Eur Biophys J (1997) 25: 445–454

ARTICLE

T. Aerts · J. Clauwaert · P. Haezebrouck · E. Peeters
H. Van Dael

Interaction of detergents with bovine lens α-crystallin: evidence for an oligomeric structure based on amphiphilic interactions

Accepted: 18 October 1996

Abstract We have studied the quaternary structure of α-crystallin in the presence of increasing concentrations of amphiphilic and neutral detergents using gel filtration, light-scattering, boundary and equilibrium sedimentation. We observed a continuous reduction of the molar mass of the polymeric α-crystallin on increasing the concentration of sodium dodecyl sulphate from 0.1 mM to 5 mM, ending up with the monomeric peptides. Dodecyltrimethylammonium bromide also disrupts the oligomeric structure of α-crystallin but the interaction appears to be cooperative: in the sharp transition region (for a 1 mg/ml protein solution) from 3 to 8 mM of the detergent, only the native protein and a mixture of monomeric and dimeric peptide-DTAB complexes can be observed. Concomitant studies of the circular dichroism in the far UV revealed a substantial decrease of the β-sheet and increase of the α-helix secondary structure. The latter can be related to the presence of amphiphilic polypeptide sequences in the constituent αA and αB peptides. These studies reveal for the first time a direct relation between changes in the secondary structure of the αA and αB peptides and the formation of the oligomeric α-crystallin structure: the binding of the amphiphilic detergent reduces the β-sheet content, induces the formation of α-helix secondary structure and reduces the tendency of the peptide to form large aggregates. The different mechanisms for reducing the oligomeric size by anionic and cationic detergents with identical apolar parts stresses the importance of charge interactions. Our findings support some aspects of the micelle model of α-crystallin and can be related to its chaperone activity.

T. Aerts · J. Clauwaert (✉) · E. Peeters
Biophysics Research Group, Department of Biochemistry, University of Antwerp, B-2610 Antwerp, Belgium
(Fax: 03/8202248; E-mail: clauwaer@uia.ua.ac.be)

P. Haezebrouck · H. Van Dael
Interdisciplinary Research Center, K.U. Leuven, Campus Kortrijk, B-8500 Kortrijk, Belgium

Key words Lens protein · Micellar structure · Quaternary structure · Secondary structure · Light scattering · Boundary and equilibrium sedimentation · Circular dichroism · Detergent

Introduction

The crystallins are the main components of the lens fibre cells and their solution properties combine a high refractive index, resulting from the high concentration of soluble proteins, and a low light scattering power.

α-crystallin is the largest protein and it is present in the highest concentration (about 45% of the total protein fraction) in the cytoplasm. It is an oligomeric protein which mainly contains 4 peptides αA_1, αA_2, αB_1, αB_2 where the A peptides have an isoelectric point below pH 7 (Acidic) and the B peptides have an isoelectric point above pH 7 (Basic). αA_2, the major α-crystallin peptide, and αB_2 are the only primary gene products. αA_1 and αB_1 arise from these peptides by a specific postsynthetic process (Spector et al. 1985; Voorter et al. 1989). In addition to these "intact" peptides, the α-crystallin contains degraded peptides; these degraded peptides arise on maturing and or aging by specific cleavages of the A or B peptides. For example $\alpha A_{2.1–169}$ represents a peptide identical to αA_2 but only containing the first 169 amino acids.

The tertiary and quaternary structure is still a matter of controversy. The characterization of the native α-crystallin suggests a relation between peptide composition and quaternary structure. α-crystallin, isolated from the cytoplasm of newly synthesised fibre cells mainly contains the 4 intact A and B peptides: the α-crystallin proteins also form a quite homogeneous population with a mean molar mass around 650,000 g/mole. The α-crystallins, isolated from older cells, contain a broad pattern of peptides: the 4 intact peptides and a whole set of degraded and modified peptides, originating from the 4 peptides by quite specific degradation and chemical modification such as phosphorylation and deamidation. These α-crystallin solutions now

contain a quite broad distribution of aggregates, whose molar mass ranges from 600,000 to 1,500,000 g/mole (Aerts et al. 1995). It is still an open question if this broad population of protein molecules is formed by a continuous collection of proteins each differing by one peptide, or if there are some discrete subclasses. Spectroscopic and light scattering studies have suggested a multi-layer tetrahedral model (Tardieu et al. 1986) which could give rise to a continuous set of sizes by filling up the different layers continuously or to a discrete set of molecules by giving the proteins with filled layers an extra stability. Some years ago, a micellar model was suggested for the quaternary structure of α-crystallin (Augusteyn and Koretz 1987): this includes the idea that hydrophobic forces are responsible for the aggregation of the peptides and that polar interactions with solvent molecules keep the aggregates soluble and limit their size. Augusteyn and Koretz also claim arguments for a two-dimensional arrangement of the peptides instead of a more globular arrangement as suggested by hydrodynamic and light scattering methods (Andries et al. 1982; Tardieu et al. 1986) but the experimental evidence for a two-dimensional arrangement is rather indirect (Radlick and Koretz 1992). A three layer structure model has been proposed for the native α-crystallin (Walsh et al. 1991): this model combines the multilayer and the micelle model. The inner layer (core) forms a micellar structure containing 12 αA subunits: the polar groups are distributed in different parts of the molecule and the apolar region forms the micelle core. Six more subunits can easily be added to form a second layer: the apolar surfaces are oriented toward the hydrophobic inner core. The third layer just adds more subunits and can accommodate 24 αA or αB in a cuboctahedron type symmetrical structure. This model easily explains the changes of the molecular mass according to the solvent conditions: the third layer peptides are loosely bound to the inner two layers by weak polar interactions and these peptides can be easily removed.

Transient electric-birefringence measurements have proven the existence of a fraction of α-crystallin in a more extended quaternary structure (van Haeringen et al. 1992; van Haeringen et al. 1993).

