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The apparent molecular size of native α-crystallin B in non-lenticular tissues

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The apparent molecular size of the native α-crystallin B in cytosol preparations from rat heart, brain and retina was determined by gel permeation chromatography, detecting the protein by immunochemical assay (ELISA), using an α-crystallin specific antiserum. Native α-crystallin from cytosol preparations of rat lens cortex was used as a reference. α-Crystallin B present in all three cytosol preparations from non-lenticular tissues eluted in a single symmetrical peak, with the same elution volume as α-crystallin from lens cortex cytosol preparations, corresponding to an apparent average molecular size of 0.8×10^6 Da. No other species could be detected. The results indicate that the α-crystallin aggregates characterized by an apparent average molecular mass of 0.8×10^6 Da, and considered to be the native, physiological form of the protein in the lens, are indeed not specific to lens tissue. Furthermore, the size of these α-crystallin aggregates is independent of their polypeptide composition. Aggregates found in the lens, composed of αA and αB polypeptides and their respective phosphorylated forms αA_p and αB_p, are similar in size to those found in heart, brain and retina, containing the αB but not the αA polypeptide.

Lens protein; α-Crystallin; Protein structure; Protein aggregation

1. INTRODUCTION

α-Crystallin is a major protein found in the lenses of all vertebrate animals [1–3]. The native protein present in the soluble fractions of lens fiber cells has an apparent molecular mass of approximately 0.8×10^6 Da [4,5]. Isoelectric focusing analysis of the isolated protein resolves, under denaturing conditions, four major polypeptides, αA, αA_p, αB and αB_p, which have a molecular mass of approximately 20×10^3 [6]. The αA and αB polypeptides from the bovine lens have 57% homology in their amino acid sequences [7,8] and are the primary products from two different genes [9]. In the bovine, and probably in all mammals, specific phosphorylation reactions account for the major post-translational modification that these polypeptides undergo in vivo. Thus, αA_p and αB_p are the phosphorylated forms of the respective primary gene products [10].

The biological function of α-crystallin in the lens is not known. It is believed that the protein has a structural function related to the physical properties of the transparent lens tissue [11], a function thought to be associated with the particular molecular structure of this protein [12,13]. Because α-crystallin has not been crystallized, its tridimensional molecular structure remains unknown. Based on theoretical analysis of the amino acid sequences of the αA and αB polypeptides and nucleotide sequences of their respective genes, models have been proposed to describe the tertiary structure of these polypeptides [14,15]. Furthermore, based on physiochemical studies, models have been proposed to describe a quaternary structure in the native protein [16–18].

Whereas the in vivo structure of α-crystallin in the lens has been conceptualized in terms of specific arrangements of both αA and αB polypeptides, the role that each polypeptide plays in the 0.8×10^6 Da α-crystallin aggregate is unknown. Work from several laboratories has demonstrated that the αB polypeptide is expressed in various non-lenticular tissues [19–21]. In contrast, the expression of the αA polypeptide has only been detected in the lens and is, therefore, considered lens specific. Thus, the non-lenticular tissues that express α-crystallin B provide an attractive experimental system to study the structure of a native 'α-crystallin macromolecule' composed of a single type of polypeptide chain.

The present communication describes the determination of the apparent average molecular size of native α-crystallin B in cytosol preparations from three non-lenticular rat tissues, heart muscle, brain and retina, using gel permeation chromatography.

