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A Dynamic Quaternary Structure of Bovine α-Crystallin As Indicated from Intermolecular Exchange of Subunits†

Piet J. M. van den Oetelaar,‡,§ Petri F. H. M. van Someren,‡ John A. Thomson,∥ Roland J. Siezen,∥,⊥ and Herman J. Hoenders*,‡

Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands, and Department of Physics, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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ABSTRACT: The structural bovine eye lens protein α-crystallin was dissociated in 7 M urea and its four subunits, A_1, A_2, B_1, and B_2, were separated by means of ion-exchange chromatography. Homopolymeric reaggregates of these subunits were prepared by removal of the denaturant via dialysis. It was found that subunits were exchanged upon incubation of mixtures of two homopolymers under native conditions. New hybrid species were formed within 24 h as demonstrated by isoelectric focusing. Moreover, native α-crystallin molecules also exchanged subunits when incubated with homopolymeric aggregates of B_2 subunits. Subunit exchange between native α-crystallin molecules is postulated, and a "dynamic quaternary structure" is presented that allows the polydisperse protein to adapt to changes in cytoplasmic conditions upon aging of the lens tissue.

Calf lens α-crystallin is a multisubunit protein composed of about 40 A- and B-type 20 000-Da subunits that occur in an average ratio of about 3:1 (Siezen et al., 1978). The primary gene products A_2 and B_2 are nearly 60% identical in primary structure (Van der Ouderaa et al., 1973, 1974), and hence they are likely to have closely similar secondary and tertiary structures. The A_1 and B_1 subunits are formed from A_2 and B_2, respectively, by a phosphorylation step (Spector et al., 1985; Voorter et al., 1986; Chiesa et al., 1987a-c). Many studies have dealt with the quaternary assembly of α-crystallin. A model for the architecture of the polydisperse molecules has been proposed with 14 ± 2, 13 ± 3, and 15 ± 2 subunits in three concentric layers (Bindels et al., 1979; Siezen et al., 1980). However, in the past few years controversy has arisen about the molecular weight of native α-crystallin. Thomson and Augusteyn (1983, 1984) described an α-crystallin of 320 000 Da, isolated at 37 °C. Hydrodynamic studies and symmetry considerations led to dodecameric models of α-crystallin, either as a protein with an intermediate tetrahedral shell organization of its subunits or as a micelle-type aggregate (Thomson, 1985; Augusteyn & Koretz, 1987). On the other hand, others presented further evidence in favor of a molecular mass of about 800 000 Da (Van den Oetelaar et al., 1985; Tardieu et al., 1986). The latter authors presented an extended version of the three-layer model, characterized by tetrahedral symmetry and 12, 24, and 24 sites occupied in the first, second, and third layer, respectively.

The subunits of α-crystallin are very prone to form aggregates. In fact, single subunits only exist in concentrated solutions of denaturing agents, and they aggregate upon dilution of these agents or after their removal by dialysis. The ratio of the subunits is not critical in order to form reaggregates. Not only dissociated native α-crystallin (Bloemendal et al., 1962; Li & Spector, 1973; Siezen & Bindels, 1982; De Block et al., 1986; Tardieu et al., 1986) but also isolated A and B subunits mixed in various ratios could be reassociated (Van Kamp et al., 1974; Bindels, 1982; Thomson & Augusteyn, 1989). Moreover, it was possible to form homopolymers by reassociation of purified subunits (Li & Spector, 1972, 1973, 1974; Bindels, 1982; Thomson, 1985; Thomson & Augusteyn, 1989). Reaggregated α-crystallins and these homopolymers are smaller than the native α-crystallin and generally do not exceed about 20-22 subunits (Bloemendal et al., 1975; Siezen et al., 1978; Siezen & Berger, 1978; Thomson & Augusteyn,

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* Address correspondence to this author at the Department of Biochemistry, University of Nijmegen, Kapittelweg 46, 6525 EP Nijmegen, The Netherlands.
‡ University of Nijmegen.
§ Present address: Scientific Development Group, Organon International, RP2203, P.O. Box 20, 5340 BH Oss, The Netherlands.
∥ Massachusetts Institute of Technology.
⊥ Present address: Division of Biophysical Chemistry, Netherlands Institute of Dairy Research, P.O. Box 20, 6710 BA Ede, The Netherlands.