Chaotropic agents such as urea and guanidine hydrochloride reduce the size of the α-crystallin and addition of high concentrations of these agents to α-crystallin solutions results in a mixture of denatured αA and αB peptides. The finding that intermediate concentrations of urea and guanidine hydrochloride resulted in a step-wise dissociation from oligomers of 800,000 to 20,000 g/mole, supported the above mentioned multilayer model (Siezen and Bindels 1982). For a long time it has not been possible to renature the denatured peptides to the original 800,000 g/mole oligomers (Thomson and Augusteyn 1984) but it has been shown that by carefully controlling the conditions such as protein concentration, pH and/or ionic strength and/or temperature of the solvent, a denatured sample can be renatured to every predetermined mean molar mass population (Clauwaert et al. 1989): an increase in repulsive interactions, obtained by a lower ionic strength or a pH further removed from the isoelectric pH, decreases the size of the renatured proteins; conditions which favor hydrophobic interactions, such as higher temperature, increase the size of renatured α-crystallins.

The study of interaction of detergents with α-crystallin can help to clear some aspects of the importance of polar and apolar interactions in the secondary, tertiary and quaternary structure: detergents contain a hydrophobic part which can match the hydrophobic interactions between the peptides of the oligomeric structure; the importance of polar interactions can be probed by using different classes of polar detergents by making a selection between cationic, neutral and anionic detergents.

We have used gel filtration chromatography for a qualitative study of the protein-detergent complexes. Light scattering, boundary sedimentation and equilibrium sedimentation allow the determination of the molar mass and some hydrodynamic parameters of the particles. So the application of these complementary techniques allows a better characterization of the α-crystallin in the presence of the different types of detergents.

We have further used circular dischroism measurements to probe concomitant changes of the secondary structure.

Material and methods

Preparation of α-crystallin

The lenses of 6-month (±2 weeks) old calves were freshly obtained at a local slaughterhouse within 3 h after slaughtering and were subsequently stored at 4 °C. The lens capsule was removed and the lenses were mixed with a sixthfold quantity of buffer (containing 10 mM Hepes, 120 mM KCL, 25 mM NaCL, 0.02% NaN_3, pH = 7.0) and gently stirred at 4 °C for 20 minutes. In this way only the outer cortical cells were dissolved. This suspension was centrifuged at 12,000 g for 30 minutes to remove the insoluble material.

About 20 ml of cortical protein solution, dissolved in the above mentioned buffer (containing about 2000 $A^{\frac{1\ cm}{280\ nm}}$ units), was loaded on a Bio-Gel A-5 m column (∅ 5 cm × 85 cm, Pharmacia) at 4 °C and the eluent was collected in 15 ml fractions. The top fractions of the low molecular mass α-crystallin elution zone were collected and eventually concentrated by using an Amicon concentration cell (model 52, Amicon Corp., Lexington MA) and a XM-100 filter system (Amicon Corp.) (Andries et al. 1982).

Detergents

The detergents, used in our experiments, can be classified into two classes:

– the nonionic detergents OG (octylglucopyranoside) and triton X-100 (octylphenolpolyethylene glycolether)

– the ionic detergents: the anionic detergent SDS (sodium dodecyl sulphate) and the cationic detergent DTAB (dodecyl-trimethyl-ammonium bromide).

Gel filtration

About 0.3 ml α-crystallin sample at a protein concentration of about 6 mg/ml was loaded on a Superose 6 (Pharmacia) gel filtration column ($\varnothing$ 1×46 cm, Pharmacia). Before loading the sample, the gel column was equilibrated at the same detergent concentration as the sample buffer. The flow rate of the gel column was 0.5 ml/min and was accurately controlled by a constant step speed peristaltic pump (10200 Perpex, LKB). The protein eluting from the gel column was detected and monitored with an UV detector (Model SP 8300, Spectra-physics, Inc). The position at which a substance elutes from a gel filtration column is usually expressed in terms of the elution fraction number. When the eluting buffer contains detergent, the surface tension of elution buffer and the drop size decrease so the fraction size will also decrease: for that purpose we weighed the fractions and as the density of the eluent is hardly influenced by its protein content, all other factors remaining constant, this weighting procedure gave quite accurate values for the elution volume V_e.

The elution volume V_e, which corresponds to the peak concentration of eluting solute, was used to normalize the data from different runs. The gel filtration profile is characterized by the distribution coefficient K_d

$$K_d = \frac{V_e - V_0}{V_i - V_0} \tag{1}$$

where V_0: the void volume
V_i: the volume of solvent immobilized within the stationary gel phase

There is a procedure for an accurate column calibration in which no structural shapes are assumed for the calibration proteins (Ackers 1967). The basic assumption is that the penetrable volumes within the gel are randomly distributed with respect to the sizes of the molecules they can accommodate. It can be proved that under these conditions the distribution coefficient Kd of a particle is related to the error function complement of the hydrodynamic radius a of the particle

$$K_d = \mathrm{erfc}\left[\frac{a - a_0}{b_0}\right] = 1 - \frac{2}{\sqrt{\pi}} \int_0^{(a-a_0)/b_0} e^{-a^2} \cdot da \tag{2}$$

where a_0 and b_0: constants.

In this way a calibration curve can be constructed for a particular gel filtration column and particles using their experimental distribution coefficient K_d and known apparent hydrodynamic radius.

Light scattering measurements

The light scattered by α-crystallin solutions was measured using a light scattering instrument in a thermostated room (Andries et al. 1983).

The light scattered by a diluted solution of particles is commonly represent by the following equation

$$\frac{Kc}{R_p(k)} = \frac{1}{P(k)} \cdot \left(\frac{1}{\langle M \rangle_w} + 2Bc + \ldots \right) \tag{3}$$

where

K: $4\pi^2 n^2 (dn/dc)^2 / N_A \lambda_0^4$; n, refractive index of reference solvent toluene; n = 1.507; dn/dc, the refractive index increment of the α-crystallin protein solution 0.195 ml · g^{-1} (Andries et al. 1982); λ_0, represents the wavelength of the laser beam in vacuum, λ_0 = 488 nm; N_A, Avogadro's number. $R_p(k) = (I_{sol}/I_{tol}) \cdot R_{tol} \cdot (n/n_{tol})^2$ where I_{sol}/I_{tol} the ratio of the scattered intensity by the protein solution to the reference solvent (toluene), R_{tol} the Rayleigh factor for toluene and n and n_{tol} are the index of refraction of the solution and the reference solvent respectively;
k: scattering vector $[4\pi n_0/\lambda_0] \cdot \sin(\theta/2)$
c: concentration of particles in mg/ml
P(k): the particle form factor
$\langle M \rangle_w$: the weight-average molar mass of the particles in solution
B: the second virial coefficient

At low concentration of particles, which are small relative to the wavelength of the incident beam so that P(k) = 1, Eq. (3) can be written in the following form

$$\frac{Kc}{R_p(k)} = \frac{1}{\langle M \rangle_w} + 2B_c = \frac{1}{\langle M \rangle_w}(1 + K_I \phi) \tag{4}$$

where ϕ: hydrodynamic volume fraction $\phi = c\upsilon$
υ: the hydrodynamic volume (ml/g)
K_I: the static coefficient

In order to obtain the $\langle M_0 \rangle_w$ value, the molar mass value extrapolated to a concentration zero, from Eq. (4) we have used a K_I of 8.54; this is the experimental value obtained for a diluted α-crystallin solution in the same ionic strength conditions (Xia et al. 1994).

