Self-Similarity Properties of α-Crystallin Supramolecular Aggregates

Francesco Andreasi Bassi,* Giuseppe Arcovito,* Marco De Spirito,* Alvaro Mordente,‡ and Giuseppe E. Martorana‡

*Istituto di Fisica and ‡Istituto di Chimica Biologica, Università Cattolica del Sacro Cuore, 00168 Roma, Italy

ABSTRACT The supramolecular aggregation of α-crystallin, the major protein of the eye lens, was investigated by means of static and dynamic light scattering. The aggregation was induced by generating heat-modified α-crystallin forms and by stabilizing the clusters with calcium ions. The kinetic pattern of the aggregation and the structural features of the clusters can be described according to the reaction limited cluster-cluster aggregation theory previously adopted for the study of colloidal particles aggregation systems. Accordingly, the average mass and the hydrodynamic radius of α-crystallin supramolecular aggregates grow exponentially in time. The structure factor of the clusters is typical of fractal aggregates. A fractal dimension $d_f \sim 2.15$ was determined, indicating a low probability of sticking together of the primitive aggregating particles. As a consequence, the slow-forming clusters assemble a rather compact structure. The basic units forming the fractal aggregates were found to have a radius about twice (~17 nm) that of the native protein and 5.3 times its size, which is consistent with an intermediate molecular assembly corresponding to the already known high molecular weight forms of α-crystallin.

INTRODUCTION

It has already been shown that a limited degree of local short-range order of crystallin proteins is sufficient to account for eye lens transparency (Benedek, 1971) and that light is principally scattered by the largest and most abundant lens protein, α-crystallin. α-crystallin is a spherically shaped molecule whose structure has not yet been ascertained, and several controversial models have been proposed to explain the wide distribution of the protein molecular weights ranging from 0.28 to 10×10^6 Da (Bindels et al., 1979; Tardieu et al. 1986; Augusteyn and Koretz, 1987; Schurtenberger and Augusteyn, 1991). α-crystallin is highly soluble, but it also shows appreciable hydrophobicity and may self-aggregate by hydrophobic interactions (Liang and Li, 1991). Intermediates in the process in which protein aggregates become insoluble or at least large enough to cause opacification are probably represented by the high molecular weight α-crystallin aggregates (Siezen et al., 1979; Srivastava, 1988).

The increase in light scattering in old and cataractous lenses can be ascribed to alterations in protein-water interactions, protein-protein interactions, and lens proteins (Latina et al., 1987; Cooper et al., 1994) due to physicochemical changes of the lens intracellular medium (Delaye et al, 1982; Xia et al., 1994) and/or to age related posttranslational modifications of α-crystallin (Garland et al., 1986; Santini et al., 1992; Luthra and Balasubramanian, 1993; Miesbauer et al., 1994) that disrupt the liquid-like molecular order and promote the formation of large scattering particles (Jedziniak et al., 1978; Guptasarma et al., 1992).

Here we report static and dynamic light-scattering measurements of heat- and calcium-induced supramolecular aggregates of α-crystallin. The experimental data have been evaluated by following the procedures already used to study the aggregation of colloidal particles (Lin et al., 1989) and occasionally of biological systems (Feder et al., 1984; Horne, 1987; Rarity et al., 1989). Clusters formed in these types of aggregation are tenuous, chain-like objects quite different from ordinary bulk matter. It has been proved that their highly disordered structure exhibits scale invariance and that they can be well described as fractals (Weitz and Oliveria, 1984). The aim of this work is the investigation of the fractal properties of α-crystallin aggregates.

Theoretical remarks

The complete description of a cluster aggregation process requires the determination of the cluster structure, of the aggregation kinetics, and of the cluster-mass distribution function (Weitz et al., 1987).

The structure of a fractal aggregate is characterized by a self-similarity symmetry upon change of length scale stated by a power law relating the mass, M, and the cluster radius of gyration, R_G, as given by:

$$M \propto R_G^{d_f} \tag{1}$$

The fractal dimension d_f defines the cluster structure (Mandelbrot, 1982), describing the spatial distribution of the primitive aggregating particles in the cluster. Its value is typically less than the Euclidean dimension $d = 3$.

Because of the random nature of the process, the aggregation dynamic is described on a statistical basis monitoring the time evolution of the cluster-mass distribution function $N(M)$.

The aggregation rate is measured by the time growth of

Received for publication 1 June 1995 and in final form 8 September 1995.

Address reprint requests to Francesco Andreasi Bassi, Istituto di Fisica, Università Cattolica del Sacro Cuore, Largo F. Vito 1, 00168 Roma, Italy. Tel.: 06-3015-4265; Fax: 0039-6-3051343; E-mail: arcovito@axcasp.caspur.it.

0006-3495/95/12/2720/08 $2.00

the average cluster mass defined as:

$$\bar{M} = \frac{\sum_M N(M)M^2}{\sum_M N(M)M} \quad (2)$$

where $\Sigma_M N(M)M$ is the number of the primary aggregating particles normalizing the cluster-mass distribution.

