Review

Alpha-crystallin

Joseph Horwitz*

Jules Stein Eye Institute, UCLA School of Medicine, 100 Stein Plaza RM B168, Los Angeles, CA 90095 7008, USA

Abstract

Alpha A and alpha B-crystallins are a major protein component of the mammalian eye lens. Being a member of the small heat-shock protein family they possess chaperone-like function. The alpha-crystallins and especially alpha B is also found outside the lens having an extensive tissue distribution. Alpha B-crystallin is found to be over-expressed in many neurological diseases, and mutations in alpha A or B-crystallin can cause cataract and myopathy. This review deals with some of the unique properties of the alpha-crystallins emphasizing especially what we don't know about its function and structure.
© 2003 Elsevier Science Ltd. All rights reserved.

Keywords: alpha-crystallin; small heat-shock proteins; chaperones; aggregation; cataract

1. Introduction

In the last decade alpha-crystallin, a major protein component of the vertebrate eye lens has been the subject of intense investigations into its structure and function. Being a key member of the small heat-shock proteins, the 'alpha-crystallin domain' is a consensus sequence that is common to all the members of the small heat-shock protein super family (de Jong et al., 1998). There are two alpha-crystallin genes, alpha A, and alpha B. In the mammalian lens, the molar ratio of alpha A to alpha B is generally three to one (Bloemendal, 1981). In humans, the alpha A gene is found on chromosome 21 and encodes for a 173 amino acid residue protein, while the alpha B gene is found on chromosome 11 encoding for 175 amino-acid residue protein. The amino-acid sequence homology between alpha A and alpha B is about 57%. Alpha A crystallin is found mainly in the lens with trace amounts in other tissues. Alpha B is essentially considered to be a ubiquitous protein (Bhat and Nagineni, 1989; Dubin et al., 1989; Iwaki et al., 1990).

In the early 1990s it was found that alpha B crystallin is a bona-fide small heat shock protein (Klemenz et al., 1991). Shortly thereafter, it was shown that alpha-crystallin as well as other small heat-shock proteins has some of the properties of molecular chaperones (Horwitz, 1992; Jakob et al., 1993). An important finding in the past decade has been the association of increased levels of alpha B crystallin with various neurological diseases such as Alexander's disease (Iwaki et al., 1989), Creutzfeldt-Jacob disease (Iwaki et al., 1992; Renkawek et al., 1992), Alzheimer's disease (Lowe et al., 1992), Parkinson's disease (Iwaki et al., 1992) and many other diseases. Several reviews on alpha-crystallin, as well as on all other small heat shock proteins have recently been published. These include reviews by Ehrnsperger et al. (1998), Derham and Harding (1999), Horwitz (2000), Jaenicke and Slingsby (2001), van Montfort et al. (2002) and Narberhaus (2002), as well as a comprehensive volume dealing with small stress proteins (Arrigo and Müller, 2002).

To understand the function of alpha-crystallin in the lens it is important to consider some of the special properties of this organ. In order to achieve the necessary refractive index, the protein concentration in the lens must be very high. In the center of a human lens for example, concentration reaches a value of 450 mg ml^{-1} (Fagerholm et al., 1981). A typical mammalian lens contains about 35% alpha-crystallin. Thus, it is one of the major protein components that produce the necessary refractive index. The eye lens continues to grow throughout life. Lens fiber cells are continuously being laid layer upon layer. In humans at birth, there are approximately 1·6 million fiber cells. At age 20 there are approximately 3 million fiber cells,

---

* Corresponding author: Dr Joseph Horwitz, Jules Stein Eye Institute, UCLA School of Medicine, 100 Stein Plaza RM B168, Los Angeles, CA 90095 7008, USA.

E-mail address: horwitz@jsei.ucla.edu (J. Horwitz).

and at 80 there are almost 3·5 million fiber cells (Kusak and Brown, 1994). Cyrstallins constitute over 90% of the proteins in each fiber cell. One of the consequences of the unique growth pattern of the lens is that in the differentiated fiber cells there is no protein turnover. Proteins do not diffuse between fiber cells. Thus, the center of a 70 year old human lens contains proteins that were synthesized during embriogenesis. Structural proteins or enzymes cannot diffuse from the center (old) part of the lens to the more recently synthesized cortical area of the lens and visa versa (Harding, 1997). It is well established that during aging, the lens proteins undergo major post-translational modifications. With normal aging there is an increase in the 'high molecular weight' protein fraction and a conversion from 'water soluble' to a 'water insoluble' protein fraction. All of these major changes occur in the normal lens without compromising too much lens transparency. With aging, and especially in the center part of the lens, old proteins unfold and denature. These proteins are prone to aggregation. Uncontrolled aggregation will cause scattering that will interfere with vision (cataract). It is the alpha-crystallin, which is responsible, in part, for maintaining lens transparency. Alpha-crystallin with its chaperone properties binds selectively the unfolded or denatured proteins and suppresses non-specific aggregation. The evidence that alpha-crystallin act as a molecular-chaperone in the intact lens is compelling. Roy and Spector (1976) were the first to note that in the nuclear region of normal old human lenses, the water-soluble alpha-crystallin fraction selectively disappeared. In humans, up to approximately 40 years of age, there is a gradual loss of the water-soluble low molecular weight alpha-crystallin in the nucleus. After 40 years there is generally a complete disappearance of the water-soluble low molecular weight alpha-crystallin fraction (McFall-Ngai et al., 1985; Rao et al., 1995). Indeed, alpha-crystallin is found together with the other major crystallin in the 'water insoluble' fraction (Harding and Crabbe, 1984; Hanson et al., 2000). Experiments, with cow, monkey and human lenses provide additional evidence that alpha-crystallin prevents non-specific aggregation in the intact lens (Wang and Spector, 1994; Boyle and Takemoto, 1994; Rao et al., 1995; Carver et al., 1996).

The current thinking is that one of the functions of the small heat-shock proteins is to trap aggregation-prone unfolded or denatured proteins and keep them in a refoldable conformation (Haslbeck and Buchner, 2002, and discussion below). The complex of the small heat shock protein with the unfolded target protein is then interacting with another chaperone system, such as HSP70 in an ATP-dependent process to refold the target protein (Ehrnsperger et al., 1997; Lee et al., 1997). However, in the center of the lens there is no evidence for the involvement of another chaperone system. The accumulated data suggests that alpha-crystallin acts as a one-way irreversible 'sink' that traps the unfolded proteins and thus controls the unavoid-able unfolding and denaturation processes during normal aging.