Boundary and equilibrium sedimentation

The Beckman Optima XL-A analytical ultracentrifuge was employed to perform sedimentation velocity experiments. Solute distributions at 20.0 °C were recorded via their absorption at 280 nm. Consecutive scans were recorded at regular intervals, utilizing the "autoscan" facility. Sedimentation coefficient ($S_{20,w}$) values were determined in the standard way by plotting the $\ln(r_{\text{first moment of the boundary}})$ versus time or from the time derivative of the sedimentation velocity concentration profile (Stafford 1992).

The conditions for the sedimentation equilibrium runs (angular velocity ω and duration of run) were calculated from the preset molecular parameters (sedimentation coefficient, molar mass range, 3 or 10 mm solution column),

using the method proposed by Yphantis (1964). After reaching the equilibrium and having taken the equilibrium absorbance profiles, the angular velocity ω was increased to high speed (25,000 or 40,000 rpm) for another 24 hours so that all the proteinous material was sedimented. The remaining absorbance profiles were considered as the best estimate of the residual blank absorbance and were subtracted from the sample absorbance profiles to obtain the c_r values as a function of r.

The standard equilibrium equation

$$c_r = c_o \cdot \exp[(M_w \cdot (1 - \nu \cdot \rho) \cdot \omega^2/2 \cdot R \cdot T) \cdot (r^2 - r_0^2)] \quad (5)$$

where c_r and c_o the concentration at the distance r respectively r_o from the rotor centre
ω: angular velocity
ν: partial specific volume of the protein
ρ: density of the solution

has been analyzed using the Beckman software, which is based on nonlinear least-squares techniques (Johnson et al. 1981) and the Equilibrium/Velocity Analysis Programs of Holladay (Kelly and Holladay 1990; Shire et al. 1991). Both equivalent methods leave the choice between different options such as an ideal solution of a single component, an ideal solution with two components, a non ideal solution of a single component, a monomer-Nmer associating system etc.; a quality factor or confidence parameter, corresponding to each option of analysis, can be used as criterion for selecting the option which matches the real situation. The XLASE method, as developed by Lechner (Lechner and Mächte 1992), uses equilibrium distribution measurements at different concentrations in order to calculate the M_w, the second virial coefficient 2B and the molar mass distribution W(M) on introducing a preset distribution function (Poisson distribution, Schulz-Flory distribution, Weslau distribution or log-normal distribution) starting from the experimental measured reduced concentration profile $C(X)/C_0$, where C(X) the solute concentration at equilibrium at the reduced distance $X = (r^2 - r_m^2)/(r^2 - r_b^2)$ where

r: distance from the rotor centre
r_m: distance of the meniscus r_b: distance of the bottom
C_0: initial concentration of the solute

The distribution function W(M) is directly calculated from the experimental measured reduced concentration profile $C(X)/C_0$ by nonlinear regression.

The initial α-crystallin solution concentrations varied from 0.2 to 0.8 mg/ml; the concentration c_r was determined from the absorbance; in accordance with the concentration, the absorbance was measured at wavelengths ranging from 295 to 220 nm; for each solution, at least 3 appropriate wavelenghts were selected for determining $c_{r,relative}$.

Circular dichroism measurements

Circular dichroism measurements were carried out with a Jasco J-600 spectropolarimeter using cuvettes of 1 mm or 0.1 mm path length. Each spectrum in the wavelength range 195–250 nm was the result of accumulation of at least 6 runs with 5 nm/min scan speed and a time constant of 8 seconds. The temperature (25 °C) was held constant by thermostated water circulating through the cell holder. After subtraction of the buffer spectrum, the results were expressed as the mean residue ellipticity $[\Theta]$ $(deg \cdot cm^2 \cdot dmol^{-1})$, which was calculated taking 115 as the mean residue weight for the α-crystallin sample. CD spectroscopy is a valuable tool for the determination of the secondary structure of proteins in solution (Johnson 1990). In analyzing far-UV CD spectra most methods make use of basic spectra originating from model systems or model proteins of known secondary structure. As a CD signal may contain contributions of the amino acid moiety, such as cystine and aromatic side-chain absorption, methods of assigning fractional indices for secondary structure types should not necessarily imply combination of reference spectra. In our analysis, therefore we used the convex constraint algorithm (CCA method) that is based on experimental curves only (Perczel et al. 1992).

Results

α-crystallin-detergent interaction: qualitative study by gel filtration

Gel filtration is a fast and easy way to study changes in the macromolecular structure of an oligomeric protein: indeed any change in the size and the form of α-crystallin as a result of interaction with a detergent will change the elution position of the protein.

For the nonionic detergents, used in this study, it became clear that their presence, even at a concentration above the CMC, hardly changes the structure of α-crystallin. The presence of ionic detergents has an important influence on the macromolecular structure of α-crystallin as can be concluded from the elution position of α-crystallin in the presence of DTAB and SDS (Fig. 1). As the K_d value of a particle is dependent on two parameters, namely the size and shape, and the elution profile further depends on the solvent properties (e.g. the presence of detergents), we have shifted to light scattering, boundary sedimentation and equilibrium sedimentation to study the influence of these detergents on the α-crystallin structure.

α-crystallin detergent interaction: quantitative study by light scattering

The detergent binding to an oligomeric protein will probably change its hydrodynamic structure. This change will be reflected in changes of its molar mass and hydrodynamic radius in the presence of the detergent. Light scattering is a suitable physical technique to monitor changes in the molar mass of oligomeric macromolecules.