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1989). It was concluded that the αA_2 and the αB_2 subunits are structurally equivalent and occupy equivalent sites in the α-crystallin aggregate.

In this paper we show that subunits are exchanged between homopolymeric molecules in the absence of denaturing agents. Moreover, subunit exchange is also demonstrated between homopolymers and native α-crystallin molecules.

Materials and Methods

Isolation of α-Crystallin. Lenses of 6-month-old calves were gently stirred at 5 °C for 15 min in a buffer containing 20 mM sodium phosphate, 100 mM sodium sulfate, and 1 mM EDTA, pH 6.9. In this way only the outer cortex dissolved. Insoluble material was pelleted by centrifugation at 10000*g* for 30 min. The water-soluble proteins were reconcentrated by dialysis in tubing with a cutoff of 3000 Da (Spectrapor) against a solution of 40% (w/v) poly(ethylene glycol) 20000 (Fluka) in the same buffer. Subsequently, the water-soluble proteins were fractionated by gel filtration at 4 °C on Bio-Gel A-5m (LKB; column dimensions 27-mm i.d., 90-cm length), and the low molecular weight α-crystallin fraction was concentrated by centrifugation at 44000*g* for 18 h (Siezen & Berger, 1978).

Isolation of α-Crystallin Subunits. Subunits were purified by anion-exchange chromatography in the presence of 7 M urea. A sample of 10 mg of α-crystallin was dialyzed against the starting buffer (5 mM Tris-HCl, 7 M urea, 0.02% dithioerythritol, pH 8.0) and subsequently layered on top of a DEAE-cellulose column (DE-52, Whatman; dimensions 16-mm i.d., 47-cm length) equilibrated with the same buffer. After the nonbonded material was eluted from the column, the subunits were fractionated by gradient elution with an increasing concentration of Tris-HCl from 5 to 100 mM. The purity of the isolated subunits was checked by isoelectric focusing in the presence of 7 M urea.

Preparation of Homopolymeric Aggregates. The isolated subunits, present in a solution of 7 M urea at a concentration of 0.5 mg/mL, were dialyzed against 5 mM Tris-HCl, pH 8.0, for 24 h, with seven renewals of the dialysis buffer. The homopolymeric reaggregates obtained in this manner were concentrated to 10 mg/mL by dialysis against a solution of 40% poly(ethylene glycol) 20000 in dialysis buffer and stored at −70 °C.

Iodination of A_2 Aggregates. The inside wall of a glass reaction vial was coated with the catalyst Iodogen (Pierce) by evaporation of a solution in trichloromethane under nitrogen. A solution of αA_2 aggregates (1 mg/mL) was incubated with $^{125}I_2$ (477 μCi, Amersham) for 15 min. The nonbound iodine was complexed with potassium iodide, and the protein fraction was isolated by gel filtration on a short Sephadex G-25 column (Pharmacia). A protein fraction with a specific activity of 52 μCi/mg was isolated.

Subunit-Exchange Experiments. Usually two types of homopolymers were filtered separately through 0.2-mm Millex filters (Millipore) and mixed in a 1:1 ratio at a final protein concentration of 5 mg/mL. Incubations were performed at both 37 and 5 °C. Samples were taken after 0, 2, 4, 6, and 24 h and stored at −70 °C until further analysis. In every experiment, pure homopolymers were incubated as a control. Buffer changes were effected prior to mixing by dialysis of individual protein solutions against the appropriate buffer. Unless otherwise stated, the experiments were carried out in 5 mM Tris, pH 6.9.