What exactly are the target proteins for alpha-crystallin in the lens? Recent analysis of the 'water insoluble' fraction of human lenses 50–65 years old show that the major component of this fraction are alpha-crystallin together with gamma S, gamma D and various beta-crystallins (Hanson et al., 2000). Thus, it is reasonable to assume that all of the crystallins are potential targets. However, many 'house-keeping' enzymes such as glyceraldehydes-3-phosphate dehydrogenase, and enolase are also potential targets for alpha-crystallin (Valasco et al., 1997). Alpha-crystallin and other small heat-shock proteins are also known to interact with various cytoskeletal elements in the cell (Quinlan, 2002). Muchowski et al. (1999) showed that when a lens cell homogenate was subjected to a mild thermal stress, alpha B-crystallin selectively binds intermediate filament proteins. The cytoskeletal proteins in the lens are most abundant in epithelium and in the cortical fiber cells. They are essentially absent in the nucleus of old human lenses. Thus, alpha-crystallin may function as a chaperone in the location where the cytoskeletal proteins are found. Another important target for alpha-crystallin is the lens plasma membrane and its associated proteins. Recent work suggests that the interaction of alpha-crystallin with the lens plasma membrane may be involved in process of cataractogenesis (Boyle and Takemoto, 1996; Cobb and Petrash, 2002).

2. The structure of alpha-crystallin

Alpha-crystallin is always found as a heterogeneous multimeric assembly with a molecular weight distribution ranging from 300 000 to over 1 million, having a monomeric molecular weight of 20 000. This means that native oligomers consist of variable complexes made up of less than 15 subunits to complexes made up of over 50 subunits. The oligomeric structure of a small heat-shock protein are different between individual super family members. The alpha-crystallins Hsp 25 and Hsp 27 form heterogeneous structures. Other small heat-shock proteins form homogeneous. For instance, Hsp 16·5 from *Methanococcus jannaschii* with a monomeric molecular weight of 16 500 are always found as a 24 mer i.e. as a homogeneous 396 000 molecular weight oligomer (Kim et al., 1998). The small heat-shock protein from wheat Hsp 16·9 also assembles into a discrete dodecamer (van Montfort et al., 2001).

The heterogeneity of alpha-crystallin was already realized almost three decades ago. Siezen et al. (1978) published a paper entitled: "The Quaternary Structure of Bovine alpha-crystallin; Size and charge Micro heterogeneity: More than 1000 Different Hybrids?" Numerous papers appeared in the literature on the 'exact' molecular weight of alpha-crystallin, with very little agreement among the various investigators (Harding, 1997). One of the major

problems for the discrepancy in the value of the native molecular weight of alpha-crystallin is that the molecular weight of the complex will depend on many parameters including concentration, temperature, pH, ionic strength, as well as the age and the species of the lens studied. An additional complication is the fact that most investigators in the field used size-exclusion chromatography to estimate the molecular weight of the alpha-crystallin. It is well know that this methodology can yield inaccurate data, as a possible consequence of protein interaction with the column matrix, the dependency of the elution volume on the shape of the eluting molecule, as well as the dependency on suitable 'standard' proteins for calibration purposes. Several investigators utilized analytical untracentrifugation and light scattering methodologies to estimate the molecular weight of alpha-crystallin, and these approaches definitely yielded more reliable data. Bessems et al. (1983) were the first to utilize gel permeation chromatography coupled to low-angle laser light scattering, and a differential refractive index detector to determine the molecular weight of various crystallins.

The most comprehensive work on the native quaternary structure of bovine alpha-crystallin was published recently by Clauwaert and his co-workers (Vanhoudt et al., 1998, 2000). They found that the average molecular weight of alpha-crystallin in a dilute solution at 37°C in vitro is about 550 000, while in the native state it is estimated to be 700 000. Earlier work by Wang and Bettelheim (1989) using light scattering measurements also yielded a weight average molecular weight of 530 000.

It should be emphasized that knowing the molecular weight of an alpha-crystallin solution accurately is extremely important if one wants to know, for example, the number of alpha-crystallin subunits that are involved while assaying the chaperone function of this protein. An accurate number of the subunits in the complex are crucial if one wants to construct a model of alpha-crystallin.

To obtain an accurate size distribution of small heat-shock proteins in a solution, we started recently to utilize size-exclusion chromatography with online light scattering, absorbance, and refractive index detectors (Wyatt, 1993; Wen et al., 1996; Folta-Stogniew and Williams, 1999). Briefly, size exclusion chromatography is carried out in a conventional way except that the column is connected to a multi-angle laser light scattering detector Down-EOS (Wyatt Technology Corp., Santa Barbara, CA, USA). In this system the scattering intensity of a very small volume ($<20 \mu l$) is continuously and simultaneously monitored at 18 different angles. The great advantage of such a system is that the molecular weight determination is based on the fundamental scattering properties of the macromolecule, and is independent of the column properties, the shape of the macromolecule, or the need to use 'standard' proteins for calibration. The results that can be obtained in such a system are shown in Fig. 1. This figure shows the elution profile and the molecular weight distribution of calf total
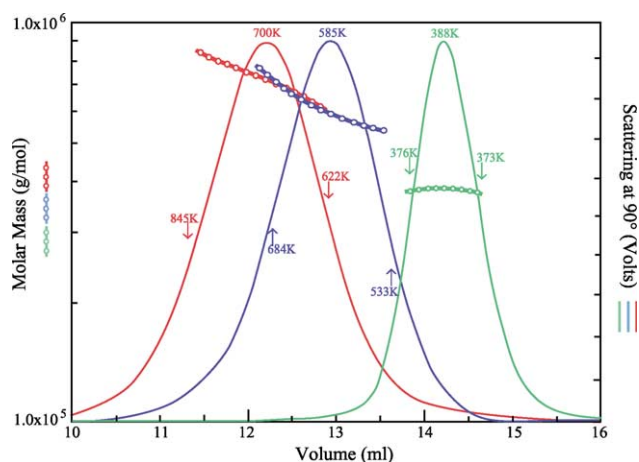