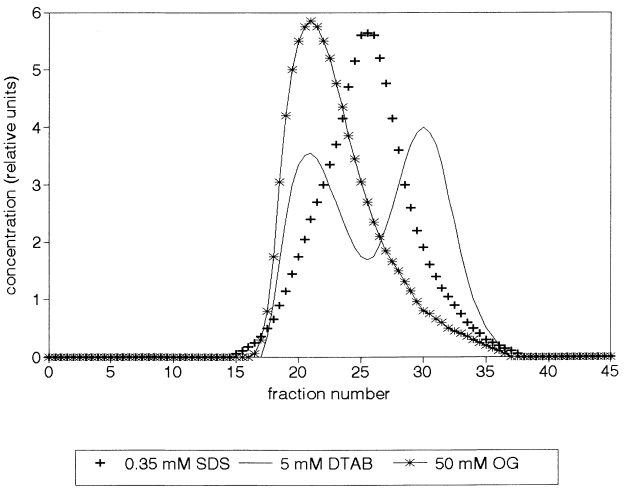


Fig. 1 Elution profile of α-crystallin in the presence of a neutral (*OG*), anionic (*SDS*) and cationic detergent (*DTAB*)

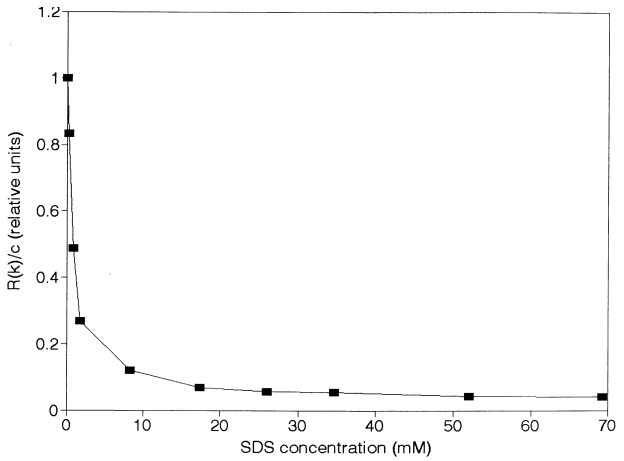


Fig. 2 Change of R(k)/c, which is proportional to the apparent relative molar mass of α-crystallin, in the presence of increasing concentrations of SDS

Figure 2 gives the results of light scattering measurements of α-crystallin in the presence of increasing concentrations of SDS. We see a dramatic decrease of the molar mass of the α-crystallin protein in the presence of SDS which suggests that α-crystallin is dissociating to smaller aggregates as a result of interaction with SDS molecules.

Equation (3) allows the calculation of the molar mass of a particle from the experimental light scattering parameter R(k), the constant K and the concentration c of the particles. When the ligand (SDS) binds to the protein, the complex formed between the protein and the ligand molecules, is the light scattering entity. So actually we have (Jones and Midgley 1984)

$$c_{app} = c\,(1 + \delta) \tag{6}$$

$$\left(\frac{\partial n}{\partial c}\right)_{app} = \left(\frac{\partial n}{\partial c}\right) + \Delta\left(\frac{\partial n}{\partial c}\right) \tag{7}$$

$$M_{app} = M\,(1 + \delta) \tag{8}$$

where

δ: mg detergent bound per mg protein

c_{app}: the concentration of the protein-detergent complex; this can be calculated from the concentration c of the protein and the detergent binding δ

$\Delta(\partial n/\partial c)$: the increase of the refractive index increment of the protein due to the binding of δ mg detergent per mg protein

M_{app}: the molar mass of the protein-detergent complex

It has been shown that the refractive index increment of a protein drastically changes on SDS binding; from data in literature (Jones and Midgley 1984) we can calculate

$$\Delta(\partial n/\partial c) = 0.100 \cdot \delta$$

So the proper use of light scattering to study protein-detergent complexes requires the knowledge of δ. This δ can be calculated if we know the binding mechanism, the binding characteristics and the total concentration of the protein and the detergent. This information is not available for the moment.

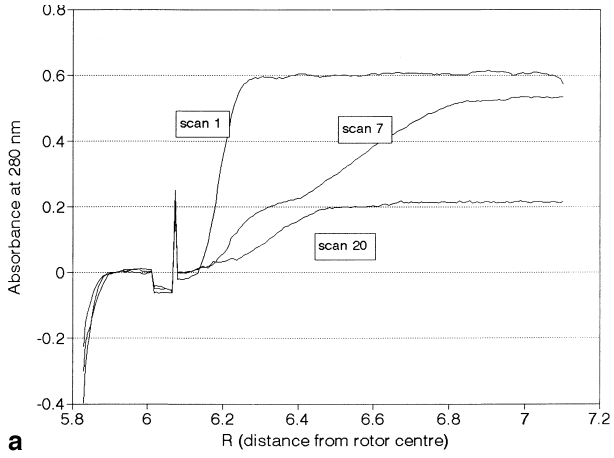
Our gel filtration data also suggest a striking difference between the way the two ionic detergents affect the quaternary structure of α-crystallin. SDS interaction results in a broad and continuous distribution of sizes, which is shifted to smaller sizes as the ratio SDS/protein increases. The DTAB interaction results in a two-state distribution: a class of proteins similar to the native one and then a class of small proteins. An increase of the ratio DTAB/protein increases the population of the smaller particles.

As light scattering, in our present setup, cannot inform us about the size distribution, we have shifted to the use of boundary sedimentation and equilibrium sedimentation, to get information on the distribution of sizes of protein-detergent complexes.

Boundary and equilibrium sedimentation

Boundary sedimentation studies confirm the differences in impact of the anionic and cationic detergent on the macromolecular structure of α-crystallin (Fig. 3a, b). In the presence of 5 mM DTAB, two discrete classes of sizes can clearly be seen (sedimenting with a S value of (18 ± 1)s and (2 ± 0.5)s respectively; in the presence of 0.45 mM SDS, the protein particles sediment as a broad population of particles with an average S value of (11.5 ± 0.5)s. The same difference of behaviour is observed by equilibrium sedimentation of α-crystallin in the presence of increasing concentrations of SDS and DTAB.