Isoelectric Focusing. Homopolymeric aggregates and hybrid aggregates resulting from subunit-exchange experiments were analyzed on 3% T, 3% C acrylamide gels containing 13% (v/v) glycerol and an ampholyte mixture of the following composition: 1.81% (v/v) Ampholines 6–8 and 2.26% (v/v) Ampholines 3.5–10 (LKB) and 1.81% (v/v) Pharmalytes 2.5–5 (Pharmacia). The subunit composition of samples was analyzed on 5% T, 3% C acrylamide gels containing 7 M urea and the same ampholyte mixture. Gels of 10 × 20 cm with a thickness of 0.3 mm were cast between a glass plate treated with γ-(methacryloxy)propyltrimethoxysilane (Aldrich) and another one treated with dimethyldichlorosilane, as described by Tegelström and Wyöni (1986). In this way the gel was covalently bound to a glass support. Flat-bed electrophoresis was carried out in a Desaphor electrophoresis unit (Desaga), using strips soaked in 1 M phosphoric acid and 1 M sodium hydroxide and an LKB 2297 Macrodrive 5 as power supply. Limit settings were 2500 V and 70 mA. Prefocusing was carried out for 45 min at 15-W constant power. Subsequently, the samples were focused for 30 min at 8-W and 105 min at 15-W constant power. Tap water cooling was used to ensure a temperature of about 15 °C. The pH gradients were determined by cutting the gel in pieces of 0.5 cm and extracting the ampholytes with 2 mL of water for 48 h. The pH of the resulting solution was measured.

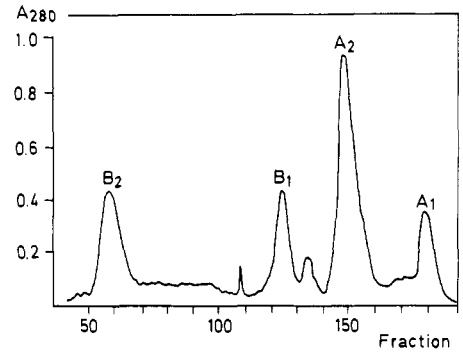

FIGURE 1: Fractionation of α-crystallin subunits. Subunits from 10 mg of α-crystallin were isolated by anion-exchange chromatography on DEAE-cellulose in Tris buffer, pH 8.0, containing 7 M urea. In the part of the chromatogram shown in this figure, the concentration of Tris linearly increases from 30 to 100 mM. Fractions of 10 mL were collected and the top fractions of the four main peaks were pooled.

After the gels were fixed for 1 h at 60 °C in an aqueous solution of 30% (v/v) ethanol and 11.5% (w/v) trichloroacetic acid and rinsed with 2% (v/v) acetic acid, they were stained for 45 min at 60 °C in a solution of 1.15% (w/v) PAGE Blue 83 (BDH Chemicals) in 25% (v/v) ethanol and 8% (v/v) acetic acid. Gels were destained in a solution of 25% (v/v) ethanol and 8% (v/v) acetic acid until a clear background was obtained and subsequently dried under a stream of hot air. Autoradiographs were made by exposing Sakura X-ray films to the gel at −70 °C. An LKB UltroScan gel scanner was used for Coomassie-stained gels as well as for autoradiographs.

Analytical Ultracentifugation. Sedimentation velocity analyses were carried out in a Beckman Spinco Model E analytical ultracentrifuge at 56000 rpm near 20 °C using UV optics.

Results

Preparation and Characterization of Homopolymers. Separation and isolation of the α-crystallin subunits A_1, A_2, B_1, and B_2 were carried out by anion-exchange chromatography on DEAE-cellulose (Figure 1). The purity of the subunits was analyzed by isoelectric focusing in the presence of 7 M urea, as shown in Figure 2. Purity was >95% for A_1, A_2, and B_2 and >85% for B_1. The purified subunits formed reaggregates upon removal of urea through dialysis against 5 mM Tris, pH 8.0; these will be referred to as $\alpha_r(A_1)$, $\alpha_r(A_2)$, $\alpha_r(B_1)$, and $\alpha_r(B_2)$ homopolymers. The reaggregates are

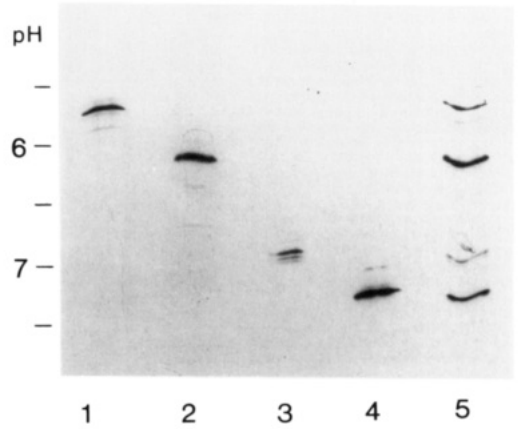

FIGURE 2: Isoelectric focusing of purified subunits. Homogeneity of the isolated subunits was analyzed by isoelectric focusing in the presence of 7 M urea. Lane 1, A_1; lane 2, A_2; lane 3, B_1; lane 4, B_2; lane 5, α-crystallin.