Fig. 1. Molar mass distribution plot and light scattering signal from the 90° detector for Bovine α-crystallin, recombinant human αB-crystallin and Hsp 16·5 from *Methanococcus jannaschii*. Size exclusion chromatography was carried out on a Pharmacia Superose HR6 column connected in line with an absorption detector, a multi-angle laser light scattering detector and a refractive index detector (Wyatt-Technology Corp. Santa Barbara, CA, USA) The solid red, blue, and green lines represent the signal obtained from the laser light scattering apparatus at 90°. The scattering intensity is presented in arbitrary units (volts) on the right ordinate. The signals from the refractive index detector and absorption detectors have a similar shape to the scattering profile. The lines with the circles represented the molecular weight obtained as a function of the elution volumes (left ordinate). Calf alpha-crystallin red plot; Recombinant alpha B-crystallin blue plot; Hsp 16.5 green plot.

alpha-crystallin, human recombinant alpha B crystallin, and the small heat-shock protein Mj Hsp 16·5 from the hyperthermophilic Archaeon *Methanococcus jannaschii*. The molecular weight distribution of Mj Hsp 16·5 is not changing much across the elution profiles (Fig. 1). The molecular weight at the peak obtained from the light scattering measurements is 388 000, which is in excellent agreement with the predicted molecular weight of 394 848. The molecular weight obtained at the half bandwidth of the elution profile is 3–4% lower. The accuracy of this light scattering system is 2–5% (Folta-Stogniew and Williams, 1999). The profile obtained for Mj Hsp 16·5 is what one would expect from a homogeneous sample.

In contrast to the homogeneous nature of Hsp Mj 16·5 (which is made up from 24 subunits), the heterogeneity of alpha-crystallin is evident (Fig. 1). For bovine native alpha-crystallin, which is comprised of three alpha A subunits to one alpha B, the molecular weight obtained at the peak is about 700 000. However, the molecular weight distribution varies significantly across the elution profile. At the bandwidth point of the leading edge (~ 11.4 ml) the molecular weight obtained is 845 000, whereas the molecular weight at the trailing edge (12·8 ml) is 622 000. Using a molecular weight of 20 000 per monomer, we can see that the alpha-crystallin complex is made up of a variable number of subunits ranging from less than 30 to more than 42.

The heterogeneity of recombinant alpha B crystallin is also evident in Fig. 1. In fact, the slope of the molecular

mass is similar to that of the native alpha-crystallin. Recombinant alpha A crystallin exhibits a similar slope (data not shown). While native alpha-crystallin undergoes many post-translational modifications, it was hoped that the recombinant protein would be more homogeneous. This however, is not the case (Fig. 1) and it seems that the polydispersed nature of alpha-crystallin is one of its innate properties.

3. Three dimensional structure of alpha-crystallin

In spite of the many efforts by several laboratories to crystallize alpha-crystallin, at the present, no crystal structure of this protein is available. It is becoming clear that the polydisperse nature of these members of the small heat-shock protein is the culprit. However, the three dimensional structure of other members of this family were solved. Kim et al., 1998 solved the three dimensional structure of Mj Hsp 16·5 from *Methanococcus jannaschii*, and more recently van Montfort et al. (2001) solved

Fig. 2. Cryo-EM reconstructions and models of recombinant αB-crystallin and αB-crystallin with bound α-lactalbumin as a model target protein. (A) A cryo-EM reconstruction of αB-crystallin showing the irregular surface of the protein assembly. (B) A cropped view of the αB-crystallin reconstruction showing the internal cavity. The crop plane is shown with red representing the strongest density and green the weakest. The surface is shown in blue. (C) A cryo-EM reconstruction of αB-crystallin with bound α-lactalbumin. (D) A model of αB-crystallin (magenta) with bound α-lactalbumin (red). The reconstructions of αB-crystallin and the αB-crystallin/α-lactalbumin complex are both average representations of variable protein assemblies. The scale bar represents 100 Å. (Haley, D.A., Hildebrant, I.J., Horwitz, J. and Stewart, P.L., UCLA School of Medicine, unpublished results.).

the structure of Hsp 16·9 from wheat. Even though there is a relatively low sequence identity between these proteins and alpha-crystallin, these structures give us, at present, the best glimpse into the quaternary structure of alpha-crystallin. For a comprehensive review of the structure of the small heat-shock proteins the reader is referred to van Montfort et al., 2002. The basic picture that emerges is that although the 'Beta-sandwich fold' of the alpha-crystallin domain is conserved throughout the small heat-shock protein family, different proteins will form completely different oligomeric assemblies with different biophysical and biochemical properties (van Montfort et al., 2002).

In the absence of X-ray crystal structure of alpha-crystallin, many different models have been proposed (*please see previous reviews cited above*). In recent years cryo-electron microscopy has become a powerful method for studying large protein complexes and proteins that cannot be crystallized (Frank, 2001; Saibil et al., 2002). Even though the resolution of this technology is not as good as X-ray crystallography, useful information can be obtained. When cryo-electron microscopy was applied to study the structure of alpha-crystallin, we found that alpha B crystallin has a variable quaternary structure and a central cavity (Haley et al., 1998). In more recent work, this technology was applied to probe Hsp 16·5, Hsp 27, human alpha B crystallin, and a complex of alpha B crystallin with unfolded alpha-lactalbamin (Haley et al., 2000). In agreement with the crystallography data it was found that the cryo-electron microscopy images of Hsp 16·5 yielded regular and symmetric assemblies. However, no clear symmetry was observed for alpha-crystallin or Hsp 27. The data obtained indicates that there are always variable assemblies of these proteins. A model of alpha-crystallin based on cryo-electron microscopy is shown in Fig. 2. With future crystallographic solutions of other members of the small heat-shock protein family, the increased resolution of cryo-electron microscopy, and with the utilization of other powerful technologies such as site directed spin labelling (Koteiche and Mchaourab, 1999, 2002), we should be able in the near future to get a better picture of the quaternary structures of alpha-crystallin.
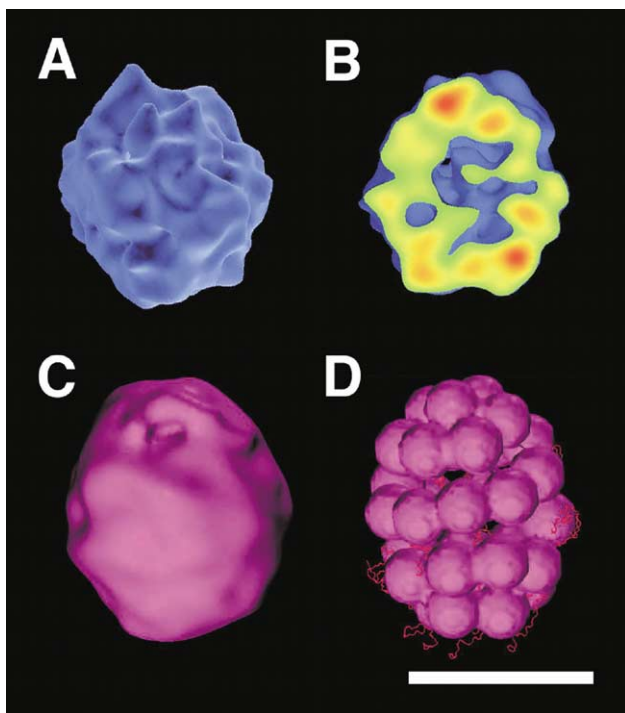
4. Effects of ATP and phosphorylation on the function and structure of alpha-crystallin