In the presence of increasing concentrations of SDS, the α-crystallin sample monotonously decreases in molar mass from 800,000 ($M(1-\nu\cdot\rho) = 330{,}000$) g/mol in the absence of detergents to a $M(1-\nu\cdot\rho)$ value of 6,500 g/mol at high concentration of SDS (see Fig. 4a; the logarithmic scale overestimates the broadness of the distribution of the low masses and underestimates the broadness of medium and high mass distributions).



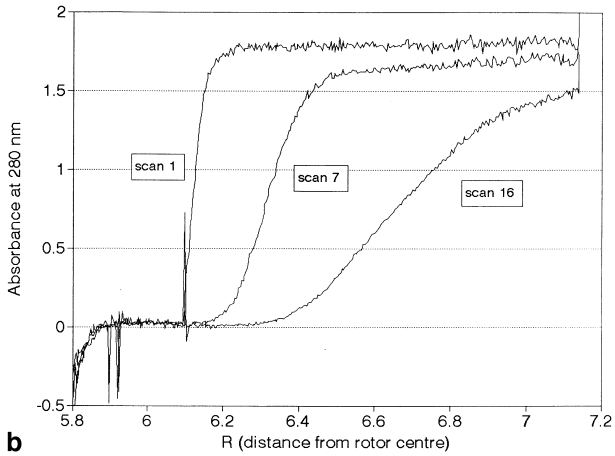
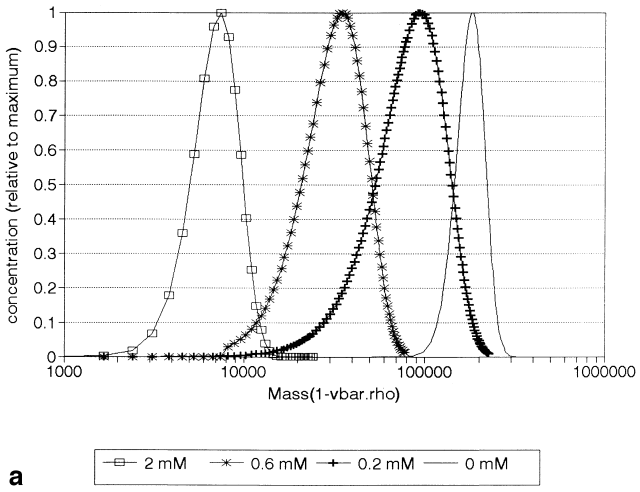


Fig. 3 **a** Boundary sedimentation of α-crystallin in the presence of 5 mM DTAB; rotor speed 35,000 rpm; scan at 666 sec (*scan 1*), 2436 sec (*scan 7*) and 6334 sec (*scan 20*). **b** Boundary sedimentation of α-crystallin in the presence of 0.45 mM SDS; rotor speed 25,000 rpm; scan at 1816 sec (*scan 1*), 5356 sec (*scan 7*) and 10757 sec (*scan 16*)



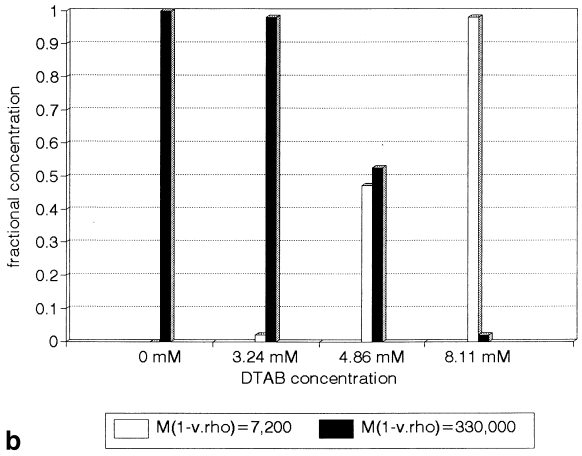


Fig. 4 **a** Molar mass distribution of α-crystallin solutions in the absence and presence of increasing concentrations of SDS; the mass distributions have been calculated using the XLASE program (Lechner and Mächtle 1991) and accepting a Poisson distribution. **b** Molar mass distribution of α-crystallin solutions in the absence and presence of increasing concentrations of DTAB; the mass distributions have been calculated using the Equilibrium/Velocity/Analysis Programs of Holladay (Kelly and Holladay 1990; Shire et al. 1991) and accepting a ideal solution of two components

The presence of DTAB causes a drastic reduction of the mass of α-crystallin in a narrow concentration range (at 1 mg/ml of protein in the concentration range 3.24 to 8.11 mM of DTAB). The solution also contains two discrete sets of particles: the native proteins with a $M(1-\nu\cdot\rho)$ value of 330,000 g/mol and a smaller unit with a $M(1-\nu\cdot\rho)$ value of 7,200 g/mol (Fig. 4b). The transition can be considered as an all or none effect: up to 3 mM DTAB, only the larger species are present; at 8 mM and higher, only the smaller units are present. Although the mechanism of interaction turns out to be different, both detergents reduce the oligomeric α-crystallin to a solution of particles with a similar $M_{w,app}(1-\nu\cdot\rho)$ value of (7,200 ± 700) g/mol for DTAB and (6,500 ± 600) g/mol for SDS and a M_w/M_n of 1.05.

In order to calculate the molar mass of the protein moiety, we have to know δ: g detergent bound per g protein: we have approximately

$$M_{app} = M_{prot}(1+\delta) \text{ and } \nu_{app} = (\nu_{prot} + \delta\cdot\nu_{deter})/(1+\delta).$$

Figure 5 gives the range of the molar mass of the protein moiety of the (protein · SDS) or (protein · DTAB) complex, with a $M(1-\nu\cdot\rho)$ value of 6,500 and 7,200 respectively, accepting binding ranges δ from 0 to 2 g detergent/g protein and ν values of 0.726 for the protein, 0.935 for DTAB and 0.814 for SDS respectively.

