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The necessity of functional proteomics: protein species and molecular function elucidation exemplified by in vivo alpha A crystallin N-terminal truncation

W. Hoehenwarter[1], **R. Ackermann**[1], **U. Zimny-Arndt**[1], **N. M. Kumar**[2], and **P. R. Jungblut**[1]

[1] Max Planck Institute for Infection Biology, Core Facility Protein Analysis, Berlin, Germany
[2] Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, Illinois, U.S.A.

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Summary. Ten years after the establishment of the term proteome, the science surrounding it has yet to fulfill its potential. While a host of technologies have generated lists of protein names, there are only a few reported studies that have examined the individual proteins at the covalent chemical level defined as protein species in 1997 and their function. In the current study, we demonstrate that this is possible with two-dimensional gel electrophoresis (2-DE) and mass spectrometry by presenting clear evidence of in vivo N-terminal alpha A crystallin truncation and relating this newly detected protein species to alpha crystallin activity regulation by protease cleavage in the healthy young murine lens. We assess the present state of technology and suggest a shift in resources and paradigm for the routine attainment of the protein species level in proteomics.

Keywords: Alpha crystallin – Lp82 – Protein species – Proteomics – Tertiary structure

Introduction

The advent of nano LC techniques for peptide separation represents a milestone and seemingly a turning point in proteomics biosciences. The online coupling of nano LC systems to ESI mass spectrometers and the development of automated spotting techniques onto MALDI templates allow high throughput analysis of whole proteomes, often without prefractionation. A number of free or commercially available mass spectrometry software suites expedite automated data evaluation, reducing proteome characterization at the protein level to a matter of days. Indeed, the speed at which an automated nano LC workflow produces results can lead one to overlook the significant preeminence 2-DE offers: the separation and visualization of the protein species, allowing the user to grasp and selectively analyze the actual biological effector molecules much better than in a black box nano LC workflow.

2-DE delivers the power to resolve samples into up to 10000 sample constituents discriminated by a single amino acid or posttranslational modification in individual spots (Klose and Kobalz, 1995). Mass spectrometric analyses of spot digests and rigorous data evaluation can yield the full sequence and posttranslational modifications of the inherent polypeptide (Okkels et al., 2004). Mass spectrometry software is invaluable for data processing, but it is by no means a substitute for skilled manual spectral analysis, leaving the routine attainment of the molecular level momentarily out of reach with 2-DE and MS.

While rapid detection of modifications is possible with conventional high throughput LC-MS techniques, the assignment of the modifications to individual molecules and thus the discrimination of the protein species is difficult. However, this is exactly what is necessary for appreciation of their effects on function and for proteomics to realize its true potential.

Materials and methods

We analyzed the murine 129/SvJ ten day old lenticular proteome as part of a larger research project using high resolution 2-DE as described by Klose and Kobalz (1995), combining carrier ampholyte IEF and SDS-PAGE on 23×30 cm gels for protein species separation. Individual spot analysis by LC/ESI-tandem mass spectrometry (MS/MS) as well as full lenticular proteome analysis was performed on an Agilent Technologies (Palo Alto, CA) XCT ion trap instrument. Individual spot analysis was also performed on an Applied Biosystems 4700 Proteomics Explorer MALDI-TOF/TOF instrument (Foster City, CA, USA).

Results and discussion

The lens is an ideal organ for proteomics research as it is readily accessible, easy to prepare and has exceptionally high protein content dominated by the crystallin protein super family (Mörner, 1894). Furthermore, due to a loss of organelles, most of the lens is biosynthetically quiescent. In addition, the lens proteins have a low rate of turnover but undergo extensive post-translational modifications and proteolytic processing. Hence, other approaches defining the protein content, such as DNA microarrays, are not as meaningful as in other tissues. Alpha crystallin is the major lenticular protein constituent, comprising 30–50% of total lens structural protein mass (de Jong et al., 1981). In vivo alpha crystallin is found as an 800 kDa high molecular weight aggregate made up of alpha A and B crystallin subunits (Bon, 1961; Schoenemakers et al., 1969) the primary gene products of the alpha A and alpha B crystallin genes. The protein exhibits chaperone activity (Horwitz, 1992) and due to its high concentration contributes to the refractive index gradient and overall transparency of the lens (Delaye and Tardieu, 1983). A model for the secondary structure for alpha A crystallin monomer has been proposed based on its sequence and gene structure (Siezen, 1981; Wistow, 1985). The molecule has a 63 amino acids long globular hydrophobic N-terminal domain, a somewhat longer C-terminal domain subdivided into two motifs in beta conformation and a C-terminal extension.