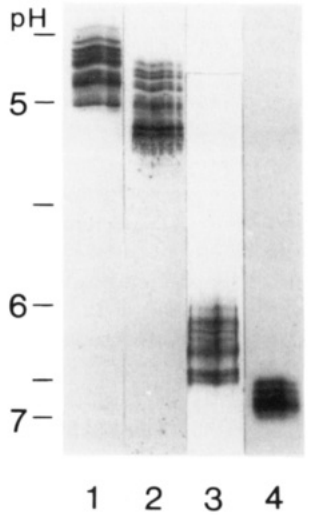

FIGURE 3: Isoelectric focusing of homopolymers. Aggregates of purified subunits were analyzed by isoelectric focusing in the absence of denaturants. Lanes 1–4: $\alpha_r(A_1)$, $\alpha_r(A_2)$, $\alpha_r(B_1)$, and $\alpha_r(B_2)$, respectively. The pH gradient is nonlinear because of the mixture of ampholytes used.

slightly polydisperse with respect to size and charge. Mean molecular weights of 360000 for $\alpha_r(A_2)$ and 420000 for $\alpha_r(B_2)$ were calculated from light scattering measurements (Thomson & Augusteyn, 1989). Isoelectric focusing in the absence of urea reveals that the homopolymers show charge heterogeneity (Figure 3). Although the reaggregates focus in a broader range than the unfolded subunits, several sharp bands are present in each population of reaggregates. The following p*I* ranges were found: $\alpha_r(A_1)$, 4.5–5.0; $\alpha_r(A_2)$, 4.8–5.2; $\alpha_r(B_1)$, 6.1–6.5; $\alpha_r(B_2)$, 6.5–6.9. It is remarkable that all of these p*I* ranges are lower than the p*I*'s of the respective subunits (Van den Oetelaar et al., 1987): 0.6–1.1 units for $\alpha_r(A_1)$ and $\alpha_r(A_2)$ and 0.3–0.7 unit for $\alpha_r(B_1)$ and $\alpha_r(B_2)$. Apparently, positively charged groups are masked either by folding or by aggregation of the subunits. The broad p*I* ranges of the homopolymeric aggregates indicate a charge heterogeneity that may be caused by a heterogeneity in structure of these aggregates with respect to either number, folding, or packing of subunits.

Subunit Exchange between Homopolymers. The differences in p*I* range of the four homopolymeric aggregates were used as a tool to study subunit exchange in mixtures of two types of homopolymers in the absence of denaturant. In the first experiment, $\alpha_r(B_2)$ was incubated at 37 °C with iodinated $\alpha_r(A_2)$ in a 1:1 ratio. Isoelectric focusing (Figure 4A) shows