The effects of phosphorylation and the binding of alpha-crystallin and other small heat-shock proteins to ATP have been reviewed previously (Gaestel, 2002; Kato et al., 2002). There are conflicting results of the effect of ATP and/or phosphorylation on the function and structure of alpha-crystallin. An earlier report suggests one binding site for ATP per two monomers and a Ka of $8·1 \times 10^3$ M-1 at 37°C. At lower temperature no significant binding was observed (Palmisano et al., 1995). The exact conformational changes that occur in alpha-crystallin upon binding to ATP are not

clear. Using trypsin proteolysis Muchowski et al. (1999b) showed that in the presence of ATP several domains in the 'core' alpha-crystallin region were shielded from the action of chymotrypsin. Other studies utilized fluorescence spectroscopy as evidence for conformational changes in the structure of alpha-crystallin upon binding to ATP (Palmisano et al., 1995; Muchowski and Clark, 1998; Rawat and Rao, 1998). It should be emphasized that alpha-crystallin can bind to various other nucleotides as well as many unrelated small molecules such as 1-anilinonaphthalene-8-sulfonic acid, or dexamethasome (Smulders and de Jong, 1997; Stevens and Augusteyn, 1997; Jobling et al., 2001). We found that alpha B-crystallin binds the dye cibacron Blue F3GA. This dye is commonly used in the affinity chromatography application to bind various enzymes or other macromolecules requiring adenyl-containing co-factors. We have observed that an affinity cibacron blue column will bind alpha B crystallin with a very high binding constant. Using high ionic-strength buffers (>2 M NaCl) did not release alpha B-crystallin from the column. Only by using detergents we could release alpha B-crystallin from this affinity column. Interestingly, alpha A-crystallin or native calf alpha-crystallin do not bind this dye strongly (Horwitz unpublished results).

Recent works by Ehrnsperger et al. (1997) on Hsp-25, and by Lee et al. (1997) on Hsp 18·1 provide evidence that the binding of these sHsps to non-native proteins create a reservoir of unfolded proteins. The interaction of this reservoir with Hsp 70 or with rabbit reticulocyte and with ATP will result in renaturation of the target protein. In a similar experiment Wang and Spector (2001) provide evidence that ATP causes alpha-crystallin to release denatured target protein that can consequently interact with the Hsp 70 chaperone system. It should be noted, however, that the renaturation efficiency with alpha-crystallin is very low. Using citrate synthase as a target protein with alpha-crystallin and Hsp 70, only 6% of the activity was recovered (Wang and Spector, 2001). This level of reactivation can be obtained by using other non-specific proteins such as IgG. However with Hsp-25, Ehrnsperger et al. (1997) achieved a respectable 32% reactivation.

While there are still questions as to the ability of alpha-crystallin to refold denatured proteins, there is evidence that various enzymes can be protected from inactivation by alpha-crystallin (Hess and Fitzgerald, 1998; Derham and Harding, 1999; Marini et al., 2000; Santhoshkumar and Sharma, 2001a,b).

The effect of phosphorylation on the chaperone-like activity of alpha-crystallin is also not clear. Earlier work by Nicholl and Quinlan, (1994) and by Wang et al., (1995) suggest that phosphorylation does not have any effect on the chaperone like function of alpha-crystallin. In a more recent work, Ito et al. (2001) used site-directed mutagenesis to mimic phosphorylation. They observed a reduction in the size of the alpha B-crystallin oligomer from 500 000 for the native protein to an apparent molecular weight of 300 000 for the mimic phosphorylated protein. These authors show that the chaperone-like function of this mimic was reduced significantly.

One of the key questions yet to be answered is if alpha crystallin interacts specifically with ATP, does it hydrolize ATP? To date there is no good evidence that alpha-crystallin has any ATPase activity. In our laboratory we have tried to detect ATPase activity by using highly purified recombinant alpha A, alpha B, or native calf or human alpha-crystallin. We used reaction mixtures of up to 2 mg ml^{-1} of highly purified alpha-crystallin, and a sensitive spectrophotometric assay (Upson et al., 1996), but we failed to detect any hydrolysis of ATP (Horwitz unpublished results). It seems therefore, that similar to the properties of Hsp 70, ATP (and some other analogs) causes alpha-crystallin to undergo conformational changes without hydrolysis (Fink, 1998). Nothing is known about the conformational changes in alpha-crystallin upon binding to ATP.

5. Mutations in alpha-crystallin that cause cataract

Using site-directed mutagenesis and in vitro assays, several investigators have found that alpha-crystallin is generally stable and can tolerate many amino-acid substitutions in its primary structure (Derham and Harding, 1999; Derham et al., 2001). Several mutations that lead to cataract in the human population were reported. The first by Litt et al. (1998) describes a family with autosomal dominant cataract due to a single missense mutation R116C in alpha A-crystallin. The second case was reported by Vicart et al. (1998), who showed that a desmin-related myopathy and cataract is caused by a missense mutation R120G in alpha B-crystallin. Identification of these human mutations resulted in an outburst of site-directed mutagenesis papers by multiple investigators. Physical-chemical analysis of recombinant alpha A, and alpha B-crystallin containing these mutations demonstrated a dramatic effect on the chaperone function of alpha-crystallin. A nonsense mutation W9X in alpha A-crystallin was found to cause cataract in an inbred family (Pras et al., 2000). In addition, new, dominant V124E mutation in mouse alpha A-crystallin was also discovered (Graw et al., 2001). An interesting mutation in the alpha B-crystallin gene that causes a dominant congenital posterior polar cataract in humans was found recently (Berry et al., 2001). This mutation resulted in the expression of an aberrant alpha B-crystallin containing 184 residues in which the 35 residues at the C-terminus are totally different from the native of protein. In the future, we can expect additional critical mutations to be discovered. For example, using site-directed mutagenesis, Santhoshkumar and Sharma (2001a,b) have shown that Phe[71] in alpha A crystallin is essential for its chaperone function. We can expect therefore, that this mutation will also cause cataract.