Unmodified and previously described (de Jong et al., 1974; Nakamura et al., 2000; Ueda et al., 2001, 2002) C-terminally truncated alpha A crystallin was identified in SSP 4121, 4103 and 4114 by automated and manual mass spectrometric raw data analysis with the MASCOT mass spectrometry software suite (Fig. 1a, the full gel image is available in our 2-DE database under the URL http://www.mpiib-berlin.mpg.de/2D-PAGE/ under the heading 2D-PAGE, *Mus musculus*, C57BL/6J, ten day old lens). The software also identified alpha A crystallin as the protein constituent of SSP 6003, correlating MS/MS spectra of fragmented ions abundant in the total count of all ions subjected to MS/MS fragmentation (TICallMSn) with three tryptic peptides on the alpha A crystallin amino acid sequence and one on porcine trypsin (Fig. 1b). Two of the three assignments were falsely scored below the software's identity threshold, a tendency we also observed

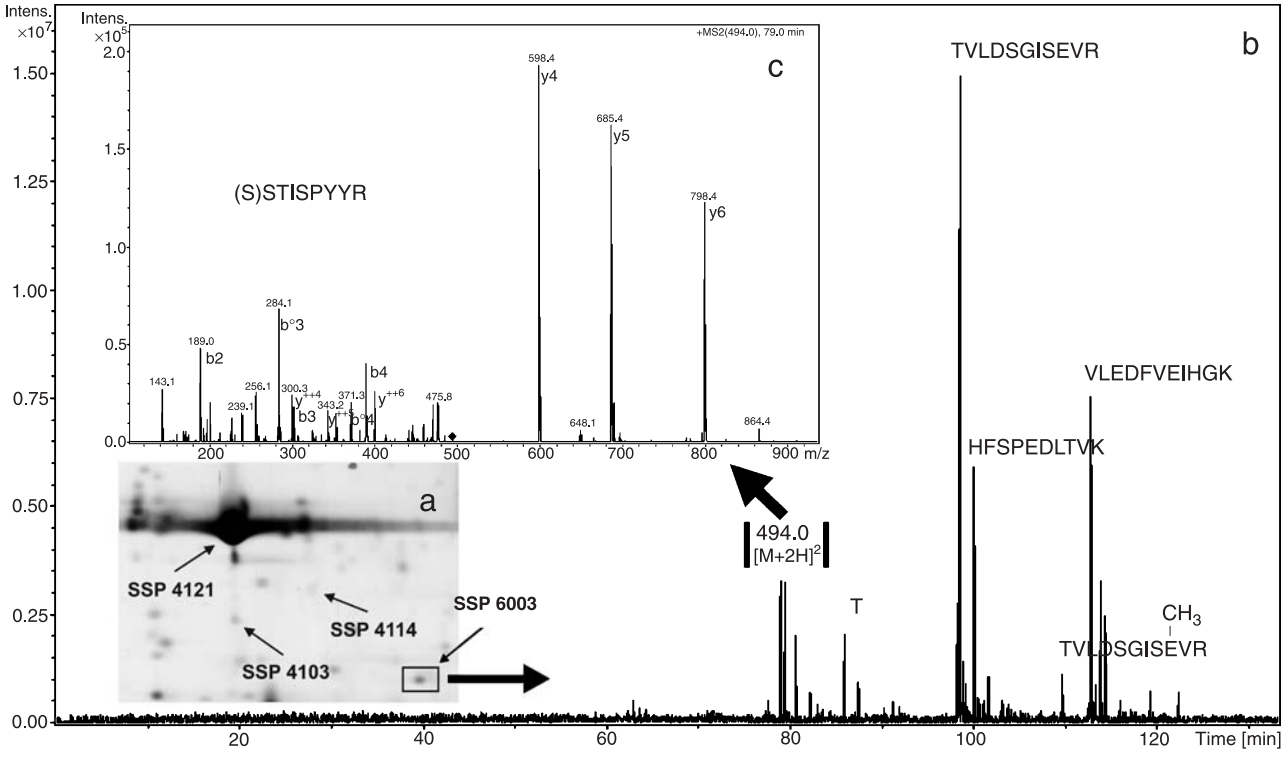

Fig. 1. Elucidation of the N-terminally truncated alpha A crystallin protein species residues 42–173 by 2-DE and mass spectrometry. **a** Cut-away of a 2-DE gel separating the ten day old 129/SvJ lenticular proteome. Arrows denote investigated spots, SSP 6003, containing the alpha A crystallin protein species residues 42–173, is boxed. **b** TICallMSn chromatogram from the LC/ESI-MS/MS analysis of spot SSP 6003. The abundant ions were identified with the exception of $[M+2H]^{2+}$ 494.0. T, trypsin autocatalysis product. **c** MS/MS spectrum of $[M+2H]^{2+}$ 494.0

in an LC/ESI-MS/MS analysis of the entire murine lenticular proteome. This is likely due to setting the threshold high to maximize identification confidence which, while justified, at the same time leads to a number of valid spectra being scored as negatives. Manual spectral interpretation is therefore always advisable for comprehensive results.