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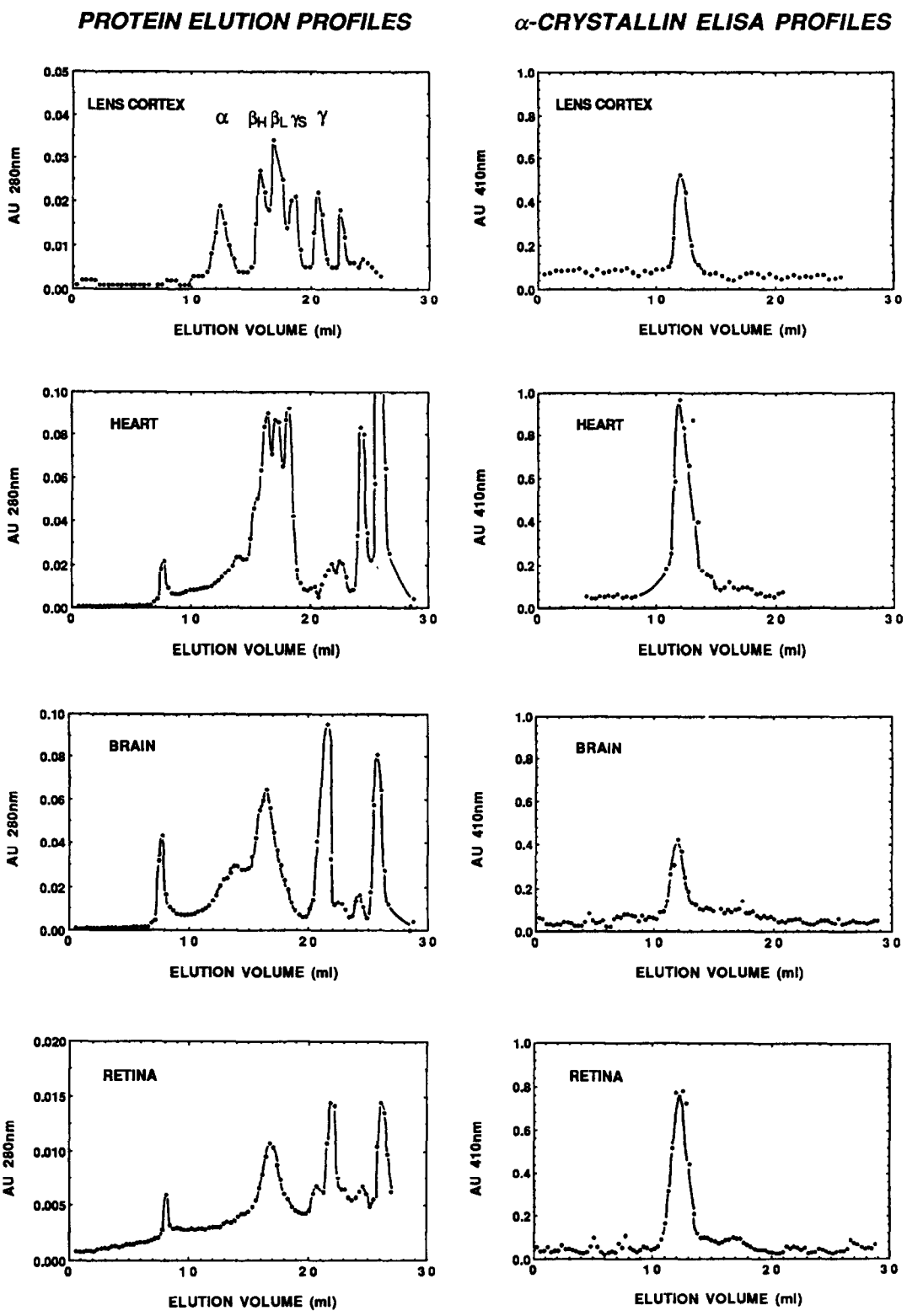

Fig. 1. Gel permeation chromatography elution profiles of the cytosol proteins from four rat tissues. The amounts of protein fractionated were: lens cortex, 25 μg; heart and brain, 250 μg; retina, 50 μg. The protein elution profiles were obtained by absorbance at 280 nm. The α-crystallin ELISA profiles were obtained by immunochemical analysis of the column eluent, using an α-crystallin specific antiserum. Eluents from heart, brain and retina preparations were analyzed directly whereas the eluent from the lens cortex preparation was diluted 1:100. The absorbance at 410 nm is proportional to the amount of α-crystallin antigen in the eluent. The crystallins elution peaks are indicated in the protein elution profile from lens cortex. The peak eluting at 8 ml observed in the chromatograms of all three non-lenticular tissues corresponds to the void volume of the column and, according to the specifications of the manufacturer, contains components with apparent molecular size larger than 25×10^6 Da. The elution volume of the standard ferritin (M_r 0.45×10^6) was 14 ml.

2. MATERIALS AND METHODS

2.1. *Tissue preparation*

All tissues were obtained from Sprague-Dawley rats weighing approximately 250 g (Charles River). After euthanasia by cervical dislocation, brain, heart and eyes were dissected and placed in beakers containing 10 ml of ice-cold phosphate-buffered saline (PBS). Brain, heart and retina were minced into approximately 2–3 mm pieces, and rinsed 2 times with 10 ml of ice-cold PBS to remove any blood. Lenses were dissected from the eyes and also rinsed in PBS. Brain and heart tissues (approximately half the total organ wet weight), whole retinas (neuroretina), and the outer cortex of the lenses (excluding the lens capsule and composed of approximately 30% of the wet weight) were homogenized at 4°C by hand with an all glass Potter Elvejhem homogenizer in 1.5 ml of 10 mM Hepes, 0.25 M sucrose, pH 7.5, previously filtered through a 0.2 μm filter. The homogenates were centrifuged at 105000 × g for 45 min at 4°C. Protein concentrations of the resultant supernatants (cytosol fractions) were estimated by absorbance measurement at 280 nm.