It has been shown (Vicsek and Family, 1984) that cluster-mass distribution exhibits dynamic scaling, i.e., its shape does not change during aggregation. Indeed, it can be written as

$$N(M) = \bar{M}^{-2}\psi(M/\bar{M}) \quad (3)$$

where the time dependence of the cluster-mass distribution is contained only in $\bar{M}$, whereas the scaling function $\psi(x)$ and as a consequence the shape of the cluster-mass distribution are time independent.

Two regimens of aggregation kinetics have been observed and theoretically described (Lin et al., 1989), both determined by the short-range interparticle interactions that affect the sticking probability p upon the approach of two particles. Each of these two regimens is characterized by a different time evolution of the average cluster mass $\bar{M}$, by the shape of cluster-mass distribution function, and by the fractal dimension of the resulting clusters. It has been shown (Ball et al., 1987) that these behaviors are strictly interconnected and related to the different physical mechanisms governing the kinetics of the aggregation.

The first mechanism, called diffusion-limited cluster aggregation (DLCA), which results in the most rapid aggregation possible, occurs when two particles always stick together in a collision ($p = 1$) so that the aggregation rate is limited solely by the time between two collisions. In this regimen, clusters are essentially monodisperse, in that their mass distribution is bell shaped and well peaked around the average cluster mass, which grows linearly with time. Computer simulation and several different experimental techniques have shown a fractal dimension $d_f = 1.8$.

The second regimen, the reaction-limited cluster aggregation (RLCA), occurs when a large number of collisions is required before two particles can stick together ($p \ll 1$). Clusters formed in this slow aggregation regimen have a structure less tenuous than in the DLCA with a typical $d_f = 2.1$. The average cluster mass is an exponential function of the time, $\bar{M} \propto e^{At}$, where A is a constant dependent on the sticking probability and the time between collisions. Clusters formed in the RLCA regimen show an extremely wide mass polydispersity. A power law describes the cluster-mass distribution up to a cutoff mass M_c, after which it again decreases exponentially as given by:

$$N(M) \propto M^{-\tau}e^{-M/M_c}. \quad (4)$$

These two regimens must be considered universal in that their features are independent of the nature of interacting particles and then on the details of particle interactions.

MATERIALS AND METHODS

Static light scattering

Static light scattering (Kerker, 1969) measures the time-averaged intensity $I(q)$ scattered from a sample as a function of the scattering wave vector:

$$q = (4\pi n/\lambda)\sin(\theta/2) \quad (5)$$

where λ is the incident light wavelength, n is the refractive index of the solution, and θ is the scattering angle.

The measured scattering intensity from aggregating particles can be written as:

$$I(q) \propto \sum_M N(M)M^2S(qR_G) \quad (6)$$

where the contribution $M^2S(qR_G)$ from a single cluster of mass M and radius of gyration R_G is weighted over cluster-mass distribution $N(M)$. The structure factor S of the aggregates can be obtained analytically by Fourier transforming the pair-correlation function of fractal objects (Chen and Teixeira, 1986).

Its normalized form with $S(0) = 1$ is given by the equation:

$$S(qR_G) = \frac{\sin[(d_f - 1)arctg(qR_G)]}{(d_f - 1)qR_G(1 + q^2R_G^2)^{(d_f-1)/2}} \quad (7)$$

where the dependence on the product qR_G only follows the scale invariance of the cluster.

Two asymptotic behaviors of the structure factor, corresponding to different experimental conditions, can be found during aggregation:

$$S(qR_G) \propto \begin{cases} 1 & qR_G << 1 \\ (qR_G)^{-d_f} & qR_G >> 1 \end{cases} \quad (8)$$

When clusters can be considered like point sources, i.e., $qR_G \ll 1$, static light-scattering intensity measurements can be used to determine the time evolution of the average cluster mass: $I(t) \propto \Sigma_M N(M)M^2 = \bar{M}$. When clusters are large enough that most of them have $qR_G \gg 1$, the fractal dimension d_f can be directly determined by measuring scattered intensity versus wave vector q: $I(q) \propto q^{-d_f}$.

In the cross-over region $qR_G \sim 1$, the full expression (7) must be used.

Dynamic light scattering

Dynamic light scattering (Berne and Pecora, 1976) measures the time autocorrelation function of the scattering intensity $I(t)$. The normalized autocorrelation function is defined as:

$$G_2(\tau) = \langle I(0)I(\tau)\rangle/\langle I\rangle^2 \quad (9)$$

where t is the delay time and the angular brackets indicate the ensemble average.

The time dependence of the scattered intensity results from local density fluctuations as a consequence of the diffusive motion of the clusters. The autocorrelation function of these density fluctuations $g_1(\tau)$ can be derived from G_2 using the Siegert relation:

$$G_2(\tau) = 1 + Bg_1(\tau)^2 \quad (10)$$

where B is an instrumental constant.

For monodisperse point particles, the density autocorrelation function decays exponentially in time as $g_1(\tau) = e^{-\Gamma\tau}$, where the decay rate Γ depends on the particle translational diffusion coefficient according to $\Gamma = D\,q^2$.