One abundant ion on the TICallMSn remained unassigned. Manual interpretation of the corresponding MS/MS spectrum (Fig. 1c) revealed a conspicuous serine, leucine/isoleucine sequence tag with high intensity ion signals at around m/z 600 and beyond. Evidence for strong MS/MS fragmentation N-terminal and weak MS/MS fragmentation C-terminal to a proline residue (Breci et al., 2003) led us to consider a possible proline C-terminal to serine and to incorporate a hypothetical quenched peak at m/z 501.4 into calculations. These considerations resulted in the following MASCOT sequence query: 494 tag(501.4, PS[IL], 798.4). It was searched against NCBInr (MS/MS tolerance 0.2 Da) with enzyme set to no enzyme and taxonomy set to *Mus musculus* and unambiguously assigned the mass to the peptide sequence STISPYYR concurrent with residues 42–49 on the alpha A crystallin amino acid sequence and identified the high intensity ion signals as the peptides Y_4, Y_5 and Y_6 ions. Additionally most of the remaining prominent MS/MS signals were assigned to other major ion series. This was corroborated by a MALDI-TOF/TOF-MS/MS analysis which in addition to detecting the Y_4, Y_5 and Y_6 ions detected the Y_1 C-terminal arginine as well as the peptides Y_2 ion (data not shown).

While evidence points to truncation in the N-terminal domain (Kamei et al., 1997; Kapphahn et al., 2003; Harrington et al., 2004), this peptide clearly defines an N-terminal truncation site on alpha A crystallin producing a 132 amino acids long polypeptide comprising residues 42–173 of the alpha A crystallin chain with a theoretical molecular weight of 14.8 kDa and a pI of 6.1. The respective spots position on calibrated 2-DE gels (Aksu et al., 2002) is within 91.9% of these values on twelve 2-DE gels of twelve independent sample preparations of ten day old murine lenticular proteomes. An alpha A crystallin in vivo N-terminal truncation site is unambiguously characterized here by proteomics evidence which was only possible by manual examination of the MS/MS spectra.

Alpha A crystallin residues 41 and 42 are likely a truncation site for the lens specific calpain protease Lp82. A number of studies indicate the Lp82 cleavage site is not defined by an exclusive amino acid sequence. However cleavage at serine residues is most prominent and clea-