This figure shows us that for the (protein · SDS) complex, the protein moiety has a molar mass of about 20,000 g/mol if accepting a binding δ in the range 0.6 to 1 g detergent/g protein; this means that the SDS molecules

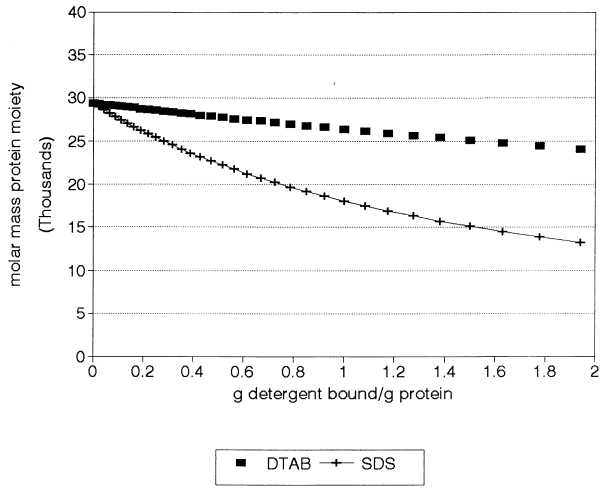


Fig. 5 Apparent molar mass of α-crystallin, which can be calculated from the experimental apparent $M(1-\nu\cdot\rho)$ values, as a function of δ:g detergent bound per g protein, in the presence of 8 mM DTAB and 2 mM SDS

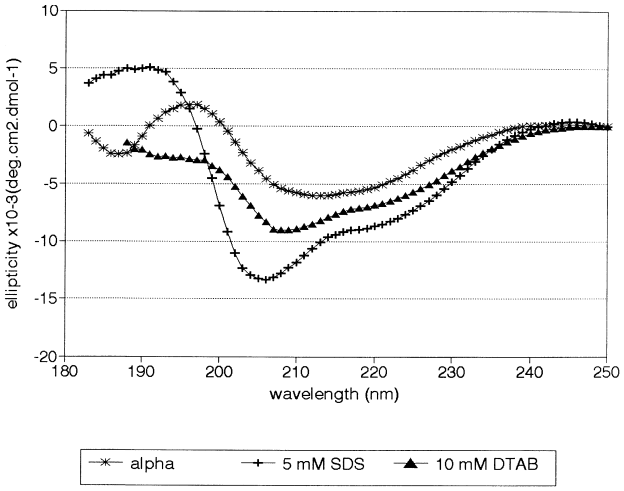


Fig. 6 CD spectra (185–250 nm) of calf α-crystallin at 25 °C, and in the presence of DTAB (10 mM) and SDS (5 mM)

dissociate the oligomeric α-crystallin to the single peptides. In the presence of DTAB and accepting detergent binding δ in the same range, the molar mass of the protein moiety is about 28,000 g/mol: this means that we probably now have a mixture of monomers (20,000 g/mol) and dimers (40,000) g/mol.

Circular dichroism measurements

The far-UV CD spectrum of native α-crystallin shows a single minimum just below 220 nm and a positive band with maximum around 200 nm (Fig. 6). These general features correspond very well with those published in literature (Siezen and Argos 1983; Horwitz 1993). The shape and intensity of this spectrum suggest that the native state of α-crystallin mainly has the β-pleated sheet structure as secondary structure motif and much less α-helical structure.

Table 1 Secondary structure analysis of the far-UV spectra of α-crystallin and in the presence of SDS and DTAB (percentage contribution)

Component	1	2	3	4	5
α-crystallin	24.2	10.5	29.3	11.1	24.8
α-crystallin + 5 mM SDS	10.2	18.1	6.3	18.6	46.7
α-crystallin + 10 mM DTAB	9.5	17.4	15.6	14.8	42.7

component 1: contribution of tryptophan side chains (Woody 1994); component 2: α-helix structure; component 3: β-sheet structure; component 4: α-helix structure and or unordered form; component 5: unordered form

Upon addition of SDS, in a first stage the minimum at 220 nm has deepened; on adding more SDS, this effect slows down and is overtaken by a prominant minimum appearing at 206 nm. The addition of DTAB has a similar effect on the CD spectrum of α-crystallin as SDS although there are small differences: the deepening at 220 nm is more pronounced and the minimum is now at about 209 nm.

In order to estimate the respective contributions of the various secondary structure elements, the spectra from Fig. 6 were submitted to the convex constraint algorithm (Perczel et al. 1992). A good fit of the experimental data was obtained by the addition of five basic spectra, which can be interpreted as:

- the contributions of the tryptophan side chains, which are related to the tertiary structure where these side chains are involved in (Woody 1994)
- the α-helix secondary structure,
- the β-sheet structure,
- others which are α-helix and or unordered structures,
- the unordered random coil structure

As a result of our analysis (see Table 1), we can conclude that in the presence of SDS, the β-sheet structure decreases from 29.3 to 6.3% and also the contribution of the tryptophan side chains is decreased: both effects are probably related to the disturbance of the peptide-peptide interaction in the oligomeric protein, due to the peptide-detergent interaction. The α-helix content increases from 10.5 to 18.1% and also the unordered random coil content increases. In the presence of DTAB, similar changes are noticed: now the β-sheet structure only decreases to 15.6% but the other components change similarly.

Discussion

Our studies on changes in secondary and quaternary structure of α-crystallin on binding single SDS or DTAB molecules, suggest a complicated interaction pattern. These results can be explained by and support some aspects of the micelle hypothesis (Augusteyn and Koretz 1987). This hypothesis is based on several observations: namely the variability in α-crystallin particle size, the apparent depen-

dence of this parameter on certain environmental factors (e.g. temperature), the absence of specific requirements for either α-crystallin isoform (containing either only αA or αB peptides) in aggregation; also the sharp division in the amino acid sequence between a strong hydrophobic region and a sharply hydrophilic one is in accordance with the micelle hypothesis.

Micelles are self-assembled aggregates of amphiphilic molecules oriented such that the non-polar regions of the molecules are segregated from the solvent. The micelles are stabilized by a combination of entropic effects in the hydrophobic core and interactions of the hydrophilic regions of the molecules with the polar solvent. There are no specific interactions between the molecules in the hydrophobic core. Although these characteristics have been described for small model compounds such as phospholipids and extended linear amphiphilic molecules, it must be possible that peptides with a similar distribution of polar and non-polar regions could behave in a similar manner. The amphiphilic peptides in a polar solvent have to arrange themselves so that the non-polar regions would be segregated from the solvent and that the polar region would be exposed to the solvent.