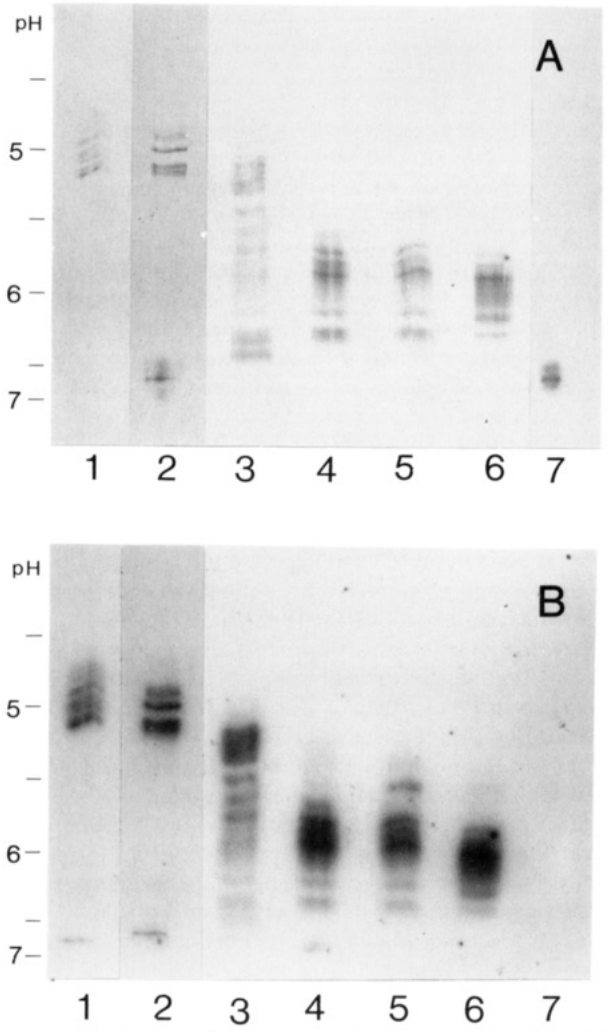

FIGURE 4: Subunit exchange between ^{125}I-$\alpha_r(A_2)$ and $\alpha_r(B_2)$. The pure homopolymers and mixtures thereof were analyzed by isoelectric focusing. The Coomassie-stained gel is shown in panel A, while panel B depicts the autoradiograph. Lane 1, $\alpha_r(A_2)$; lane 7, $\alpha_r(B_2)$; lanes 2–6, the focusing pattern of the 1:1 mixture of these two homopolymers after incubation at 37 °C for 0, 2, 4, 6, and 24 h. In lanes 1, 2, and 4, some labeled protein has precipitated at the application site in the lower part of the gel.

that within 2 h of incubation the bands focusing in the $\alpha_r(A_2)$ region become more alkaline, whereas those in the $\alpha_r(B_2)$ region shift toward a more acidic p*I*. The new hybrid aggregates focusing in the alkaline region of the gel have incorporated a few labeled A_2 subunits, as is indicated by the autoradiograph of this gel (Figure 4B).

As the incubation proceeds, more and more bands are observed with intermediate p*I*'s and the label is more uniformly distributed over these bands. Within 24 h a steady-state situation is reached, and no changes in the focusing pattern were observed when incubation was extended to 7 days at 37 °C. The newly formed hybrid aggregates in the steady state have isoelectric points in the p*I* range from 5.7 to 6.4, and the major part (87%) of the Coomassie staining is observed in the narrow p*I* 5.9–6.0 region, which falls within the average calculated from the p*I* regions of $\alpha_r(A_2)$ and $\alpha_r(B_2)$, namely, 5.7–6.1. It is important to note here that when we mixed these homopolymers in other ratios (3:1 and 1:3), the resulting hybrid population again showed a narrow p*I* range around that of the number average of the p*I*'s of the homopolymers (not shown).

Although the staining intensities and resolution of the various bands did not allow for an exact calculation of the

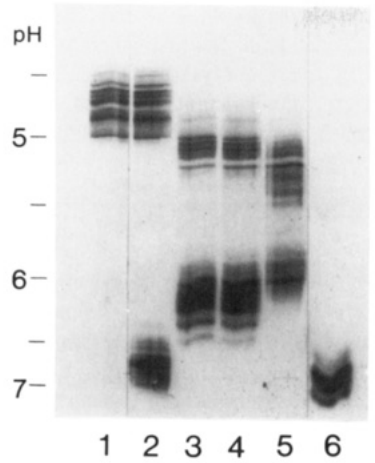

FIGURE 5: Subunit exchange between $\alpha_r(A_1)$ and $\alpha_r(B_2)$. Isoelectric focusing gel of the pure homopolymers of A_1 and B_2 and 1:1 mixtures thereof. Lanes 1 and 6 show $\alpha_r(A_1)$ and $\alpha_r(B_2)$, respectively. Lanes 2–5 show the focusing pattern of their 1:1 mixture after incubation at 37 °C for 0, 4, 6, and 24 h.

subunit composition of the bands indicated in Figure 4, arithmetic comparison of the gel scans of the Coomassie-stained gel and the autoradiograph gave a rough indication. In this way it turned out that the subunit composition is reflected in the isoelectric point as a number average of the isoelectric points of both homopolymers. In the 24-h steady-state situation the subunit composition of the major band (lane 6) showed a more or less equal amount of A_2 and B_2 chains, which is in line with the equimolar ratio in which the homopolymers were mixed.