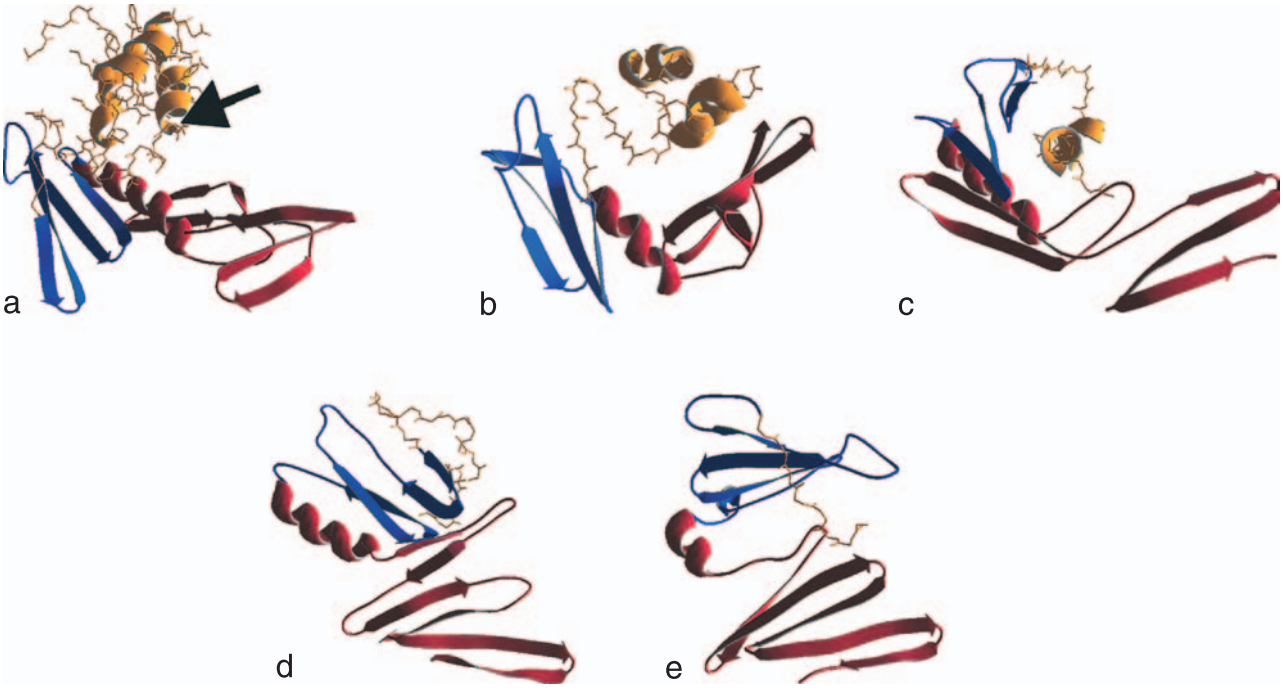

Fig. 2. *Ab initio* alpha A crystallin tertiary structure models. 3D models of **a** alpha A crystallin; **b** alpha A crystallin truncation product lacking 19 N-terminal residues; **c** alpha A crystallin truncation product lacking 35 N-terminal residues. **d** *In vivo* truncated alpha A crystallin protein species described here lacking 41 N-terminal residues. **e** Alpha A crystallin truncation product lacking 55 N-terminal residues. N-terminal regions are colored orange with functional domains displayed as ribbons, C-terminal regions are colored red and are entirely displayed as ribbons

vage after hydroxylated or carboxylated amino acid residues is frequent (Takemoto 1995; Nakamura et al., 2000; Ueda et al., 2001, 2002; Baruch et al., 2001; Fukiage et al., 2002; Azuma et al., 2003). Other evidence has determined Lp82 to be the predominantly active calpain protease in the young lens (Ma et al., 1999; Ueda et al., 2001) especially around day ten. The N-terminal alpha A crystallin residues 41 and 42 focused on here are both serines making in vivo Lp82 cleavage at this position probable. The 2-DE spot containing the N-terminal truncation product is faint and not in proximity of the other alpha A crystallin containing spots, perhaps making it difficult to detect by 2-DE analysis of *in vitro* treated soluble lens proteins (Ueda et al., 2002; Azuma et al., 2003).

Presently the tertiary structure of the alpha crystallin structure is unknown. To examine the relationship of this truncation product to the functional alpha crystallin oligomer, we constructed *ab initio* alpha A crystallin 3D tertiary structure models for a systematic comparison of molecular morphology (Fig. 2). The Rosetta algorithm on the HMMSTR server (http://www.bioinfo.rpi.edu/~bystrc/hmmstr/server.php) (Bystroff and Shao, 2002) available on the ExPASY (http://au.expasy.org/) homepage was used for molecular modeling. Full length alpha A crystallin secondary structure was calculated at 29.5% alpha helix and 32% beta sheet content from the 3D model (Fig. 2a). The N-terminal globular domain is organized into three helices with hydrophobic side chains buried. Structure function regions identified earlier (Smith et al., 1996; Pasta et al., 2003) make up the first two of these N-terminal alpha helices. The putative N-terminal Lp82 cleavage site at residues 41 and 42 (indicated by an arrow) is located precisely behind the third N-terminal alpha helix, suggesting deliberate removal of functional elements at this point. The highly conserved residues 102–117 of the alpha crystallin domain (Caspers et al., 1995), containing the substantial first part of a DNA binding motif (Singh et al., 1998) as well as an arginine residue 116 shown to be critical for molecular integrity (Bera et al., 2002) also are predicted to have an alpha helical conformation. This is consistent with an older 3D model (Farnsworth et al., 1998) and makes the alpha helical prediction, which is somewhat higher than previous calculations (Farnsworth et al., 1997; Horwitz et al., 1998; Bova et al., 2000), seem plausible. However, it is inconsistent with site directed spin label studies that demonstrate beta sheet conformation for residues 109–120 (Berengian et al., 1997). Our model confirms the beta sheet secondary structure of residues 67–101 determined to be an alcohol dehydrogenase (ADH) and 1,1′-bi (4-anilino) naphtalene-5,5′-disulfonic acid (bis-ANS) binding site and to exhibit extensive chaperone activity (Farnsworth and Singh, 2004).