In the case of aggregating particles, deviations from the monoexponential decay are observed because of cluster polydispersity and rotational diffusion effects.

In this condition, the derivative of g_1 for $t \to 0$ measures the average decay rate of the clusters:

$$\bar{\Gamma} = \frac{\partial ln\, g_1(t)}{\partial t}\Big|_{t=0} \tag{11}$$

To determine $\bar{\Gamma}$ experimentally, we fitted the logarithm of the measured autocorrelation function g_1 to a third-order polynomial, according to the cumulant expansion (Koppel, 1972):

$$ln\, g_1(t) = -\Gamma_1 t + \frac{1}{2!}\Gamma_2 t^2 - \frac{1}{3!}\Gamma_3 t^3 \tag{12}$$

where we assumed $\bar{\Gamma} = \Gamma_1$.

When the cluster is larger than q^{-1}, the different parts of its highly asymmetric and anisotropic structure are seen as individual scatterers, so that changes of cluster orientation, resulting from rotational diffusion, contribute significantly to the fluctuations of the scattered intensity (Lindsay et al., 1988). In this condition, the increased decay rate due to the contribution of rotational diffusion can be accounted for by means of a cluster-effective diffusion coefficient $D_{eff} = \Gamma/q^2$.

In aggregating systems, because of cluster-mass polydispersity, what we actually measure is an average effective diffusion coefficient that can be expressed as:

$$\bar{D}_{eff} = \frac{\bar{\Gamma}}{q^2} = \frac{\sum N(M)M^2S(qR_G)D_{eff}}{\sum N(M)M^2S(qR_G)} \tag{13}$$

Experimental procedure

Light scattering. Static and dynamic light-scattering measurements were performed concurrently during α-crystallin aggregation by using a computer-interfaced scattering system ALV-125 (ALV GmbH, Langen, Germany).

A vertically polarized monochromatic light source at 632.8 nm produced by an NEC He-Ne 50 mW laser was used. The sample was contained in a cylindrical quartz cuvette (1-cm diameter) enclosed in a vat filled with toluene as optical matching fluid. Sample temperature was controlled within ± 0.01°C by means of a Julabo HC Thermostat and measured with a Pt100 thermometer. Photons scattered by the sample were revealed by a single photon photomultiplier mounted on the rotating arm of the goniometer. The photopulses were sent to a 256-channel digital autocorrelator (ALV-5000) that performed a hardware autocorrelation function of the photopulses with a logarithmic spacing of delay times starting from 0.2 μs. Counts per second were used to measure the scattered intensity during the aggregation.

Data were collected from several scattering angles (usually eight) ranging from 20° to 150°, corresponding to wave vectors $0.46 \le q \le 2.5 \times 10^5$ cm^{-1}. Because the measurements were performed during the aggregation process, data are a function of both scattering vector q and aggregation time t. The slow rate of the α-crystallin aggregation and the high values of scattered intensity usually allowed an average collecting time of 30 s, sufficient to obtain a good measure of the intensity autocorrelation function before the system could change significantly.

Preparation and aggregation of α-crystallin. α-crystallin from bovine eye lens was prepared according to Santini et al. (1992). The α-crystallin fractions suspended in 10 mM Tris-HCl buffer, pH 7.4, were thoroughly mixed and pooled together. The purified protein was divided into aliquots and kept in the same buffer at −20°C until used. Just before the experiment, the samples were thawed and centrifuged at 50,000 × g for 30 min at 4°C, and the supramolecular aggregates already formed were discarded. The supernatant was filtered through a 3-mm Millipore low-retention filter (0.2-μm pore diameter) directly into the measuring cuvette.

Protein concentration was determined by using an absorption coefficient of $A_{1cm}^{0.1\%}$=0.81 at 280 nm (Delaye and Gromiec, 1983).

Aggregation was induced by increasing temperature above 45°C and by the addition of sufficient amounts of calcium ions because heating provokes the generation of particularly reactive isoforms of α-crystallin (Walsh et al., 1991), and calcium ions stabilize the aggregates while they are forming and allow their continuous growth (Jedziniak et al., 1972).

Aggregation of α-crystallin was performed at different protein and calcium concentrations and temperatures to find a suitable measuring time (data not shown).

The aggregation of α-crystallin (1.6 mg/ml) was monitored at 55°C for ~5 h after the addition of 16 mM $CaCl_2$.

RESULTS AND DISCUSSION

Static scattering intensity and intensity autocorrelation function were measured concurrently during the aggregation process.

In the first phase, lasting ~0.5 h, a fast enhancement of scattering intensity is observed (Fig. 1). This could be ascribed to the initial conversion of the protein from the native to the heat- and calcium-induced conformers and to the consequent fast binding to form high molecular weight species. Later on, the scattering intensity shows an exponential increase, with evident deviations from this pattern only at the highest values of the wave vector and particularly toward the end of the measurement. This trend at each q was used to interpolate the data in order to obtain sets of concurrent intensity values, as shown in Fig. 2.