6. Gene knockout studies of alpha A and alpha B-crystallin

Brady et al. (1997) were the first to produce an alpha A crystallin knockout mouse. These animals develop a cataract that starts in the nucleus and progresses with age to encompass the whole lens. An additional interesting finding in these animals is the presence of dense inclusion bodies (1–3 μm in diameter) in the central lens fiber cells. Using antibodies against various crystallins the authors found that the inclusion bodies are made up of alpha B-crystallin. Thus, it was concluded that alpha A crystallin is clearly needed for maintaining lens transparency, and in addition, alpha A is essential in maintaining the solubility of alpha B in the lens.

Because alpha A and alpha B are highly homologous, it is difficult to understand why alpha A is needed to maintain alpha B soluble in the lens. Especially since in any other cell or tissues alpha B is found without alpha A. Recombinant alpha B-crystallin is also soluble at very high concentrations. We therefore decided to take a second look at the alpha A knockout mouse (Horwitz et al., 2002). Using 2D-gel electrophoresis we found in agreement with the original observation of Brady et al. (1997), that the inclusion bodies are made up mostly of alpha B crystallin. However, they also contain a significant amount (10–15%) of gamma-crystallin. A more dramatic new finding is the difference in the soluble crystallin distribution between the wild type mouse lens and the alpha A knockout lens. This is seen in Fig. 3 for a 25 week old lens. A significant decrease in the amount of the soluble gamma is observed for the alpha A knockout, whereas the beta crystallins are not significantly changed. The 'lost' soluble gamma crystallin is found in the urea-insoluble fraction of this lens. Thus, it seems that alpha A is needed to protect the gamma-crystallin from becoming

insoluble, and it is the accelerated insolubility of gamma-crystallin that is responsible for the total opacity at a later age. A recent publication by Kannan et al. (2001) implicates alpha-crystallin as controlling the glutathione levels in the alpha A knockout lenses. These authors show that the levels of reduced glutathione in the alpha A knockout lenses ages 11–14 months old are about 50% lower than the wild type. However, a 50% drop in the level of reduced glutathione is not enough to cause protein aggregation. It was shown previously that glutathione protects lens proteins down to a level of about 1 mM (Giblin, 2000; Padgaonkar et al., 2000). The mechanisms by which alpha A is protecting the other crystallins and in particular the gamma-crystallin are at present not known.

A gene knockout study of alpha B crystallin yielded some unexpected results. Brady et al. (2001) have shown that alpha B is not essential for the normal development of a transparent lens. It has been noted however, that the life span of these animals is about one half of the wild type. The alpha B knockout mouse showed 'normal' growth curve up to approximately 40 weeks of age. After that they lose weight, develop degenerative osteoarthritis and die prematurely. Thus, we cannot rule out the essential need for alpha B later in life. While the knockout of the alpha B gene apparently did not have any effect on lens transparency and development, the R120G mutation in alpha B can cause desmin-related myopathy, cardiomyopathy and cataract (Vicart et al., 1998; Wang et al., 2001). This mutation is a dominant negative mutation, and in vitro experiments using a recombinant R120G alpha B crystallin show that not only did this mutation cause a decrease in chaperone protection, but with some target proteins it also became an 'anti-chaperone' promoting aggregation (Bova et al., 1999).

7. Conclusions

Alpha-crystallin has been the 'flag-ship' of the small heat-shock proteins. In the lens it plays a critical role as a major refractive element as well as a molecular chaperone. We still don't understand its function outside the lens in the various ocular and non-ocular tissues where it is found. At present we don't have the answers to many fundamental questions such as: what is the quaternary structure? What is the significance of its polydispersivity? What are its target proteins in vivo? How does it interact with other proteins? Is ATP important for its function? Does it have additional regulatory roles? Future studies will clarify the structure and function of this essential multifunctional protein.
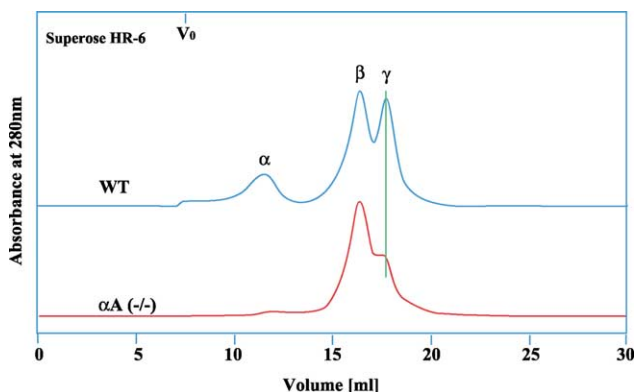

Fig. 3. Gel filtration chromatography of lens soluble proteins from an alpha A knockout lens, and from a control wild type lens. Lenses from 25 weeks old animals were homogenized in a phosphate buffer pH 7, and the soluble fraction was chromatographed on a Pharmacia Superose HR6 column. Blue chromatograph: wild type lens. Red chromatograph: alpha A knockout lens. Notice the loss of gamma crystallin in the knockout lens (from Horwitz et al., 2002).

Acknowledgements

References

Arrigo, A.P., Müller, W.E.G., 2002. Small Stress Proteins, Springer, Berlin.

Berry, V., Francis, P., Reddy, M.A., Collyer, D., Vithana, E., Mackay, I., Dawson, G., Carey, A.H., Moore, A., Bhattacharya, S.S., Quinlan, R.A., 2001. Alpha-B crystallin gene (CRYAB) mutation causes dominant congenital posterior polar cataract in humans. Am. J. Hum. Genet. 69, 1141–1145.

Bessems, G.J.H., Haenders, H.J., Wollensak, J., 1983. Variation in proportion and molecular weight of native crystallins from single human lenses upon aging and formation of nuclear cataract. Exp. Eye Res. 37, 627–637.