No clear consensus as to alpha crystallin quaternary structure has been reached although several models (Bindels et al., 1979; Thomson and Augusteyn, 1983, 1984; Tardieu et al., 1986; Augusteyn and Koretz, 1987; Walsh et al., 1991; Wistow, 1993; Carver et al., 1994; Groth-Vasselli et al., 1995; Smulders et al., 1998) agree on the amphipathic character of the monomeric subunits (van der Ouderaa et al., 1973; Puri et al., 1983) which dynamically exchange (van den Oetelaar et al., 1990; Gesierich and Pfeil, 1996) and form small multimers as the building blocks of functional higher molecular order (Bova et al., 2000). Earlier studies indicate alpha A crystallin lacking 19 and 35 N-terminal residues retains its function as a monomeric subunit *in vitro* (Augusteyn, 1998; Bova et al., 2000). Conversely, alpha A crystallin lacking 55 N-terminal residues has lost this ability and is likely to be non functional (Bova et al., 2000). It has been shown that large regions of the alpha A crystallin N- and C-terminal domain are essential for subunit multi- and oligomerization (Merck et al., 1992; Augusteyn, 1998; Bova et al., 2000, Pasta et al., 2003; Thampi and Abraham, 2003) quaternary structure dynamics (Augusteyn and Koretz, 1987; Bova et al., 2000; Pasta et al., 2003) and chaperone activity (Takemoto et al., 1993; Derham and Harding, 1999; Pasta et al., 2003).

Models of the native protein species lacking 41 N-terminal residues and of the truncation products described above lacking 19, 35 and 55 N-terminal residues were constructed in addition to the tertiary structure model of alpha A crystallin (Fig. 2b–e). The degeneration of alpha A crystallin structural integrity accompanying successive N-terminal truncation is evident. Alpha A crystallin lacking 19 and 35 N-terminal residues retains closed C-terminal beta sheet organization and a generally intact tertiary structure quite similar to the full length monomer (Fig. 2a–c). The 3D model of the native molecule lacking 41 N-terminal residues however shows extensively opened C-terminal beta conformation and a loose, unorganized tertiary structure very much like the short, non functional alpha A crystallin lacking 55 N-terminal residues (Fig. 2d, e). This reinforces our concept that in vivo truncation at residue 41 and 42 leads not only to a loss of defined N-terminal functional domains but also to a marked change in tertiary structure. This is likely to negatively affect the monomeric structure as a whole and subsequently higher order organization and oligomer function.

C-terminal truncation at the major Lp82 cleavage sites on alpha A crystallin does not affect oligomeric size, secondary

or tertiary structure or chaperone function (Carver et al., 1996; Thampi and Abraham, 2003). Together with the emerging role of Lp82 as the more dominant calpain protease in rodent lenticular development (Ma et al., 1999; Ueda et al., 2001), further investigation of this N-terminally truncated protein species and its function in connection with lenticular protease activity as well as previously described C-terminal cleavage is warranted (Emmons and Takemoto, 1992; Takemoto et al., 1993; Takemoto, 1994, 1995). The intrinsic relationship between molecular structure and function and recent results attesting to alpha A crystallin robustness even after extensive chemical modification *in vitro* (Horwitz et al., 2004) suggest that functional regulation of the alpha crystallin via Lp82 cleavage at N-terminal residues 41 and 42 of the alpha A chain may be of significance in the healthy organism.

Using this investigation as a reference we assess the present state of technology and first consider 2-DE as a proteomics method estimating 3000 spots for an average proteome. Complete proteome coverage with protein identification as protein name takes about 30–90 days but can mean an incomplete characterization of important functional changes (Fig. 3). We calculated the duration of complete proteome coverage at the protein species level based on our analysis of one 2-DE spot. We achieved 50% sequence coverage for the alpha A crystallin protein species including elucidation of the N-terminal peptide in five days. We estimate digestion with alternate enzymes and further analysis would optimally achieve 100% sequence coverage in another five days or in total in about two weeks. Thus, it would take an individual 30000 days or about 75 years to elucidate all of the wealth of information already contained in one 2-DE gel, an unacceptable duration.