In the time course, the curves assume the shape of the cluster structure factor, isotropic at small q and approaching a power law at large q (Lin et al., 1990), as expected from fractal aggregates (Eq. 7). However, the scattering intensity

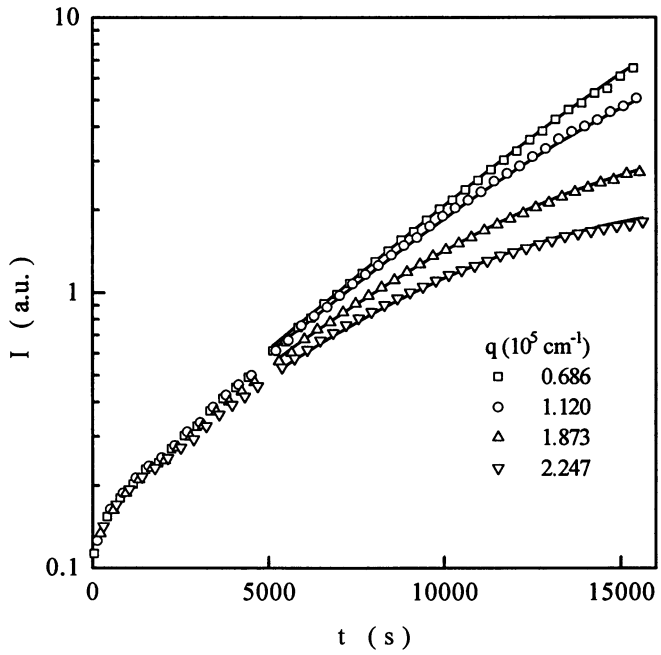

FIGURE 1 Static light scattering intensity I (in arbitrary units) versus aggregation time t of 1.6 mg/ml α-crystallin in 10 mM TRIS-HCl buffer, pH 7.4, with 16 mM $CaCl_2$ at 55°C. For the sake of clarity, data at only four q values are shown. Solid lines represent the fit of the data, as explained in the text.

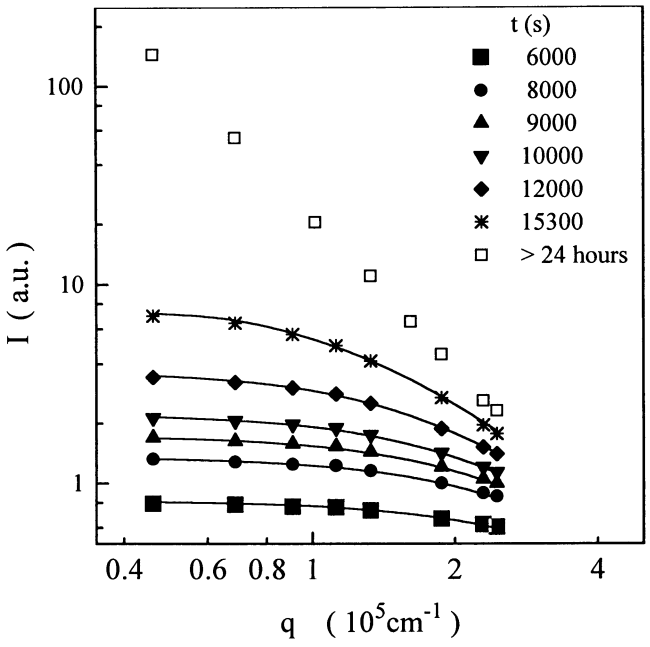

FIGURE 2 Static light scattering intensity I versus wave vector q at different aggregation times t. Concurrent values at each q were obtained by interpolating from the data plotted in Fig. 1. Solid lines represent the dependence on q of the function used to fit the experimental data. Open squares represent the data obtained after >24 h from the beginning of the aggregation, with the sample mixed to resuspend the sedimented clusters.

curves as a function of q in the crossover region $\bar{R}_G q \sim 1$ are more rounded off than was predicted for the structure factor of the individual clusters. This could be ascribed to cluster-mass polydispersity, which smooths the total scattered intensity (Eq. 6), as suggested by Lin et al. (1990).

The data at aggregation time long enough to reach the fractal region were not used because of interference from sedimentation effects. When the aggregation process is studied over a long period of time, a wide distribution of cluster sizes is produced and the clusters will sediment differentially, depending on size (Lin et al., 1990). This effect substantially changes the cluster-mass distribution along the height of the sample, which in turn modifies the aggregation kinetics. Mixing or tilting the sample can determine restructuring of the delicate framework of the clusters (Carpineti and Giglio, 1993). Despite this, we report a set of scattering intensity values versus q (see Fig. 2, open symbols) obtained after more than 24 h from the beginning of the aggregation. The measurements were taken after resuspension and mixing of the aggregates because the sample showed remarkable sedimentation. In this case a time sufficient to reach the fractal region ($qR_g \gg 1$) was allowed, and the scattering intensity follows a power-law dependence from q (Eq. 8 and following discussion), as predicted for fractal aggregates of very large size. Such behavior supports our cluster-cluster aggregation model. However, these data can not be used for quantitative analysis because they are strongly influenced by sedimentation and restructuring effects, which cannot be estimated accurately. Therefore, mechanical mixing of the sample during aggregation was not performed, and only unspoiled data from the first few hours of the aggregation were taken into account.