Bhat, S.P., Nagineni, C.N., 1989. Alpha B subunit of lens specific protein alpha-crystallin is present in other occular and non-occular tissues. Biochem. Biophys. Res. Commun. 158, 319–325.

Bloemendal, H., 1981. Molecular and cellular biology of the eye lens, John Wiley, New York.

Bova, M.P., Yaron, O., Huang, Q.L., Ding, L.L., Haley, D.A., Stewart, P.L., Horwitz, J., 1999. Mutation R120G in alpha B-crystallin which is linked to a desmin-related myopathy, results in an irregular structure and defective chaperone-like function. Proc. Nat. Acad. Sci. USA 96, 6137–6142.

Boyle, D., Takemoto, L., 1994. Characterization of the alpha-gamma and alpha-beta complex: evidence for an in vivo functional role of alpha-crystallin as a molecular chaperone. Exp. Eye Res. 58, 9–16.

Boyle, D., Takemoto, L., 1996. EM immunolocalization of alpha-crystallins: association with the plasma membrane from normal and cataractous human lenses. Curr. Eye Res. 15, 577–582.

Brady, J.P., Garland, D., Douglas-Tabor, Y., Robinson, G. Jr, Groome, A., Wawrousek, E.F., 1997. Targeted disruption of the mouse alpha A-crystallin gene induces cataract and cytoplasmic incluiion bodies containing the small heat shock protein alpha B-crystallin. Proc. Nat. Acad. Sci. USA 94, 884–889.

Brady, J.P., Garland, D., Green, D.E., Tamm, E.R., Giblin, F.J., Wawrousek, E.F., 2001. Alpha B-crystallin in lens development and muscle integrity: a gene knockout approach. Invest. Ophthalmol. Vis. Sci. 42, 2924–2934.

Carver, J.A., Nicholls, K.A., Aquilina, J.A., Truscott, R.J.W., 1996. Age-related changes in bovine alpha-crystallin and high molecular-weight protein. Exp. Eye Res. 63, 639–647.

Cobb, B.A., Petrash, J.M., 2002. Alpha-crystallin chaperone-like activity and membrane binding in age-related cataracts. Biochemistry 41, 483–490.

Derham, B.K., van Boekel, M.A.M., Muchowski, P.J., Clark, J.I., Horwitz, J., Hepburne-Scott, H.W., de Jong, W.W., Crabbe, J.C., Harding, J.J., 2001. Chaperone function of mutant versions of alpha A- and alpha B-crystallin prepared to pinpoint chaperone binding sites. Eur. J. Biochem. 268, 713–721.

Derham, B.K., Harding, J.J., 1999. Alpha-crystallin as a molecular chaperone. In: Osborne, N.N., Chader, G. (Eds.), Progress in Retinal and Eye Research, 18. Pergamon: Elsevier EB, pp. 431–462.

Dubin, R.A., Wawrousek, E.F., Piatigorsky, J., 1989. Expression of the murine alpha B-crystallin is not restricted to the lens. Mol. Cell Biol. 9, 1083–1091.

Ehrnsperger, M., Gaestel, M., Buchner, J., 1998. Structure and function of small heat-shock proteins. In: Fink, A.L., Goto, Y. (Eds.), Molecular Chaperones in the Life Cycle of Proteins, Marcel Dekker, New York, pp. 553–577.

Ehrnsperger, M., Graber, S., Gaestel, M., Buchner, J., 1997. Binding of non-native protein to Hsp 25 during heat shock creates a reservoir of folding intermediates for reactivation. EMBO J. 16, 221–229.

Fagerholm, P.P., Philipson, B.T., Lindström, B., 1981. Normal human lens—the distribution of proteins. Exp. Eye Res. 33, 615–620.

Fink, A.L., 1998. The Hsp 70 reaction cycle and its role in protein folding. In: Fink, A.L., Goto, Y. (Eds.), Molecular Chaperones in the Life Cycle of Proteins, Marcel Dekker, New York, pp. 123–150.

Folta-Stogniew, E., Williams, K.R., 1999. Determination of molecular masses of proteins in solutions: implementation of an HPLC size exclusion chromatography and laser light scattering service in a core laboratory. J Biomolec. Tech. 10, 51–63.

Frank, J., 2001. Cryo-electron microscopy as an investigative tool: the ribosome as an example. Bio. Essays. 23, 725–732.

Gaestel, M., 2002. sHsp-phosphorylation: enzymes, signaling pathways and functional implications. In: Arrigo, A.P., Müller, W.E.G. (Eds.), Small Stress Proteins, 28. Springer, Berlin, pp. 151–184.

Giblin, F.J., 2000. Glutathione: a vital lens antioxidant. J. Ocular Parm. Therap. 16, 121–135.

Graw, J., Loster, J., Soewarto, D., Fuchs, H., Meyer, B., Reis, A., Wolf, E., Balling, R., Hrabe de Angelis, M., 2001. Characterization of a new dominant V124E mutation in the mouse αA-crystallin-encoding gene. Invest. Ophthal. Vis. Sci. 42, 2909–2915.

Haley, D.A., Bova, M.P., Huang, Q.L., Mchaourab, H.S., Stewart, P.L., 2000. Small heat-shock protein structure reveals a continuum from symmetric to variable assemblies. J. Mol. Biol. 298, 261–272.

Haley, D.A., Horwitz, J., Stewart, P.L., 1998. The small heat-shock protein, aB-crystallin, has a variable quaternary structure. J. Mol. Biol. 277, 27–35.

Hanson, S.R.A., Hasan, A., Smith, D.L., Smith, J.B., 2000. The major in vivo modifications of the human water-insoluble lens crystallins are disulfide bonds, deamidation, methionine oxidation, and backbone cleavage. Exp. Eye Res. 71, 195–207.

Harding, J.J., 1997. Lens. In: Harding, J.J., (Ed.), Biochemistry of the Eye, Chapman and Hall, London, pp. 94–135.

Harding, J.J., Crabbe, M.J.C., 1984. The lens: development, protein, metabolism and catract. In: Davson, H(Ed.), The Eye, 3rd Ed., Vol.1B, Academic Press, Orlando, FL, pp. 207–492.

Haslbeck, M., Buchner, J., 2002. Chaperone function of sHsps. In: Arrigo, A.P., Müller, W.E.G. (Eds.), Small Stress Proteins, Springer, Berlin, pp. 37–52.