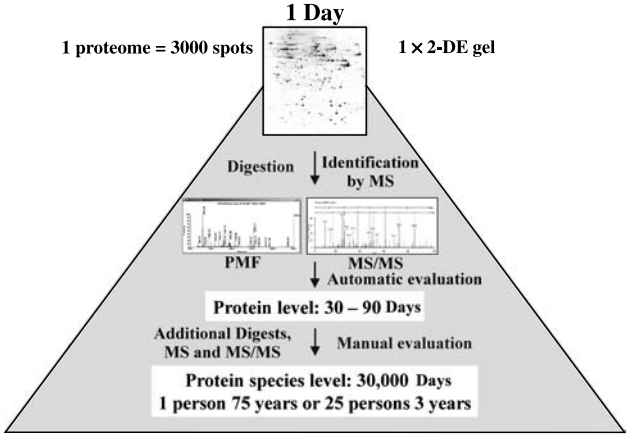

Fig. 3. Estimation of efforts to reach the complete protein species level

Alternatively, a nano LC/ESI-MS/MS analysis of the murine lenticular proteome produced around 7000 MS/MS spectra in one week. This technique however suffers from a number of limitations that include i) that peptides coelute from the nano LC column, ii) that discriminate peptides can have highly similar masses and iii) that only the more or less abundant peptides reach the stage of MS/MS fragmentation and detection. Thus, it is unlikely that the defining N-terminal alpha A crystallin truncation site described above would be obtained even upon manual interpretation of all 7000 MS/MS spectra. Sophisticated multi-dimensional LC-MS/MS approaches utilizing up to 15 or more chromatographic steps deliver the required specifity. However with outputs of up to 100000 MS/MS spectra, this methodology rapidly becomes highly labor intensive (Swanson and Washburn, 2005). Furthermore, while capable of detecting post translational modifications, it can not necessarily distinguish between protein species.

In conclusion, this investigation and the investigation of *Mycobacterium tuberculosis* ESAT6 protein species (Okkels et al., 2004) show that the functional level of proteomics is much better represented if the protein species are investigated in detail. Because the protein species are already separated by 2-DE, the task remains to improve the elucidation of the posttranslational modifications in a moderate time scale. However, current methods are cumbersome and uneconomical on a large scale in a true proteomics sense. We suggest that efforts and resources need to be focused now to develop a technology that facilitates the routine attainment of the protein species level if proteomics is not to crash land before it soars.

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References

Aksu S, Scheler C, Focks N, Leenders F, Theuring F, Salnikow J, Jungblut PR (2002) An iterative calibration method with prediction of post-translational modifications for the construction of a two-dimensional electrophoresis database of mouse mammary gland proteins. Proteomics 2: 1452–1463

Augusteyn RC (1998) Alpha-crystallin polymers and polymerization: the view from down under. Int J Biol Macromol 22: 253–262

Augusteyn RC, Koretz JF (1987) A possible structure for alpha-crystallin. FEBS Lett 222: 1–5

Azuma M, Tamada Y, Kanaami S, Nakajima E, Nakamura Y, Fukiage C, Forsberg NE, Duncan MK, Shearer TR (2003) Differential influence of

proteolysis by calpain 2 and Lp82 on in vitro precipitation of mouse lens crystallins. Biochem Biophys Res Commun 307: 558–563

Baruch A, Greenbaum D, Levy E, Nielsen P, Gilula N, Kumar N, Bogyo M (2001) Defining a link between gap junction communication, proteolysis, and cataract formation*. J Biol Chem 276: 28999–29006

Bera S, Thampi P, Cho WJ, Abraham EC (2002) A positive charge preservation at position 116 of alpha A-crystallin is critical for its structural and functional integrity. Biochemistry 41: 12421–12426

Berengian AR, Bova MP, Mchaourab HS (1997) Structure and function of the conserved domain in alphaA-crystallin. Site-directed spin labeling identifies a beta-strand located near a subunit interface. Biochemistry 36: 9951–9957

Bindels JG, Siezen RJ, Hoenders HJ (1979) A model for the architecture of alpha-crystallin. Ophthalmic Res 11: 441–452

Bon WF (1961) Physicochemical investigations on a complex protein: the soluble protein of the eye lens, alpha-Crystallin*. J Biol Chem 236: 81–85

Bova MP, McHaourab HS, Han Y, Fung BK (2000) Subunit exchange of small heat shock proteins. Analysis of oligomer formation of alphaA-crystallin and Hsp27 by fluorescence resonance energy transfer and site-directed truncations. J Biol Chem 275: 1035–1042

Breci LA, Tabb DL, Yates 3rd JR, Wysocki VH (2003) Cleavage N-terminal to proline: analysis of a database of peptide tandem mass spectra. Anal Chem 75: 1963–1971

Bystroff C, Shao Y (2002) Fully automated ab initio protein structure prediction using I-SITES, HMMSTR and ROSETTA. Bioinformatics 18 [Suppl 1]: S54–61

Carver JA, Aquilina JA, Truscott RJ (1994) A possible chaperone-like quaternary structure for alpha-crystallin. Exp Eye Res 59: 231–234

Carver JA, Nicholls KA, Aquilina JA, Truscott RJ (1996) Age-related changes in bovine alpha-crystallin and high-molecular-weight protein. Exp Eye Res 63: 639–647