In this period, the weak dependency of the intensity on q at low angle indicates that most of the clusters in solution have a size such as $qR_G < 1$. In this condition, scattering intensity is proportional to the average mass of the clusters, and its exponential growth is typical of an RLCA process.

The experimental data were henceforth fitted according to this model using Eq. 6. The mass term in this equation was considered as the number of primitive aggregating units forming a cluster. With this assumption, the experimental scattered intensity is described by

$$I(q) = I_0 \frac{\sum_M N(M) M^2 S(qR_G)}{\sum_M N(M) M} \qquad (14)$$

where the normalization term $\Sigma_M N(M)M$ accounts for the conservation of the protein total mass, and the zero time intensity I_0 must be considered only a best fit parameter, which may be different from the experimental value.

For the cluster-mass distribution, a power-law form with an exponential cutoff given by Eq. 4 has been used. We adopted a value of $\tau = 1.5$, established for colloidal RLCA aggregates (Lin et al., 1989; Broide and Cohen, 1990). With this exponent, the cutoff mass becomes such that $\bar{M} \approx 0.51\ M_c$. The growth of the cutoff mass M_c as a function of time t is described by the equation $M_c = exp(At)$, where the aggregation rate constant A is a best fit parameter. The cluster structure factor $S(qR_G)$ was determined using the full form of Eq. 7. The cluster gyration radius R_G is related to the mass by $R_G = R_{G0} M^{1/d_f}$ where R_{G0} is a fitting parameter representing the dimension of the basic aggregating unit. Total scattering intensity $I(q)$ was obtained by summing the contributions from the clusters up to a cluster mass equal to 10-fold the current value of the cutoff mass when further contributions to the scattering intensity become negligible.

The fit to Eq. 14 is shown by the solid lines in Figs. 1 and 2 and is in excellent agreement with the data. The fitting parameter for the aggregation rate A is $(2.72 \pm 0.05)\ 10^{-4}\ s^{-1}$, and the value we determined for the fractal dimension $d_f = 2.18 \pm 0.04$ is consistent with that expected for clusters undergoing a reaction-limited aggregation.

At time 0, a gyration radius R_{G0} of 17.8 ± 0.5 nm is about twice the value reported for the α-crystallin molecule (Xia et al., 1994). At the same time, the fitted value for scattering intensity I_0 is 5.3 times that measured with the native protein at the same concentration.

It should be recalled at this point that static light scattering cannot provide a determination of the functional form of cluster-mass distribution (Lin et al., 1989). This negative feature is further evidenced in the first stages of the aggregation process because of the limited spread of the cluster-mass distribution.

Therefore, to verify the results obtained in these conditions, we report in Table 1 the values of the best fit param-

TABLE 1 Results of fits to Eq. 14 of static light scattering data with different cluster mass distribution functions

	a	b	c	b	b
$\sigma^* \times 10^2$	4.75	4.14	4.00	4.11	6.04
τ		1	1.30 ± 0.12	1.5	2
d_f	2.23 ± 0.03	2.07 ± 0.04	2.13 ± 0.06	2.18 ± 0.05	2.36 ± 0.08
R_{G0} (nm)	23.5 ± 0.03	13.9 ± 0.4	15.9 ± 1.0	17.8 ± 0.5	24.9 ± 0.8
$\Gamma \times 10^4$ (s^{-1})	2.72 ± 0.05	2.74 ± 0.05	2.73 ± 0.05	2.72 ± 0.05	2.69 ± 0.05
I_0 (arbitrary units)	1.99 ± 0.02	1.93 ± 0.02	1.96 ± 0.02	1.99 ± 0.02	2.12 ± 0.03

a, Monodisperse cluster-mass distribution; b, polydisperse cluster-mass distribution according to Eq. 4 at a given τ value; c, polydisperse cluster-mass distribution according to Eq. 4 with τ as a free-fit parameter.
*Standard error of estimate.

eters by using Eq. 14 with different mass distribution $N(M)$ forms. It can be inferred from Table 1 that the values of Γ, I_0, and d_f do not change significantly even when considering the monodisperse mass distribution as in a DLCA process, which is clearly not our case. The robustness of d_f demonstrates straightforwardly the sensitivity of static light scattering in determining the internal structure of the clusters. Only the value of R_{G0} varies significantly because it is strongly dependent on the systematic errors due to the fitting function. Moreover, although the τ coefficient obtained from the best fit is 1.3, we have adopted the value of 1.5 because (as said above) it is an established value verified with more sensitive techniques for this type of aggregation process (Lin et al., 1989; Broide and Cohen, 1990). Furthermore, it is worth noting that with $\tau = 1.5$ a better resolution of R_{G0} could be obtained.

Dynamic light scattering provides information both independent of and related to static light scattering about the aggregation kinetics, the cluster dimension, and their evolution in time as the aggregation proceeds.

