMP                              | 3,907 ± 0       | 3,906 70–103      |

37°C over a 30-min time course (Fig. 3). In the absence of added nucleotide, intact aB-crystallin was degraded by chymotrypsin, similar to the results observed with trypsin (Fig. 3A). After 30 min of chymotrypsin proteolysis nearly all the aB-crystallin (~20 kDa) was digested and appeared as relatively small peptide fragments on Coomassie Blue-stained SDS-polyacrylamide gels (Fig. 3A). In contrast, ATP protected aB-crystallin against proteolysis by chymotrypsin (Fig. 3B). The prominent bands observed in gels after 30 min of chymotrypsin digestion were identified as intact aB-crystallin (~20 kDa), a ~14-kDa fragment, a ~12-kDa fragment, and smaller fragments of ~3–6 kDa (Fig. 3B). In control experiments using chemically similar analogs of ATP, ADP was observed to have a modest protective effect. AMP and the non-hydrolyzable ATP analogs ATPyS and AMP-PNP provided minimal protection against proteolysis of aB-crystallin over the 30-min time course of the experiment (Fig. 1, D, E, and F). Large proteolytic fragments were absent from the SDS-polyacrylamide gels and the pattern of proteolysis resembled that of nucleotide-free aB-crystallin (Fig. 1, D, F).

**Peptide masses observed by ESI-MS**

- ATP: 18,224 ± 4
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**Predicted masses**

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- AMP: 3,983 83–116
- AMP: 3,906 70–103

**Peptide sequences**

- ATP
- AMP
- ADP

**Mapping of tryptic cleavage sites on aB-crystallin in the presence and absence of ATP elucidated from ESI-LCMS.** The linear amino acid sequence for human aB-crystallin can be divided into two domains (75). Residues in bold indicate amino acids that are conserved among ~80 different sHSPs and have been used to define the core a-crystallin domain, spanning residues Glu67–Ile161 (2). Arrows below the linear amino acid sequence indicate all potential tryptic cleavage sites. Filled triangles above the linear amino acid sequence indicate tryptic cleavage sites of aB-crystallin observed in the presence of ATP. Open triangles above the linear amino acid sequence indicate four additional tryptic cleavage sites in aB-crystallin observed only in the absence of ATP. The difference in the gel patterns of proteolytic fragments of aB-crystallin with and without ATP was due to protection at residues Lys82, Lys103, Arg116, and Arg123 on aB-crystallin. These residues are contained in the core a-crystallin domain, spanning residues Glu67–Ile161, which is conserved in nearly all identified sHSPs (2, 5, 6).

Protein bands could also be detected: intact aB-crystallin (mass ~20 kDa), a ~18-kDa fragment, a ~8–kDa fragment, and small protein fragments of ~3–6 kDa (Fig. 1C). In contrast, AMP and the non-hydrolyzable ATP analogs ATPyS and AMP-PNP provided minimal protection against proteolysis of aB-crystallin over the 30-min time course of the experiment (Fig. 1, D, E, and F). Large proteolytic fragments were absent from the SDS-polyacrylamide gels and the pattern of proteolysis resembled that of nucleotide-free aB-crystallin (Fig. 1, D, F). Lys82, Lys103, Arg116, and Arg123 were shielded from proteolysis by trypsin in the presence of ATP—ESI-LCMS was used to identify tryptic peptides of aB-crystallin generated in the absence and presence of ATP. The primary sequence of human aB-crystallin includes all Arg and Lys cleavage sites for trypsin (Fig. 2). In the absence of ATP, 12 aB-crystallin peptides were identified by ESI-LCMS that matched precisely the masses of tryptic fragments predicted from the cleavage at Arg11, Arg22, Arg56, Arg69, Lys82, Lys92, Lys103, Arg116, Arg123, and Arg157 in the linear amino acid sequence of human aB-crystallin (Table I and Fig. 2). In the presence of ATP, nine proteolytic fragments of aB-crystallin were identified corresponding to protection of cleavage sites at Lys1, Lys103, Arg116, and Arg123 (Table I and Fig. 2). The pattern of tryptic digestion products observed on SDS-PAGE gels suggested that proteolysis occurred at the most accessible cleavage sites in the absence of ATP. The cleavage sites protected in the presence of ATP were in the region of the core a-crystallin domain between Glu67 and Ile161 (Fig. 2).