The importance of hydrophobic interactions in the formation of the α-crystallin oligomeric structure is based on experimental data and theoretical considerations resulting mainly from sequence studies and sequence comparisons. The contributions of hydrophobic interactions to the stability of oligomeric α-crystallin have been emphasised by solubility studies of cortical and nuclear low-molecular-mass α-crystallin (Coopman et al. 1984) and denaturation-renaturation studies of α-crystallin (Clauwaert et al. 1989); hydrophobic interactions are also critical in the interaction of α-crystallin with plasma membranes (Mulders et al. 1985; Ifeanyi and Takemoto 1990; Ifeanyi and Takemoto 1991) and crystallin-crystallin interaction (Liang and Li 1991).

The behaviour of α-crystallin under increased hydrostatic pressure and the effect of its concentration on the aqueous surface tension have also been explained in the framework of a micellar structure (Radlick and Koretz 1992; Carver et al. 1994).

Recombinant αA-peptides from bovine lens fibre cells, the αA1D peptide containing the amino acids 1 to 63 of this peptide and the αA2D peptide containing the amino acids 64 to 173, have been expressed in Escherichia Coli. The αA peptide and the C-terminal domain are expressed in water-soluble form: the αA peptide forms aggregates which are similar to the native α-crystallin. The C-terminal domain forms small aggregates (dimers to tetramers). The hydrophobic N terminal peptide is expressed in a water-insoluble form: after solubilization, denaturation and renaturation high molecular aggregates are formed: this confirms that the mainly hydrophobic interactions of the N-terminal peptide are responsible for the formation of the polymeric α-crystallin (Merck et al. 1992).

The homology and evolutionary relationship of α-crystallin to heat shock proteins has been concluded from sequence homology and from similarities in quaternary structure (Wistow 1985; Bloemendal and de Jong 1991), both classes share heterogeneity in quaternary structure and the evidence of an important contribution of hydrophobic interactions to the formation of the quaternary structure (de Jong et al. 1989). This relation has been nicknamed as the “the stress connection”.

The drastic changes in quaternary structure, as observed by gel filtration, light scattering, and sedimentation of α-crystallin in solution in the presence of SDS and DTAB molecules suggest that the amphiphilic detergent can substitute amphiphilic interactions between the peptides in the α-crystallin aggregate, resulting in smaller protein aggregates.

Likely binding sites on the αA and αB peptides can also be suggested. The formation of secondary α-helix structures is known to be induced by interaction with SDS at amphiphilic sites, having both a hydrophobic and a hydrophilic face (Parker and Song 1992). The use of the hydrophobic moment (Eisenberg et al. 1982) program reveals for the bovine αA peptide two regions, which are candidates for the formation of an amphiphilic α-helix, namely the sequences 21 to 37 and 110 to 118. The sequence 21 to 27, sometimes called the phenylalanine-rich sequence of the α-crystallin, is similar to equivalent sequences in the hamster and human hsp 27 and some other hsp proteins (Crabbe and Goode 1994). The sequence 110 to 118 is flanking the sequences 84 to 100, 114 to 121 and 129 to 145. These sequences are homologous for the HSP25 from mouse, the IbpA from E. coli and the bovine αA crystallin (Jakob and Buchner 1994).

For the bovine αB peptide, the sequences 22 to 30 and 114 to 122 are likely candidates for interaction with detergents for the same reason. This interaction can indeed increase the α-helix content from 10 to about 18%, as our CD measurements indicate.

Until now, no direct link between the secondary structure of the αA and αB peptides and the concomitant tertiary and quaternary structure has been made clear. It has not been possible to reduce the molar mass of the oligomeric α-crystallin to the molar mass of the constituent peptides without destroying the secondary structure. This suggests that the native secondary structure of αA and αB peptide is only stable in the oligomeric structure.

Our experiments indicate that a change in the secondary structure of the αA and or αB peptides, more specifically a formation of an α-helix structure, is allied to a reduction of the molar mass of the oligomeric structure. This change in secondary structure at room temperature is induced by the interaction with the amphiphilic detergent.

Our concomitant studies of the quaternary and secondary structure of α-crystallin in the presence of SDS and DTAB have given the first direct evidence of amphiphilic interactions between the peptides in this polymeric α-crystallin structure.

This finding can be important in view of the structure and function of α-crystallin as a chaperone. Indeed it has been suggested that this chaperone activity is related to a reorganization of hydrophobic surfaces (Raman and Rao 1994). The difference in interaction mechanism between

the anionic SDS and the cationic DTAB is consistent with results from mutation studies (Smulders et al. 1995), which indicated that charged residues in α-crystallin influence the efficiency of heat-protection capacity of α-crystallin.

Acknowledgements This research was supported by grants from the Fund for Joint Basic Research (FKFO) and the Fund for Medical Scientific Research (FGWO). This research was performed within the framework of the EU Concerted Action "The Role of membranes in lens Ageing and Cataract". We thank Dr. L. Holladay and Dr. M.D. Lechner for providing us their program of analysis equilibrium sedimentation profiles and assisting us in using it.

References

Ackers GK (1967) A new calibration procedure for gel filtration columns. J Biol Chem 242:3237–3238

Aerts A, Wang QH, Tatarkova S, Clauwaert J (1995) Physical-chemical characterization of the different individual cortical alfa-crystallin fractions from bovine lenses. Progr Colloid Polym Sci 99:94–100

Andries C, Backhovens H, Clauwaert J, De Block J, De Voeght F, Dhont C (1982) Physical-chemical studies on bovine eye lens proteins. I. Light-scattering and viscosity studies of low-molecular weight α-crystallin isolated from adult and embryonic bovine lenses. Exp Eye Res 34:239–255

Andries C, Guedens W, Clauwaert J, Geerts H (1983) Photon and fluorescence correlation spectroscopy and light scattering of eye-lens proteins at moderate concentrations. Biophys J 43:345–354

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

Bloemendal H, de Jong WW (1991) Lens proteins and their genes. In: Cohen WE, Moldave K (eds) Progress in nucleic acid research and molecular biology, vol 41. Academic Press, San Diego, pp 259–281

Carver JA, Aquilina JA, Cooper PG, Williams GA, Truscott RJW (1994) α-crystallin: molecular chaperone and protein surfactant. Biochim Biophys Acta 1204:195–206

Clauwaert J, Ellerton HD, Koretz JF, Thomson K, Augusteyn RC (1989) The effect of temperature on the renaturation of α-crystallin. Curr Eye Res 8:397–403