Although the effects of both rotational diffusion and cluster-mass distribution contribute to determine the intensity autocorrelation function, only small deviations from a strictly exponential decay could be observed until the end of the measurements, as confirmed by the low value found for the variance Γ_2/Γ_1^2 (< 0.07), as well as predicted by calculations when $qR_G \gg 1$ (Lin et al., 1990).

At each q, the average effective diffusion coefficient $\bar{D}_{eff}$, obtained from the first cumulant $\bar{D}_{eff} = \Gamma_1/q^2$, decreases exponentially in time (Fig. 3). This trend was used to interpolate the data in order to obtain sets of concurrent values for each q (Fig. 4). As can be seen, in the first phase of α-crystallin aggregation the clusters can be considered point scatterers, i.e., $\bar{D}_{eff}$ is independent of q. Later on, as soon as cluster dimension increases, $\bar{D}_{eff}$ becomes dependent on q, mostly because of the cluster-mass distribution effect. The rotational diffusion contribution, albeit present, does not significantly affect $\bar{D}_{eff}$ in the region $qR_G < 1$ and was thus neglected. However, this approximation is quite good, inasmuch as the aggregates formed by RLCA are so highly polydisperse that the shape of the intensity autocorrelation function is dominated by the effects of the cluster-mass distribution (Lin et al., 1990). Under our conditions, therefore, a linear extrapolation of $\bar{D}_{eff}$ at $q = 0$ could be performed to obtain the average translational diffusion coefficient $\bar{D}$. The average hydrodynamic radius of the clusters $\bar{R}_h$ obtained from the Stokes-Einstein relation displays an exponential behavior (Fig. 5) from the very beginning of the aggregation; this kind of time dependence is again consistent with an RLCA process, as already shown by static scattering measurements. The fit of the experimental data to the equation $\bar{R}_h = \bar{R}_{h0} exp(k\,t)$ gave a value for the rate constant $k = (1.34 \pm 0.02)\ 10^{-4}\ s^{-1}$. The $\bar{R}_{h0}$ value is equal to 16.7 ± 0.4 nm, obtained from the fit, and is consistent with the value of the gyration radius at time 0. Both values are indicative of the dimension of the basic aggregating units, which constitute the fractal clusters and strongly support the view of the binding of two or more α-crystallin molecules (Liang and Li, 1991), intermediate in the formation of truly high molecular weight α-crystallin (Kramps et al., 1975).

Our results, namely the values of the radii (~17 nm) and the ratio between scattering intensities (5.3), suggest a struc-

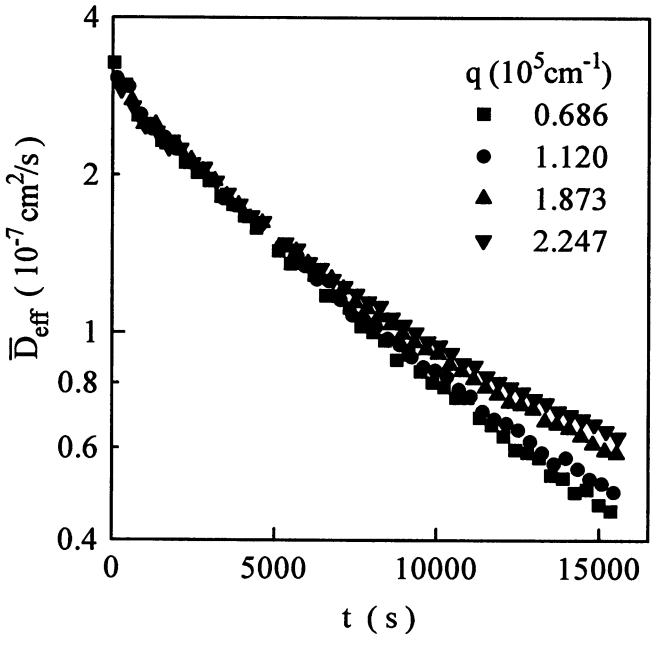

FIGURE 3 Average effective diffusion coefficient $\bar{D}_{eff}$ versus aggregation time t (data at only four q values). Experimental conditions as in Fig. 1.

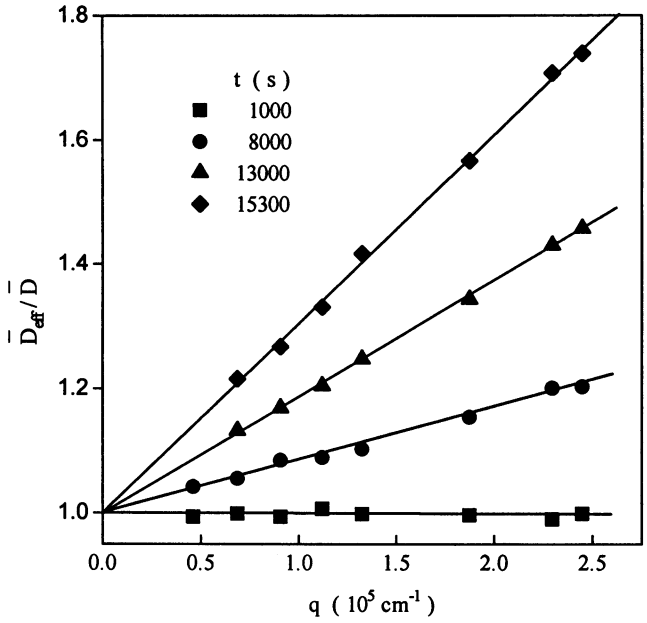

FIGURE 4 The ratio of average effective diffusion coefficient over the average diffusion coefficient $\bar{D}_{eff}/\bar{D}$ versus q at the aggregation times t indicated. The concurrent values of $\bar{D}_{eff}$ at each q were obtained by interpolation from the data in Fig. 3. The $\bar{D}_{eff}$ values were obtained at q = 0 from the linear best fit of $\bar{D}_{eff}$ (*solid lines*).