The ability to form hybrids is not just limited to a mixture of $\alpha_r(A_2)$ and $\alpha_r(B_2)$. We also tested the following combinations of two homopolymers: $\alpha_r(A_1)/\alpha_r(B_1)$, $\alpha_r(A_2)/\alpha_r(B_1)$, and $\alpha_r(A_1)/\alpha_r(B_2)$. In each case steady-state situations were obtained within 24 h, which did not change if incubation was extended up to 7 days. In the combinations $\alpha_r(A_1)/\alpha_r(B_1)$ and $\alpha_r(A_2)/\alpha_r(B_1)$ a broad band was observed after 24 h, focusing at the average intermediate p*I*, very similar to the results observed for $\alpha_r(A_2)/\alpha_r(B_2)$ in Figure 4. On the other hand, subunit exchange was incomplete in the combination $\alpha_r(A_1)/\alpha_r(B_2)$: Figure 5 shows that at 24 h the homopolymers have exchanged some subunits, since the clusters of both protein populations are shifted toward each other. However, absence of Coomassie staining in the intermediate p*I* region indicates that subunit exchange was incomplete. This did not change upon prolonged incubation at 37 °C.

Exchange of subunits between homopolymers did not seem to depend on pH or ionic strength, since identical results were obtained when the pH of the buffer was 8.0 instead of 6.9 or when the Tris buffer concentration was increased from 5 to 50 mM. Subunit exchange was also observed in a pH 8.0 buffer containing 20 mM sodium phosphate, 25 mM sodium chloride, and 120 mM potassium chloride. Subunit exchange between $\alpha_r(A_1)$ and $\alpha_r(B_1)$ was studied at protein concentrations of 1, 5, and 10 mg/mL, and no differences were observed in hybrid formation.

Temperature, however, plays a crucial role in subunit exchange. While exchange reached a steady state within 24 h at 37 °C, it was undetectable upon incubation at 5 °C for all mixtures tested even after 7 days.

Subunit Exchange between Native α-Crystallin and Homopolymers. In order to study whether subunit exchange also occurred with native α-crystallin molecules, we incubated α-crystallin with $\alpha_r(B_2)$ homopolymers at 37 °C in a 1:1 ratio. The progression of the reaction was analyzed by isoelectric focusing and is depicted in Figure 6. Within 2 h numerous new bands have appeared that cover the whole p*I* region between the most acidic α-crystallin and the $\alpha_r(B_2)$ species. All of the original $\alpha_r(B_2)$ aggregates and nearly all of the α-crystallins have exchanged at this stage. After 24 h the p*I* range of the hybrids has narrowed down and most Coomassie-staining material is now concentrated in a small region at an intermediate p*I* of 5.6–6.2. Unfortunately, the reaction of α-crystallin with $\alpha_r(A_1)$ or $\alpha_r(A_2)$ cannot be analyzed by IEF, since the isoelectric points of the two initial populations are too similar.

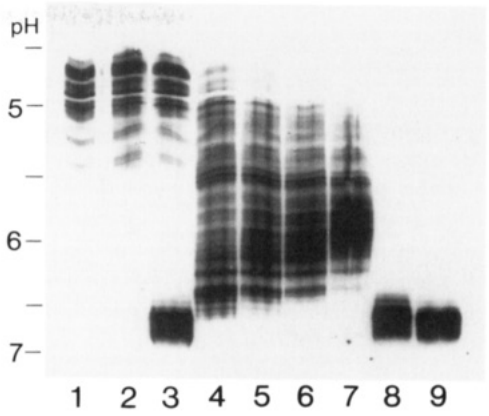

FIGURE 6: Subunit exchange between α-crystallin and $\alpha_r(B_2)$. Isoelectric focusing gel of α-crystallin and $\alpha_r(B_2)$ and mixtures thereof. Lanes 1 and 2, α-crystallin before and after 24 h of incubation at 37 °C; lanes 8 and 9, $\alpha_r(B_2)$ after and before incubation; lanes 3–7, 1:1 mixture after incubation at 37 °C for 0, 2, 4, 6, and 24 h.