**ATP Protected aB-crystallin against Proteolysis by Chymotrypsin—** To control for the possibility that the protective effect of ATP against trypsin proteolysis was limited to the action of trypsin on Arg and Lys peptides, proteolysis was repeated using chymotrypsin, a serine protease thatcleaves after the bulky aromatic residues Phe, Trp, and Tyr (Fig. 3) (50). SDS-PAGE was used to resolve the pattern of chymotryptic cleavage products of aB-crystallin generated in the absence and presence of ATP and chemically similar adenine nucleotides at 37°C over a 30-min time course (Fig. 3). In the absence of added nucleotide, intact aB-crystallin was degraded by chymotrypsin, similar to the results observed with trypsin (Fig. 3A). After 30 min of chymotrypsin proteolysis nearly all the aB-crystallin (~20 kDa) was digested and appeared as relatively small peptide fragments on Coomassie Blue-stained SDS-polyacrylamide gels (Fig. 3A). In contrast, ATP protected aB-crystallin against proteolysis by chymotrypsin (Fig. 3B). The prominent bands observed in gels after 30 min of chymotrypsin digestion were identified as intact aB-crystallin (~20 kDa), a ~14-kDa fragment, a ~12-kDa fragment, and smaller fragments of ~3–6 kDa (Fig. 3B). In control experiments using chemically similar analogs of ATP, ADP was observed to have a modest protective effect. AMP and the non-hydrolyzable ATP analogs ATPyS and AMP-PNP provided minimal protection against proteolysis of aB-crystallin over the 30-min time course of the experiment (Fig. 3, D, E, and F). In this regard, the protective effects of ATP and AMP analogs were similar for both trypsin and chymotrypsin proteolysis.
FIG. 3. SDS-PAGE analysis of chymotryptic digestion of αB-crystallin in the presence and absence of nucleotides at 37 °C. The 30-min time courses for the proteolysis of αB-crystallin are shown in the absence of added nucleotides (A), and in the presence of 3.5 mM ATP (B), ADP (C), AMP (D), ATPγS (E), and AMP-PNP (F). Molecular size standards (in kDa) are listed adjacent to scanned gels. After 30 min of chymotryptic digestion in the presence of added nucleotide, intact αB-crystallin (~20 kDa) was not observed in the Coomassie Blue-stained gels and the majority of the protein remained as small protein fragments. After 30 min of chymotryptic digestion in the presence of ATP five prominent protein fragments remained: intact αB-crystallin (~20 kDa), a ~14-kDa fragment, a ~12-kDa fragment, and two fragments of ~3–4 kDa. When chemically similar ATP analogs were used in the chymotryptic digestion, large proteins were retained in the presence of ADP (C) and were barely visible in gels containing AMP (D) and the non-hydrolyzable ATP analogs ATPγS (E) and AMP-PNP (F). The results are consistent with the concept that the core α-crystallin domain contained cleavage sites for chymotrypsin that were inaccessible to proteolysis in the presence of ATP.

DISCUSSION

In the presence of ATP, trypsin cleavage sites inside the core α-crystallin domain of the sHSP, human αB-crystallin, were shielded from proteolysis. Although conserved among nearly all identified sHSPs (2, 5, 6, 53) the function of the core α-crystallin domain has not been identified. Human αB-crystallin contains 14 Arg residues and 10 Lys residues that are cleavage sites for trypsin and are distributed throughout its primary sequence of 175 amino acids. SDS-PAGE analysis of tryptic fragments of αB-crystallin demonstrated that many Lys and Arg residues on αB-crystallin were exposed to trypsin in the absence of added nucleotides and the majority of the native ~20-kDa protein was degraded to small proteolytic fragments. ESI-LC/MS analysis of the tryptic digestion products of αB-crystallin identified four residues, Lys52, Lys103, Arg116, and Arg123, that were shielded from the proteolysis in the presence of ATP. Chemically similar ATP analogs were less effective than ATP in protecting against proteolytic cleavage at the sites located inside the core α-crystallin domain of αB-crystallin. This finding suggested that the protected trypsin cleavage sites in the core α-crystallin domain were less accessible in the presence of ATP than in the presence of ATP analogs. A structural modification involving the core peptides, Glu67 to Ile161, may be associated with the effect of ATP on the molecular chaperone activity of the sHSP protein, human αB-crystallin.