ture of increased size that can accommodate six native α-crystallin molecules or about 12 heat-induced forms (Walsh et al., 1991), with whatever composition and arrangement of subunits they may have (Wistow, 1993). We make no inference about shape and composition of the basic aggregating units, but our data compare well with those reported by Schurtenberger and Augusteyn (1991), who also measured by light scattering the dimensions of the α-crystallin aggregates of intermediate size obtained after gel filtration and suggested a semiflexible ("wormlike") chain of ~1.5–4 particles, depending on the particle molecular size.

Our experimental data indicate that these initial aggregating units are the smallest supramolecular aggregates of α-crystallin that will inevitably be driven together simply because the system becomes thermodynamically more stable, perpetuating the aggregation until very large clusters are formed (Liang and Li, 1991).

The scattering intensity and the average effective diffusion coefficient data can be used to obtain the fractal dimension d_f of the cluster. From Eqs. 2 and 6 at $q = 0$ the following relation must hold:

$$I \propto D_{eff}^{-d_f}. \tag{15}$$

We remark that even at very low q values, but different from zero, d_f obtained from Eq. 15 is always underestimated (Pusey and Rarity, 1987).

In Fig. 6 the scattering intensity is plotted versus the reciprocal of $\bar{D}_{eff}$. The linear tracts of the curves fit very well a power law from which an approximate value of the fractal dimension can be calculated. As can be seen from Fig. 7, d_f values are always underestimated compared with the true fractal dimension. However, because the dependence on q is weak, a linear extrapolation (the solid line in Fig. 7) is sufficient to assess robustly the parameter d_f = 2.13 ± 0.05, consistent with that previously found by static

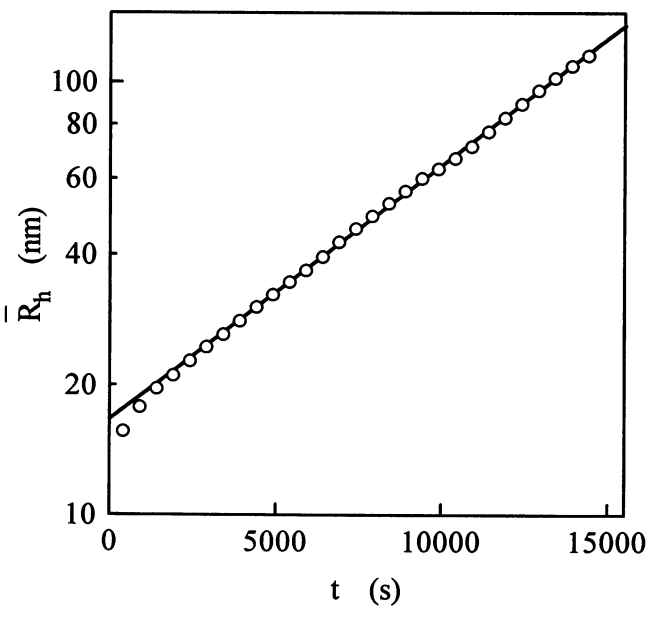

FIGURE 5 Average cluster hydrodynamic radius $\bar{R}_h$ versus α-crystallin aggregation time t calculated from the Stokes-Einstein relation using the value of $\bar{D}$ obtained as shown in Fig. 4. The solid line represents the time exponential fit of the experimental data.

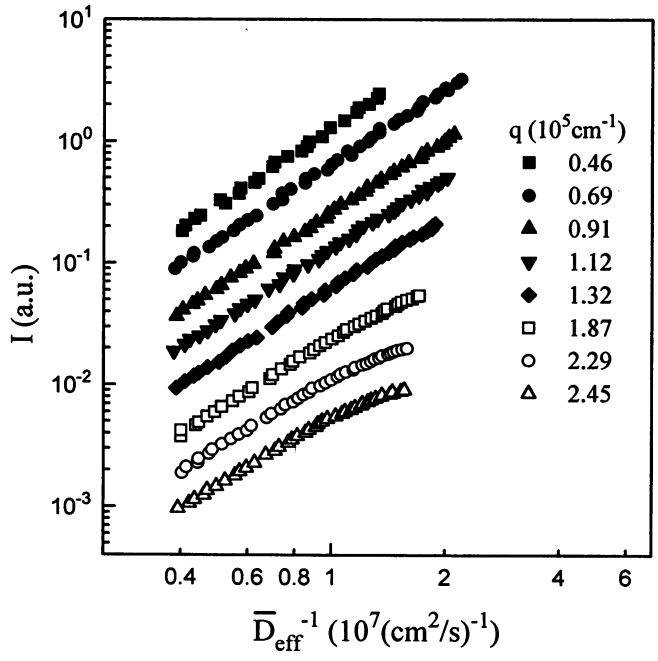

FIGURE 6 Static light scattering intensity I versus the concurrent values of $\bar{D}_{eff}^{-1}$ at each q. Intensity data at each q were multiplied by a different arbitrary factor for clarity.