Caspers GJ, Leunissen JA, de Jong WW (1995) The expanding small heat-shock protein family, and structure predictions of the conserved ''alpha-crystallin domain''. J Mol Evol 40: 238–248

de Jong WW, van Kleef FS, Bloemendal H (1974) Intracellular carboxy-terminal degradation of the alpha A chain of alpha-crystallin. Eur J Biochem 48: 271–276

de Jong WW, Zweers A, Goodman M (1981) Relationship of aardvark to elephants, hyraxes and sea cows from alpha-crystallin sequences. Nature 292: 538–540

Delaye M, Tardieu A (1983) Short-range order of crystallin proteins accounts for eye lens transparency. Nature 302: 415–417

Derham BK, Harding JJ (1999) Alpha-crystallin as a molecular chaperone. Prog Retin Eye Res 18: 463–509

Emmons T, Takemoto L (1992) Age-dependant loss of the C-terminal amino acid from alpha crystallin. Exp Eye Res 55: 551–554

Farnsworth P, Singh K (2004) Structure function relationship among alpha-crystallin related small heat shock proteins. Exp Eye Res 79: 787–794

Farnsworth PN, Frauwirth H, Groth-Vasselli B, Singh K (1998) Refinement of 3D structure of bovine lens alpha A-crystallin. Int J Biol Macromol 22: 175–185

Farnsworth PN, Groth-Vasselli B, Greenfield NJ, Singh K (1997) Effects of temperature and concentration on bovine lens alpha-crystallin secondary structure: a circular dichroism spectroscopic study. Int J Biol Macromol 20: 283–291

Fukiage C, Nakajima E, Ma H, Azuma M, Shearer T (2002) Characterization and regulation of lens-specific calpain Lp82. J Biol Chem 277: 20678–20685

Gesierich U, Pfeil W (1996) The conformational stability of alpha-crystallin is rather low: calorimetric results. FEBS Lett 393: 151–154

Groth-Vasselli B, Kumosinski TF, Farnsworth PN (1995) Computer-generated model of the quaternary structure of alpha crystallin in the lens. Exp Eye Res 61: 249–253

Harrington V, McCall S, Huynh S, Srivastava K, Srivastava OP (2004) Crystallins in water soluble-high molecular weight protein fractions and water insoluble protein fractions in aging and cataractous human lenses. Mol Vis 10: 476–489

Horwitz J (1992) Alpha-crystallin can function as a molecular chaperone. Proc Natl Acad Sci USA 89: 10449–10453

Horwitz J, Huang Q, Ding L (2004) The native oligomeric organization of alpha-crystallins, is it necessary for its chaperone function? Exp Eye Res 79: 817–821

Horwitz J, Huang QL, Ding L, Bova MP (1998) Lens alpha-crystallin: chaperone like properties. Methods Enzymol 290: 365–383

Jungblut P, Thiede B (1997) Protein identification from 2-DE gels by MALDI mass spectrometry. Mass Spectrom Rev 16: 145–162

Kamei A, Iwase H, Masuda K (1997) Cleavage of amino acid residue(s) from the N-terminal region of alpha A- and alpha B-crystallins in human crystalline lens during aging. Biochem Biophys Res Commun 231: 373–378

Kapphahn RJ, Ethen CM, Peters EA, Higgins L, Ferrington DA (2003) Modified alpha A crystallin in the retina: altered expression and truncation with aging. Biochemistry 42: 15310–15325

Klose J, Kobalz U (1995) Two-dimensional electrophoresis of proteins: an updated protocol and implications for a functional analysis of the genome. Electrophoresis 16: 1034–1059

Ma H, Hata I, Shih M, Fukiage C, Nakamura Y, Azuma M, Shearer T (1999) Lp82 is the dominant form of calpain in young mouse lens*. Exp Eye Res 68: 447–456

Merck KB, De Haard-Hoekman WA, Oude Essink BB, Bloemendal H, De Jong WW (1992) Expression and aggregation of recombinant alpha A-crystallin and its two domains. Biochim Biophys Acta 1130: 267–276

Mörner CT (1894) Untersuchung der Proteinsubstanzen in den leicht-brechenden Medien des Auges. Z Physiol Chem 18: 61–106

Nakamura Y, Fukiage C, Shih M, Ma H, David LL, Azuma M, Shearer TR (2000) Contribution of calpain Lp82-induced proteolysis to experimental cataractogenesis in mice. Invest Ophthalmol Vis Sci 41: 1460–1466

Okkels LM, Muller EC, Schmid M, Rosenkrands I, Kaufman SH, Andersen P, Jungblut PR (2004) CFP10 discriminates between non-acetylated and acetylated ESAT-6 of Mycobacterium tuberculosis by differential interaction. Proteomics 4: 2954–2960

Pasta SY, Raman B, Ramakrishna T, Rao ChM (2003) Role of the conserved SRLFDQFFG region of alpha-crystallin, a small heat shock protein. Effect on oligomeric size, subunit exchange and chaperone-like activity. J Biol Chem 278: 51159–51166