Coopman S, De Block J, Aerts T, Peeters W, Moens L, Clauwaert J (1983) Physicochemical studies on bovine eye lens proteins. II Comparative physical study of the low-molecular-weight α-crystallins from calf lens cortical and nuclear fiber cells. Exp Eye Res 38:463–476

Crabbe MJC, Goode D (1994) α-crystallin: chaperoning and aggregation. Biochem J 297:653–654

Eisenberg D, Weiss RM, Terwilliger TC (1984) The hydrophobic moment detects periodicity in protein hydrophobicity. Proc Natl Acad Sci USA 81:140–144

de Jong WW, Hendriks W, Mulders JWM, Bloemendal H (1989) Evolution of eye lens crystallins: the stress connection. TIBS 14:365–368

Horwitz J (1993) The function of alpha-crystallin. Invest Ophthalm Vis Sci 34:10–21

Ifeanyi F, Takemoto L (1990) Specificity of alpha crystallin binding to the lens membranes. Curr Eye Res 9:259–265

Ifeanyi F, Takemoto L (1991) Interaction of lens crystallins with lipid vesicles. Exp Eye Res 52:535–538

Jakob U, Buchner J (1994) Assisting spontaneity: the role of Hsp90 and small Hspss as molecular chaperones. TIBS 19:205–211

Jones MN, Midgley PJW (1984) Light-scattering from detergent-complexed biological macromolecules. Biochem J 219:875–881

Johnson WC (1990) Protein secondary structure and circular dichroism: a practical guide. Proteins Struc Funct Genet 7:205–214

Johnson ML, Correia JJ, Yphantis DA, Halvorson HR (1981) Analysis of data from the analytical ultracentrifuge by nonlinear least-squares technique. Biophys J 36:575–588

Kelly L, Holladay LA (1990) A comparative study of the unfolding thermodynamics of vertebrate metmyoglobins. Biochemistry 29:5062–5069

Lechner MD, Mächtle W (1991) A new procedure for the determination of the molar mass distribution of polymers in solution from sedimentation equilibrium. Prog Colloid Polymer Sci 86:62–69

Liang JN, Li X (1991) Interaction and aggregation of lens crystallins. Exp Eye Res 53:61–66

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

Moosavi-Movahedi AA, Housaindokht MR (1991) Unusual behaviour of the interaction of sodium n-dodecyl sulphate with calf thymus histone H2B in aqueous solution. Int J Bio Macromol 13:50–54

Mulders JWM, Stokkermans J, Leunissen JAM, Benedetti EL, Bloemendal H, de Jong WW (1985) Interaction of α-crystallin with lens plasma membrane. Affinity for MP26. Eur J Biochem 152:721–728

Mulders RHP, Merck KB, Aendekerk J, Horwitz J, Takemoto L, Slingsby C, Bloemendal H, de Jong WW (1995) The mutation Asp69-Ser affects the chaperone-like activity of αA-crystallin. Eur J Bioch 232:834–838

Parker W, Song P-S (1992) Protein structures in SDS micelle-protein complexes. Biophys J 61:1435–1439

Perczel A, Park K, Fasman GD (1992) Analysis of the circular dichroism spectrum of proteins using the convex constraint algorithm: a practical guide. Anal Biochem 203:83–93

Radlick JW, Koretz JF (1992) Biophysical characterization of α-crystallin aggregates: validation of the micelle hypothesis. Biochim Biophys Acta 1120:193–200

Raman B, Rao C (1994) Chaperone-like activity and quaternary structure of alpha-crystallin. J Biol Chem 269:27264–27268

Shire SJ, Holladay LA, Rinderknecht E (1991) Self-association of human and porcine relaxin as assessed by analytical ultracentrifugation and circular dichroism. Biochemistry 30:7703–7711

Siezen RJ, Bindels JG (1982) Stepwise dissociation/denaturation and reassociation/renaturation of bovine α-crystallin in urea and guanidine hydrochloride: sedimentation, fluorescence, near-ultra and far-ultraviolet circular dichroism studies. Exp Eye Res 34:969–983

Siezen RJ, Argos P (1983) Structural homology of lens crystallins III. Secondary structure estimation from circular dichroism and prediction from amino acid sequences. Biochim Biophys Acta 748:56–67

Spector A, Chiesa R, Sredy J, Garner W (1985) cAMP-Dependent phosphorylation of bovine lens α-crystallin. Proc Nat Acad Sci USA 82:4712–4716

Srinivasan AN, Nagineni ChN, Bhat SP (1992) αA-crystallin is expressed in non-ocular tissue. J Biol Chem 267:23337–23341

Stafford III WF (1992) Methods for obtaining sedimentation coefficient distributions. In: Harding SE, Rowe AJ, Horton JC (eds) Analytical ultracentrifugation in biochemistry and polymer science. The Royal Society of Chemistry, Cambridge, pp 359–393

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

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

van den Oetelaar PJM, Clauwaert J, van Laethem M, Hoenders HJ (1985) The influence of isolation conditions on the molecular weight of bovine α-crystallin. J Biol Chem 260:14030–14034

van Haeringen B, Eden D, van den Bogaerde MR, van Grondelle R, Bloemendal M (1992) α-crystallin exists in a non-spherical form. Eur J Biochem 210:211–216

van Haeringen B, Van den Bogaerde MR, Eden D, Van Grondelle R, Bloemendal M (1993) Further characterization of structural and electric properties of non-spherical α-crystallin. Eur J Biochem 217:143–150

Voorter CEM, de Haard-Hoekman WA, Roersma ES, Meyer HE, Bloemendal H, de Jong W (1989) The in vivo phosphorylation sites of bovine αB-crystallin. FEBS Lett 259:50–52

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

Wistow G (1985) Domain structure and evolution in α-crystallin and small heat shock proteins. FEBS Lett 181:1–16

Woody RW (1994) Contributions of tryptophan side chains to the far-ultraviolet circular dichroism of proteins. Eur J Biophys 23:253–262

Xia J-Z, Aerts T, Donceel K, Clauwaert J (1994) Light Scattering by bovine α-crystallin proteins in solution: hydrodynamic structure and interparticle interaction. Biophys J 66:861–872

Yphantis DA (1964) Equilibrium ultracentrifugation of dilute solutions. Biochemistry 3:297–317