2.2. *Gel filtration chromatography and detection of α-crystallin*

The protein concentrations in cytosol fractions from lens cortex, heart, brain and retina were adjusted by dilution with homogenization buffer to 0.25, 2.5, 2.5 and 0.5 mg/ml, respectively. Aliquots of each cytosol sample (100 μl) were injected onto a gel filtration column (Superose 6 HR10/30 prepacked, Pharmacia-LKB) previously equilibrated with 200 mM NaCl, 5 mM DTT, 50 mM Tris, pH 7.5. The column was eluted at 20°C, at a flow rate of 0.5 ml/min and monitored by absorbance at 280 nm. The absorbance signal was obtained in a Gilson 112 UV/vis Absorbance Detector and acquired in a Macintosh SE computer using a data acquisition system and Analog Connection Work Bench version 2.0.2. The response of the system was linear up to 1 AU at 280 nm. Fractions (250 μl) of the chromatographic eluent were collected directly into 96 well immunoplates (Nunc MaxiSorp Flat Bottom Immuno Plates). α-Crystallin was detected by the immunochemical assay ELISA. The samples from heart, brain and retina preparations were analyzed directly. The samples from rat lens cortex were diluted 1:100 with elution buffer before analysis. Antigen was bound to the well surface at room temperature overnight, blocked with a 0.05% solution of Tween 20 in phosphate buffered saline (TPBS) containing 3% bovine serum albumin (BSA/TPBS) for 30 min, and exposed to an anti α-crystallin antiserum at a 1:500 dilution in BSA/TPBS, for 60 min. The polyclonal antiserum I.D. 23.1 prepared at this laboratory was used. This antiserum was raised in rabbit against total bovine α-crystallin (0.8 × 10^6 Da aggregate), prepared from bovine lenses [22]. The analysis of this antiserum by Western blot has demonstrated that it is specific for α-crystallin, and recognizes, in addition to the αA and αB polypeptides of the bovine protein, the αA, αA_{ins} and αB polypeptides from the rat lens protein. The plates were washed with TPBS, blocked with BSA/TPBS, and then exposed to goat anti-rabbit IgG conjugated to horseradish peroxidase (BioRad), 1:2000 dilution in BSA/TPBS. The color was developed using a Peroxidase Substrate Kit (BioRad), with H_2O_2 and 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid) for 10 min and stopping the reaction by adding oxalic acid to a 1% final concentration. Quantitation was carried out by measurement of the absorbance at 410 nm, in a microplate reader.

3. RESULTS

In order to determine the molecular size of the native soluble α-crystallin B, gel permeation chromatography analysis was carried out in cytosol fractions prepared from heart muscle, brain, retina, three rat tissues known to express the αB polypeptide but not the αA [19]. Cytosol preparations from rat lens cortex, corresponding to differentiated lens fiber cells, and containing α-crystallin aggregates composed of both αA and αB polypeptide chains, were simultaneously analyzed for comparative purposes. The cytosol preparations were fractionated by gel permeation FPLC, monitoring the elution simultaneously by absorbance at 280 nm and an immunochemical assay (ELISA) using an antiserum specific for α-crystallin. The A_{280} elution profiles of the cytosol preparations from the different tissues are shown in Fig. 1. The elution profile of the proteins in the preparation from rat lens cortical fibers showed as expected, the characteristic pattern of lens soluble proteins, comprising the α-, β_H-, β_L-, γS- and γ-crystallins (Fig. 1, protein elution profile of lens cortex). Rat lens α-crystallin eluted as a single symmetrical peak at 12 ml, and coeluted with α-crystallin from bovine lens cortex (data not shown), corresponding to an average apparent molecular size of 0.8 × 10^6 Da [4,5].

The A_{280} elution profiles of the soluble proteins from heart, brain and retina preparations were very different from that of the lens cortex preparations (Fig. 1, protein elution profiles). Although significant absorbance readings were observed in the three preparations in the eluent at 12 ml, corresponding to a molecular size of 0.8 × 10^6 Da, no defined elution peak could be resolved in that range in any of the three preparations.

To determine the elution pattern of α-crystallin B in the preparations of the three non-lenticular tissues, aliquots of the chromatographic eluent from the columns were analyzed by a immunoassay (ELISA), using a specific α-crystallin antiserum (see section 2). The elution profile of the heart, brain and retina preparations, obtained by this immunoassay resolved a single symmetrical elution peak at 12 ml (Fig. 1, α-crystallin ELISA profiles), corresponding to the α-crystallin peak observed in the elution profile of the lens cortex preparations (Fig. 1, α-crystallin ELISA profile, lens cortex). Accordingly, an apparent molecular size of 0.8 10^6 Da was assigned to the α-crystallin material in the elution peaks detected in the preparations of all three non-lenticular tissues.