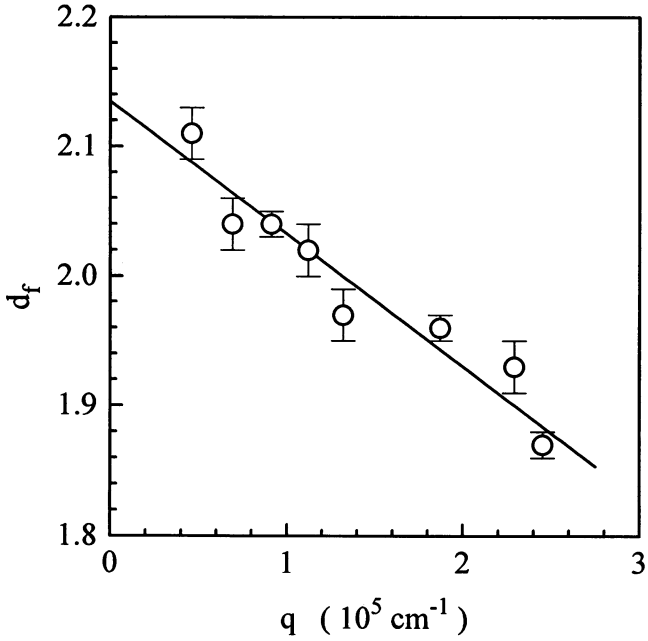

FIGURE 7 Fractal dimension d_f values as a function of q obtained from the fit of the linear tract of the curves shown in Fig. 6. The true fractal dimension of α-crystallin clusters was found by extrapolating at $q = 0$.

light scattering. This value, typical of a reaction limited process, confirms that α-crystallin, as in physiological conditions, maintains a weak propensity to form supramolecular aggregates also under stressed conditions. The aggregation of α-crystallin is, therefore, a slow process, needing a high number of collisions before binding takes place and giving time to assemble a rather compact cluster less thread-like than that formed in a diffusion-limited regimen in which the particles stick as soon as they collide.

Concluding remarks

α-crystallin is the most abundant lens protein of the mammalian eye, and its supramolecular aggregates are the main scattering elements that are strongly involved in the process of cataractogenesis.

We suggest that supramolecular aggregation of α-crystallin could be described as a cluster-cluster aggregation like that of colloidal particles.

Our data support the view that heat- and calcium-induced aggregation of α-crystallin follows a reaction-limited regimen. After the aggregation of the modified protein has occurred and the first clusters or basic aggregating units are formed with a radius of ~17 nm, corresponding to the so-called high molecular weight α-crystallins (Kramps et al., 1975; Siezen et al., 1979), the clusters themselves continue to diffuse, collide, and aggregate. As the aggregation progresses, clusters with different masses are formed and stick to one another. Because it is a naturally undesirable event, the probability of α-crystallin clusters sticking together is low and numerous collisions are required, resulting in a slow rate of aggregation. However, because the sticking probability is proportional both to p and to the number of available bonding sites, clusters with larger mass and more potential bonding sites grow faster than do the smaller ones. Therefore, although the initial aggregation rate is slow because all the clusters are small, the rate increases as the cluster size grows. In fact, the average cluster size of aggregating α-crystallin increases exponentially in time. The growth kinetics will in turn influence the structure of the cluster formation. The fractal dimension ~2.15 indicates that α-crystallin supramolecular aggregates sample all possible mutual configurations before they stick together. Thus, the smaller clusters have chances to interpenetrate the larger ones. Moreover, the polydispersity of the cluster-mass distribution, typical of an RLCA regimen, results in many collisions that involve clusters of different masses or at different stages of assembly. Both effects lead to a less tenuous cluster structure, with a resultant increase in the fractal dimension compared with that formed in a DLCA regimen.

It could be of great interest to search for fractal properties in the structural and kinetic aspects of α-crystallin supramolecular aggregation occurring in vivo under pathophysiological conditions. In this view, some of the electron microscopy features of aggregated cardiac α-crystallin after ischemic insult (Chiesi et al., 1990) and of the high molecular weight α-crystallin aggregates of the lens, previously considered artifactual (Siezen et al., 1979), are very suggestive of products derived from a fractal aggregation process.

We are grateful to Dr. Stefano A. Santini for his kind collaboration in α-crystallin purification and to Mr. Davide Bonvissuto for his skillful technical assistance. This work was supported by grants from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (M.U.R.S.T.) and the Consiglio Nazionale delle Ricerche (C.N.R.).

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