Puri N, Augusteyn RC, Owen EA, Siezen RJ (1983) Immunochemical properties of vertebrate alpha-crystallins. Eur J Biochem 134: 321–326

Schoenmakers JG, Gerding JJ, Bloemendal H (1969) The subunit structure of alpha-crystallin. Isolation and characterization of the S-carboxymethylated acidic subunits from adult and embryonic origin. Eur J Biochem 11: 472–481

Siezen RJ (1981) Reflections on the internal primary, secondary and tertiary structure homology of the eye lens proteins alpha-, beta- and gamma-crystallin. FEBS Lett 133: 1–8

Singh K, Groth-Vasselli B, Farnsworth PN (1998) Interaction of DNA with bovine lens alpha-crystallin: its functional implications. Int J Biol Macromol 22: 315–320

Smith JB, Liu Y, Smith DL (1996) Identification of possible regions of chaperone activity in lens alpha-crystallin. Exp Eye Res 63: 125–128

Smulders RH, van Boekel MA, de Jong WW (1998) Mutations and modifications support a 'pitted-flexiball' model for alpha-crystallin. Int J Biol Macromol 22: 187–196

Swanson SK, Washburn MP (2005) The continuing evolution of shotgun proteomics. Drug Discov Today 10: 719–725

Takemoto L (1994) Release of alpha-A sequence 158–173 correlates with a decrease in the molecular chaperone properties of the high molecular weight aggregate from aged lens. Curr Eye Res 13: 35–44

Takemoto L (1995) Quantitation of C-terminal modification of alpha-A crystallin during aging of the human lens. Exp Eye Res 60: 721–724

Takemoto L, Emmons T, Horwitz J (1993) The C-terminal region of alpha-crystallin: involvement in protection against heat-induced denaturation. Biochem J 294: 435–438

Takemoto LJ (1995) Identification of the in vivo truncation sites at the C-terminal region of alpha-A crystallin from aged bovine and human lens. Curr Eye Res 14: 837–841

Tardieu A, Laporte D, Licinio P, Krop B, Delaye M (1986) Calf lens alpha-crystallin quaternary structure. A three-layer tetrahedral model. J Mol Biol 192: 711–724

Thampi P, Abraham EC (2003) Influence of the C-terminal residues on oligomerization of alpha A-crystallin. Biochemistry 42: 11857–11863

Thomson JA, Augusteyn RC (1983) alpha m-Crystallin: the native form of the protein? Exp Eye Res 37: 367–377

Thomson JA, Augusteyn RC (1984) On the structure of alpha m-crystallin. The reversibility of urea dissociation. J Biol Chem 259: 4339–4345

Ueda Y, Fukiage C, Shih M, Shearer TR, David LL (2002) Mass measurements of C-terminally truncated alpha-crystallins from two-dimensional gels identify Lp82 as a major endopeptidase in rat lens. Mol Cell Proteomics 1: 357–365

Ueda Y, McCormack AL, Shearer TR, David LL (2001) Purification and characterization of lens specific calpain (Lp82) from bovine lens. Exp Eye Res 73: 625–637

van den Oetelaar PJ, van Someren PF, Thomson JA, Siezen RJ, Hoenders HJ (1990) A dynamic quaternary structure of bovine alpha-crystallin as indicated from intermolecular exchange of subunits. Biochemistry 29: 3488–3493

van der Ouderaa FJ, de Jong WW, Bloemendal H (1973) The amino-acid sequence of the alphaA2 chain of bovine alpha-crystallin. Eur J Biochem 39: 207–222

Walsh MT, Sen AC, Chakrabarti B (1991) Micellar subunit assembly in a three-layer model of oligomeric alpha-crystallin. J Biol Chem 266: 20079–20084

Wasinger VC, Cordwell SJ, Cerpa-Poljak A, Yan JX, Gooley AA, Wilkins MR, Duncan MW, Harris R, Williams KL, Humphrey-Smith I (1995) Progress with gene-product mapping of the Mollicutes: Mycoplasma genitalium. Electrophoresis 16: 1090–1094

Wistow G (1985) Domain structure and evolution in alpha-crystallins and small heat-shock proteins. FEBS Lett 181: 1–6

Wistow G (1993) Possible tetramer-based quaternary structure for alpha-crystallins and small heat shock proteins. Exp Eye Res 56: 729–732

Authors' address: P. R. Jungblut, Max Planck Institute for Infection Biology, Schumannstraße 21/22, D-10117 Berlin, Germany,
Fax: +49 30 28460 507, E-mail: Jungblut@mpiib-berlin.mpg.de