The results demonstrate that α-crystallin B polypeptides in cytosol preparations of rat heart, brain and retinal tissues, are present in an aggregated form which has an apparent molecular size of 0.8 × 10^6 Da, indistinguishable from that of rat or bovine lens α-crystallin. This appears to be the only form present in the cytosol of these non-lenticular tissues, since neither other sized aggregates nor free polypeptide chains could be demonstrated.

4. DISCUSSION

α-Crystallin has been conceptualized as a lens-specific protein with a structural function in the lens, related specifically to the fundamental physiochemical properties of the tissue: transparency and refractive in-

dex. Such a specific structural function has been thought to be determined by a specific molecular structure of the protein. It has been presumed that the in vivo structure of the protein in the lens is characterized by an apparent average molecular size of 0.8×10^6 Da where both A and B polypeptide chains are constitutive monomers. The present work demonstrates the presence of native aggregates of α-crystallin polypeptides in cytosol preparations from heart, brain and retina, indistinguishable in size from the aggregates present in lens tissue. If the property to form 0.8×10^6 Da aggregates is associated with the specific function of α-crystallin polypeptides in the lens, such a property would be expected to be specific for the lens protein, and moreover, the lens specific αA polypeptide would be expected to be required for the formation of such aggregates. Instead, aggregates of α-crystallin polypeptides are found in tissues other than the lens, and moreover, in these tissues the lens non-specific αB polypeptide alone seems to be a sufficient requirement for the formation of the aggregates.

Bhat and co-workers in two preliminary reports [23,24] described αB aggregates in heart muscle and lens preparations with apparent molecular size of 0.3×10^6 Da, determined by gel permeation chromatography. Since the preliminary reports by these investigators do not contain sufficient experimental details, it is difficult to discuss the discrepancy. It has been demonstrated that experimental conditions may affect the apparent molecular size of lens α-crystallin, giving lower apparent sizes [5]. It is, therefore, possible that the smaller apparent molecular size observed by Bhat and co-workers is due to the procedure utilized in the preparation and analysis of soluble tissue fractions.

Because bovine lens α-crystallin aggregates with apparent average molecular size of 0.8×10^6 Da have been isolated under physiological conditions of temperature, pH and ionic strength, it is believed that they occur in the intact tissue in vivo [5], a concept which is supported by indirect evidence from spectroscopic studies of the lens tissue [25]. However, whether in the lens or in the other tissues, α-crystallin aggregates are the result of particular interactions between the constitutive polypeptide molecules. Such interactions are perhaps the consequence of artificial conditions of analysis, in particular, the disorganization of the cell structure. It is then possible that the 0.8×10^6 Da α-crystallin aggregates are unrelated to the structure in vivo which conveys the specific function of the protein in a given tissue.

Native α-crystallin aggregates in soluble preparations from lens epithelial cells are indistinguishable in size from the native aggregates in lens fiber cell preparations. Despite having the same apparent molecular size, 0.8×10^6 Da, the polypeptide compositions of these aggregates are different [26]. In the aggregates from epithelial cells only non-phosphorylated polypeptides are present (less than 0.1% of αBp is present in these aggregates). In contrast, the aggregates from the fiber cell preparations, contain as much as 20% of phosphorylated polypeptide chains. These observations suggest that phosphorylation has little or no effect on the apparent molecular size of the native protein, a view supported by recent physicochemical studies involving denaturation and renaturation of isolated α-crystallin polypeptides [27]. The results presented here indicate that the polypeptide type also has no effect on the overall structure of the native protein. Aggregates containing both αA and αB chains have the same apparent average molecular size as those containing αB but not αA. It should be noted, however, that the experiments described here do not allow for determining whether the αB aggregates in the non-lenticular tissues are composed exclusively of αB polypeptides, or represent associations of αB polypeptides and other proteins such as cytoskeleton components.

Preliminary experiments from this laboratory indicate that αB phosphorylation also occurs in non-lenticular tissues. In vitro phosphorylation experiments in preparations from Alexander's disease human brain, which is known to overexpress the protein, demonstrated a phosphorylated polypeptide with molecular weight (SDS-PAGE) and isoelectric point identical to those of bovine αB_p [28]. Mouse and bovine heart extracts appear to contain a charged form of αB comparable to lens αB_p [29]. Whether the 0.8×10^6 Da aggregates found in heart, brain and retina contain phosphorylated form(s) of the protein is not known at the present time. Experiments are being conducted to determine whether the αB polypeptide chains in these aggregates are phosphorylated, and if so, to what extent.

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