The IEF observations mentioned in the preceding paragraphs would also be made if the polymers only interacted with each other to form supraaggregates, that is, larger aggregates containing multiple units of the already existing polymers that were initially mixed. Although supraaggregation is not to be expected under the applied experimental conditions (Van den Oetelaar et al., 1985), we tested this possibility by analytical ultracentrifugal analysis of a mixture of α-crystallin and $\alpha_r(A_2)$. After increasing periods of incubation at 37 °C, the two original peaks with sedimentation coefficients of 17.0 and 8.2 S, respectively, gradually shifted toward each other. After 24 h the two peaks had merged into a single peak of intermediate sedimentation velocity (9.3 S). We interpret these results as an initial conversion of 17S α-crystallin to ≈12S α_m-crystallin at 37 °C (Thomson & Augusteyn, 1983), followed by subunit exchange of the latter species with the 8.2S $\alpha_r(A_2)$ to form a hybrid population with intermediate size (9.3 S). These observations not only exclude the possibility of supraaggregation but also demonstrate that dissociation into individual subunits prior to hybridization can be excluded. At 4 °C the two original peaks remained after incubation for 24 h.

Discussion

We used homopolymers of the four subunits, A_1, A_2, B_1, and B_2, as model molecules to study the interaction of α-crystallin aggregates. There are few techniques available to study the mutual interactions of individual α-crystallin molecules directly. The homopolymers offer the advantage that they differ in p*I* and any change in their subunit composition results in a change of their p*I*, which is detectable by isoelectric focusing.

The experiment in which iodinated $\alpha_r(A_2)$ was incubated with $\alpha_r(B_2)$ shows that the changes in p*I* of the bands are accompanied by changes in the ^{125}I-label content of the molecules. This is a first indication that homopolymeric

molecules exchange their subunits and that this reaction continues until, within 24 h, a completely new population of hybrid molecules is formed that is relatively homogeneous with regard to its subunit composition. The second indication for exchange of subunits is found in the observation that the narrow p*I* range of the final hybrid population is the number average of the p*I*'s of the original two homopolymer populations.

Subunit exchange is not limited to the combination of $\alpha_r(A_2)$ and $\alpha_r(B_2)$ but was observed for several combinations of all four homopolymer aggregates. We carried out similar experiments with α-crystallin to assess whether the native population undergoes subunit exchange. Upon incubation of α-crystallin with $\alpha_r(B_2)$, we monitored a rapid redistribution of all subunits as new species with intermediate pI's appeared simultaneously with the disappearance of the original α-crystallin and $\alpha_r(B_2)$ homopolymer population. Since, on the basis of analytical ultracentrifugal experiments, supraaggregation could be excluded as an explanation for the aforementioned results, the isoelectric focusing experiments demonstrate that α-crystallin is capable of exchanging its subunits with homopolymers in the absence of denaturing agents. There is little reason to assume that the subunit exchange observed between α-crystallin and homopolymers would not also happen between two α-crystallin molecules. This kind of interaction, without the interference of dissociating agents such as urea, might even be a more universal characteristic of protein aggregates.

As far as α-crystallin is concerned, this "dynamic quaternary structure" might provide us with an explanation for several unusual and unstoichiometric properties of this protein. First, both native and reassocited α-crystallins show extensive charge microheterogeneity; our previous Monte Carlo computer simulation of the charge frequency distribution demonstrated that random combination of subunits can adequately describe the observed charge microheterogeneity (Siezen et al., 1978). At the same time it was hypothesized that this random Gaussian-like distribution could arise by exchange of subunits. Second, upon aging, the size distribution of α-crystallin gradually shifts toward a higher average molecular weight (Siezen et al., 1979; Bindels et al., 1982; Bessems et al., 1983). An M_r of 780 000 was observed in the younger calf cortex and of 1 100 000 in the older nucleus. Since this molecular growth takes place in the absence of protein synthesis, it cannot be explained by incorporation of newly synthesized subunits. Moreover, a simple oligomerization of α-crystallin molecules can be excluded because of the unstoichiometric, gradual increase of the molecular weight. Third, rechromatography of five molecular weight subpopulations of native α-crystallin, isolated by means of size-exclusion chromatography on Bio-Gel A-5m, revealed a slow reequilibration of these subpopulations toward the initial size distribution (Siezen & Owen, 1983). Fourth, another type of unstoichiometric reaction is encountered in the study of the dissociation of α-crystallin by denaturing agents such as urea or guanidine hydrochloride. It has been shown that, with increasing concentration of denaturant, the size of α-crystallin gradually decreases without the appearance of single subunits (Wisse et al., 1969; Siezen & Bindels, 1982; Thomson & Augusteyn, 1984; Bindels et al., 1986). It is obvious that these results cannot be explained by simple stoichiometric dissociation and hence other processes have to be involved. Subunit exchange may contribute to all these phenomena.