Structural domains involved with ATP functions have been characterized in several molecular chaperones using trypsin proteolysis (42, 54–63), a useful method that depends on the chemical specificity of trypsin for Arg and Lys peptides (64–66) and the absence of an effect of ATP on enzymatic activity (51). Trypsin is a serine protease having a well known chemical mechanism. Formation of an acyl-enzyme intermediate is followed by hydrolysis to release a peptide fragment (67, 68). Under the conditions used in this study, it is extremely unlikely that ATP participates in this chemical reaction as an inhibitor. The possibility that the protective effect of ATP was
Fig. 5. The effect of ATP on activity of trypsin in the BANA assay. The formation of trypsin cleavage product was recorded as the optical density, OD, at λ = 350 nm over the 30-min time course of the reaction. In the upper pair of plots, the increase in the formation of product ± ATP was nearly identical in a sample reaction using the same concentration of trypsin used in the proteolysis experiments. In the lower pair of plots, the concentration of trypsin was 50% of that used in the proteolysis experiments. At the lower trypsin concentration the formation of product was nearly identical ± ATP. ATP had no effect on the enzymatic activity of trypsin.

limited to Arg and Lys cleavage sites was evaluated in control experiments using chymotrypsin, a serine protease that cleaves at aromatic peptides, Phe, Trp, and Tyr (50, 68, 69). Using LC-MS/MS analysis of chymotrypsin fragments of αB-crystallin, seven protected chymotryptic cleavage sites, Trp60, Phe61, Phe75, Phe84, Phe113, Phe118, and Tyr122, were identified that were near or within the core α-crystallin domain of αB-crystallin. Although the cleavage sites are different for chymotrypsin and trypsin, analysis of the proteolytic fragments determined that nearly all the protected peptides were located in the same conserved core α-crystallin domain of human αB-crystallin.

In control experiments, chemically similar ATP analogs were substituted for ATP in the proteolysis reactions. ADP had a modest protective effect against trypsin and chymotrypsin proteolysis and the protective effects of AMP, ATPγS, and AMP-PNP were not as pronounced. While it may be important that the protective effect of ATP analogs was strongest with the nucleotide triphosphate, less with the diphosphate and least with the monophosphate the possibility of conversion of ADP to ATP during the assay cannot be excluded. Autophosphorylation of αB-crystallin has been reported and the protective action of phosphonucleotides may be related to phosphorylation at the serine 19, 45, and 59 sites near the core of trypsin was investigated in a kinetic study using the BANA assay in the presence and absence of ATP. BANA is a synthetic arginine substrate that releases a chromogenic product following cleavage by trypsin. No effect of ATP on trypsin activity was observed under conditions that were identical to the conditions used for proteolysis of human αB-crystallin. In a separate study the enzymatic activity of trypsin on the digestion of bovine serum albumin, was unaffected in the presence of ATP (51). ATP does not fit the structural constraints required for inhibitors of serine proteases (66, 68, 69) and it would have been a startling discovery to find that the action of ATP was on the enzymatic activity of trypsin. Rather than an inhibitory effect on the enzymatic activity of trypsin, the protective effect of ATP involved a structural modification of αB-crystallin that resulted in inaccessible cleavage sites in the core α-crystallin domain.

Having confirmed that ATP had no effect on the enzymatic activity of trypsin, the structural basis for the protective effect of ATP on proteolysis of human αB-crystallin needs to be considered. The results in this report suggested that the effect of ATP on proteolysis of human αB-crystallin resembled the effect of ATP on other molecular chaperones and may be associated with structural modifications having functional significance (54–63). Alternatively, the protective effect of ATP could be due to direct binding and steric blockage at the site of proteolytic cleavage in the core domain. Two clusters of positively charged amino acids are present in the core α-crystallin domain and they could interact with the triphosphate moiety on ATP (Lys82, His83, Lys90, and Lys92) and (Arg116, His119, Arg120, Lys121, Arg123). Four of these residues, Lys82, Lys90, Arg116, and Arg123, were accessible to trypsin in the absence of added nucleotide, suggesting that they are exposed on the surface of αB-crystallin. Both charged clusters are flanked by numerous hydrophobic residues that are potential sites of interaction with the adenine moiety on ATP. Separate kinetic studies will be necessary to determine whether or not binding of ATP is required for the protection of core residues against proteolysis. Under the conditions used in this study, it is reasonable to suggest that the protective effect of ATP against proteolytic degradation of human αB-crystallin involved structural modification of α-crystallin domain that decreased the accessibility of the cleavage sites between Gly57 and Ile61. Trypsin proteolysis has been used previously in studies of nucleotide-induced conformational changes in GroEL, DnaK, Hsp70, Hsp90, and BiP, where structural modifications have been demonstrated (42, 54–63). To our knowledge, this is the first report using trypsin proteolysis in the analysis of human αB-crystallin, a chaperone, in the presence and absence of ATP. The protection of trypsin cleavage sites in the core α-crystallin domain in the presence of ATP may indicate that the core domain is involved with the effect of ATP on the function of human αB-crystallin and other sHSPs as molecular chaperones.

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