Hess, J.F., FitzGerald, P.G., 1998. Protection of a restriction enzyme from heat inactivation by alpha-crystallin. Mol. Vision. 4, 29–32.

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

Horwitz, J., 2000. The function of alpha-crystallin in vision. Sem. Cell and Devel. Biol. 11, 53–60.

Horwitz, J., Wawrousek, E.F., Huang, Q.L., Garland, D., Ding, L.L., Brady, J.P., 2002. Gamma-crystallin abnormalities, in alpha A-crystallin gene knockout mouse lenses. Invest. Ophthalmol. Vis. Sci., 1921Abstract.

Ito, H., Kamei, K., Iwamoto, I., Inaguma, Y., Nohara, D., Kato, K., 2001. Phosphorylation-induced changes of the oligomerization state of alpha B-crystallin. J. Biol. Chem. 276, 5346–5352.

Iwaki, T., Kume-Iwaki, A., Goldman, J.E., 1990. Cellular distribution of alpha B-crystallin in non-lenticular tissues. J. Histochem. Cytochem. 38, 31–39.

Iwaki, T., Kume-Iwaki, A., Liem, R.K., Goldman, J.E., 1989. Alpha B-crystallin is expressed in non-lenticular tissues and accumulates in Alexander's disease brain. Cell 57, 71–78.

Iwaki, T., Wisniewski, T., Akiko, I., Corbin, E., Tomokane, N., Tateishi, J., Goldman, J.E., 1992. Accumulation of alpha B-crystallin in central nervous system glia and neurons in pathologic conditions. Am. J. Pathol. 140, 345–356.

Jaenicke, R., Slingsby, C., 2001. Lens crystallin and their microbial homologs: structure, stability and function. Crit. Rev. Biochem. Molec. Biol. 36, 435–499.

Jakob, U., Gaestel, M., Engel, K., Buchner, J., 1993. Small heat-shock proteins are molecular chaperones. J. Biol. Chem. 268, 1517–1520.

Jobling, A.I., Stevens, A., Augusteyn, R.C., 2001. Binding of dexamethasone by alpha-crystallin. Invest. Ophthalmol. Vis. Sci. 42, 1829–1832.

de Jong, W.W., Caspers, G.J., Leunissen, J.A.M., 1998. Genealogy of the alpha-crystallin-small heat-shock protein superfamily. Int. J. Biol. Marcomol. 22, 151–162.

Kannan, R., Ouyang, B., Wawrousek, E., Kaplowitz, N., Andley, U.P., 2001. Regulation of GSH in alpha A-expressing human lens epithelial cell line and in alpha A knockout mouse lenses. Invest. Ophthalmol. Vis. Sci. 42, 409–416.

Kato, K., Ito, H., Inaguma, Y., 2002. Expression and phosphorylation of mammalian small heat shock proteins. In: Arrigo, A.P., Müller, W.E.G. (Eds.), Small Stress Proteins, 28. Springer, Berlin, pp. 129–150.

Kim, K.K., Kim, R., Kim, S.H., 1998. Crystal structure of a small heat-shock protein. Nature 394, 595–599.

Klemenz, R., Frohli, E., Steiger, R.H., Schafer, R., Aoyama, A., 1991. Alpha B-crystallin is a small heat-shock protein. Proc. Nat. Acad. Sci. USA 88, 3652–3656.

Koteiche, H.A., Mchaourab, H.S., 1999. Folding pattern of the alpha-crystallin domain in alpha A-crystallin determined by site directed spin labeling. J. Mol. Biol. 294, 561–577.

Koteiche, H.A., Mchaourab, H.S., 2002. The determinant of oligomeric structure in Hsp 16.5 are encoded in the alpha-crystallin domain. FEBS Lett. 519, 16–22.

Kusak, J.R., Brown, H.G., 1994. Embryology and anatomy of the lens. In: Albert, D.M., Jakobiec, F.A. (Eds.), Principles and Practice of Ophthalmology: Basic Sciences, WB Saunders, Philadelphia, PA, p. 82.

Lee, G.J., Roseman, A.M., Saibil, H.R., Vierling, E., 1997. A small heat-shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state. EMBO J. 16, 659–671.

Litt, M., Kramer, P., La Morticella, D.M., Murphy, W., Lovrien, E.W., Weleber, R.G., 1998. Autosomal dominant congenital cataract associated with a missence mutation in the human alpha-crystallin gene CRYAA. Human Mol. Gen. 7, 471–474.

Lowe, J., McDermott, H., Pike, I., Speudlove, I., Landon, M., Mayer, R., 1992. Alpha B-crystallin expression in non-lenticular tissues and selective presence in ubiquinated inclusion bodies in human disease. J. Pathol. 166, 61–68.

Marini, I., Moschini, R., Del Corso, A., Mura, U., 2000. Complete protection by alpha-crystallin of lens sorbitiol dehydrogenase undergoing thermal stress. J. Biol. Chem. 275, 32559–32565.

McFall-Ngai, M.J., Ding, L.L., Takemoto, L.J., Horwitz, J., 1985. Spatial and temporal mapping of the age-related changes in human lens crystallins. Exp. Eye. Res. 41, 745–758.

van Montfort, R.L.M., Basha, E., Friedrich, K.L., Slingsby, C., Vierling, E., 2001. Crystal structure and assembly of a eukaryotic small heat-shock protein. Nature Struct. Biol. 8, 1025–1030.

van Montfort, R., Slinsgsby, C., Vierling, E., 2002. Structure and function of the small heat-shock protein/alpha-crystallin family of molecular chaperones, Adv. Protein Chem., 5a. Academic Press, San Diego, CA, pp. 105–156.

Muchowski, P.J., Clark, J.I., 1998. ATP enhanced molecular chaperone function of the small heat-shock protein human alpha B-crystallin. Proc. Nat. Acad. Sci. USA 95, 1004–1009.

Muchowski, P.J., Hays, L.G., Yates, J.R., Clark, J.I., 1999b. ATP and the core 'alpha-crystallin' domain of the small heat-shock protein alpha B-crystallin. J. Biol. Chem. 274, 30190–30195.

Muchowski, P.J., Valdez, M.M., Clark, J.I., 1999. Alpha B-crystallin selectively targets intermediate filament proteins during thermal stress. Invest. Ophthalmol. Vis. Sci. 40, 951–958.

Narberhaus, F., 2002. Alpha-crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network. Microbiol. Mol. Biol. Rev. 66, 64–93.