This exchange phenomenon may also explain the observation by Manski and Malinowski (1980, 1983) that the addition of reaggregated B chains to native α-crystallin caused complete loss of the antigenic determinants, which are dependent on the quaternary structure of α-crystallin. These authors also found that these antigenic determinants were conserved upon addition of reaggregated A chains to native α-crystallin, suggesting that subunit exchange did not occur. However, subunit exchange would not be detected in this case if the A chains predominantly determine the antigenicity of α-crystallin, as recently suggested (Thomson & Augusteyn, 1989).

The observation that most subunits with the exception of the $\alpha_r(A_1)/\alpha_r(B_2)$ combination appear to participate in the redistribution seems to contrast with the three-layer model for the architecture of α-crystallin. In this model the two inner layers are thought to form more or less stable entities (Bindels et al., 1979; Siezen et al., 1980; Tardieu et al., 1986). The nondifferentiated behavior exhibited by the subunits in our experiments lends support to the concept that they occupy (semi)equivalent positions in the α-crystallin molecule, which forms the basis of the tetrahedral shell and micelle-type models for the aggregation state of this protein (Thomson, 1985; Augusteyn & Koretz, 1987; Thomson & Augusteyn, 1989).

We emphasize, however, that complete or random exchange of subunits cannot explain all of our experimental results. That the exchange rules are complex can be appreciated from the observation that the $\alpha_r(A_2)/\alpha_r(B_2)$ mixture yields considerable exchange, while the $\alpha_r(A_1)/\alpha_r(B_2)$ mixture shows limited exchange. Possibly the phosphorylation of serine residues in A_1 and B_1 (Spector et al., 1985) may alter exchange. Furthermore, one would expect that size heterogeneity of α-crystallins and homopolymers would affect the efficiency of subunit exchange. Finally, even homopolymers exhibit limited charge heterogeneity, which makes the quantification of subunit exchange rather difficult.

With respect to the possible physiological role for subunit exchange, the following can be said. Upon aging, several changes take place in the bovine lens that might affect the stability of α-crystallin. (i) It was observed that the electrolyte composition of the cytoplasm of the fiber cells changes with age (Rink & Twenhöven, 1985; Bloemendal et al., 1985), and it has been pointed out that the quaternary structure of α-crystallin is dictated by parameters such as the ionic strength and pH of its medium (Siezen et al., 1980; Van den Oetelaar et al., 1985; Tardieu et al., 1986; De Block et al., 1986). (ii) During aging, the α-crystallin subunits undergo several posttranslational modifications like C-terminal clipping, resulting in an increase in negative charge (Van Kleef et al., 1975; Van Kleef et al., 1976). It was postulated that these degraded chains are involved in the supraaggregation of α-crystallin molecules (Siezen et al., 1979). (iii) Due to dehydration of the bovine lens nucleus, the cellular protein concentration increases with age (Rink, 1977; Rink et al., 1977; Hockwin et al., 1978). We suggest that these changes disturb the thermodynamic equilibrium of the existing α-crystallin population. Apparently, this lens protein is capable of responding to destabilizing changes in physiological conditions by means of intermolecular exchange of subunits, thereby producing new, thermodynamically stable populations. Future experiments may be directed toward simulating the age-related increase in the size of α-crystallin through subunit exchange/uptake of postsynthetically shortened A and B chains.

The molecular mechanism of the reaction is still unclear. Either subunits are transferred from one polymer molecule to another during collision or subunits may detach from one polymer molecule and adhere to another after having existed as free subunits for a short period of time. From the exper-

iments described here, no conclusions can be drawn as to the validity of either mechanism.

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