Nicholl, I.D., Quinlan, R.A., 1994. Chaperone activity of alpha-crystallins modulates intermediate filament assembly. EMBO J. 13, 945–953.

Padgaonkar, V.A., Leverenz, V.R., Fowler, K.E., Reddy, V.N., Giblin, F.J., 2000. The effects of hyperbaric oxygen on the crystallins of cultural rabbit lenses: a possible catalytic role for copper. Exp. Eye Res. 71, 371–383.

Palmisano, D.V., Groth-Vasselli, B., Farnsworth, P.N., Reddy, M.C., 1995. Interaction of ATP and lens alpha-crystallin characterized by equilibrium binding studies and intrinsic tryptophan fluorescence spectroscopy. Biochem. Biophys. Acta. 1246, 91–97.

Pras, E., Frydman, M., Levy-Nissenbaum, E., Bakhan, T., Raz, J., Assia, E.I., Goldman, B., Pras, E., 2000. A nonsense mutation (W9X) in CRYAA causes autosomal recessive cataract in an inbred Jewish Persian family. Invest. Ophthal. Vis. Sci. 41, 3511–3515.

Quinlan, R., 2002. Cytoskeletal competence requires protein chaperones. In: Arrigo, A.P., Müller, W.E.G. (Eds.), Small Stress Proteins, Springer, Berlin, pp. 219–228.

Rao, P.V., Huang, Q.L., Horwitz, J., Zigler, S.J., 1995. Evidence that alpha-crystallin presents non-specific protein aggregation in the intact eye lens. Biochem. Biophys. Acta. 1245, 439–447.

Rawat, U., Rao, M., 1998. Interaction of chaperone alpha-crystallin with the molton globule state of xylose-reductase. Implication for reconstitution of the active enzyme. J. Biol. Chem. 273, 9415–9423.

Renkawek, K., de Jong, W.W., Merk, K.B., Frenken, C.W.G.M., van Workum, F.P.A., Bosman, G.J.C.G.M., 1992. Alpha B-crystallin is present in reactive glia in Creutzfeldt-Jakob disease. Acta. Neuropathol. 83, 324–327.

Roy, D., Spector, A., 1976. Absence of low-molecular-weight alpha-crystallin in the nuclear region of old human lenses. Proc. Nat. Acad. Sci. USA 76, 3484–3487.

Saibil, H.R., Horwich, A.L., Fenton, W.A., 2002. Allostery and protein substrate conformational change during GroEL/ES-mediated protein folding. In: Horwich, A., (Ed.), Advan. Prot. Chem., 59. Academic Press, San Diego, CA, pp. 45–72.

Santhoshkumar, P., Sharma, K.K., 2001a. Analysis of alpha-crystallin chaperone function using restriction enzymes and citrate-synthase. Mol. Vision. 7, 172–177.

Santhoshkumar, P., Sharma, K.K., 2001b. Phe[71] is essential for chaperone-like function in alpha-A crystallin. J. Biol. Chem. 276, 47094–47099.

Siezen, R.J., Bindels, J.G., Holenders, H.J., 1978. The quaternary structure of bovine alpha-crystallin. Size and charge microheterogeneity: more than 1000 different hybrids? Eur. J. Biochem. 91, 387–396.

Smulders, R.H.P.H., de Jong, W.W., 1997. The hydrophobic probe 4,4′-bis (1-anilino-8-naphthalene sulfonic acid) is specifically photoincorporated into the N-terminal domain of alpha B-crystallin. FEBS Lett. 409, 101–104.

Stevens, A., Augusteyn, R.C., 1997. Binding of 1-anilinonaphthalene-8-sulfonic acid to alpha-crystallin. Eur. J. Biochem. 243, 792–797.

Upson, R.H., Hauglond, R.P., Malekzadeh, M., Haugland, R.P., 1996. A spectrophotometric method to measure enzymic activity in reaction that generate inorganic pyrophosphate. Anal. Biochem. 243, 41–45.

Valasco, P.T., Lukas, T.J., Murthy, S.N.P., Duglas-Tabor, Y., Garland, D., Lorand, L., 1997. Hierarchy of lens proteins requiring, protection against heat induced precipitation by the alpha-crystallin chaperone. Exp. Eye Res. 65, 497–505.

Vanhoudt, J., Aerts, T., Abgar, S., Clauwaert, J., 1998. Quaternary structure of bovine alpha-crystallin: influence of temperature. J. Biol. Macromolec. 22, 229–237.

Vanhoudt, J., Aerts, T., Abgar, S., Clauwaert, J., 2000. Native quaternary structure of bovine alpha-crystallin. Biochemistry 39, 4483–4492.

Vicart, P., Caron, A., Guicheney, P., Li, Z., Prevost, A.F., Chatean, D., Chapon, F., Tome, F., Dupret, J.M., Saulin, D., 1998. A missence mutation in the alpha-B crystallin chaperone gene causes a desmin-related myopathy. Nature Gen. 20, 92–95.

Wang, K., Spector, A., 1994. The chaperone acitivity of bovine alpha crystalline. Interaction with other lens crystallins in native and denatured states. J. Biol. Chem. 269, 13601–13608.

Wang, K., Ma, W., Spector, A., 1995. Phosphorylation of alpha-crystallin in rat lenses is stimulated by H_2O_2 but phosphorylation has no effect on chaperone activity. Exp. Eye Res. 61, 115–124.

Wang, K., Spector, A., 2001. ATP causes small heat shock proteins to release denatured protein. Eur. J. Biochem. 268, 6335–6345.

Wang, X., Bettelheim, F.A., 1989. Second virial coefficient of alpha-crystallin. Proteins: Struct. Funct. Genet. 5, 166–169.

Wang, X., Osinska, H., Klevitsky, R., Gerdes, M., Nieman, M., Lorenz, J., Hewett, T., Robbins, J., 2001. Expression of R120G-alpha B crystallin causes aberrant desmin and alpha B-crystallin aggregation and cardiomyopathy. Circ. Res. 89, 84–91.

Wen, J., Arakawa, T., Philo, J.S., 1996. Size-exclusion chromatography with on-line light-scattering, absorbance, and refractive index detectors for studying proteins and their interactions. Anal. Biochem. 240, 155–166.

Wyatt, P.J., 1993. Light scattering and the absolute characterization of macromolecules. Anal. Chim. Acta. 272, 1–40.