Biochemical comparison of γ-crystallins from duck and frog eye lenses

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A biochemical comparison has been made on the crystallins isolated from duck and frog lenses. Gel-permeation chromatography of lens homogenates from both classes on Fractogel TSK HW-55(S) revealed a homogeneous trimeric protein of 120 kDa in the duck lenses and a monomeric protein of 39 kDa in the frog lenses. Both crystallin fractions consist only of an approx. 38-kDa polypeptide in their subunit structures as determined by SDS gel electrophoresis. These two crystallins were compared with respect to their native molecular masses, subunit structures, peptide mapping and amino acid compositions in order to establish the identity of each crystallin. We have found differences in the protein structures of these two crystallins despite some degree of similarity in their amino acid compositions.

γ-Crystallin (Frog lens, Duck lens) Amino acid composition Peptide mapping Sequence homology

1. INTRODUCTION

The diversity and structural variants of lens crystallins have started to be recognized in lens research. In addition to the 4 main structural crystallins, i.e. α-, β-, γ- and δ-crystallins described in the literature [1–3], there are now 29-kDa squid crystallin [4,5], 48-kDa lamprey crystallin [6] and ε-crystallins recently named for both the 35-kDa frog crystallin [7] and the 38-kDa avian and reptilian crystallin [8].

The report by Tomarev et al. [7] indicated that ε-crystallin from the frog (*Rana temporaria*) lens is an oligomeric protein with an apparent molecular mass of 200 kDa, consisting of a single subunit of 35 kDa. This is in sharp contradiction with the report by Bindels et al. [9], which demonstrated a monomeric protein of 40 kDa from the frog lens of the same species without any indication of the existence of the 200-kDa protein with a 35-kDa subunit. Here, we have isolated and characterized the same protein fraction from the bull frog (*R. catesbeiana*), which also exists as a monomeric protein of approx. 40 kDa. A biochemical comparison between this crystallin and the ε-crystallin reported for the duck lens [8] is described in order to clarify the identities of these novel crystallins and their possible evolutionary relationship.

2. MATERIALS AND METHODS

Bull frog (*R. catesbeiana*) and duck (mule duck, a hybrid between *Cairina moschata* and *Anas platyrhynchos* var. *domestica*) lenses were obtained from a local meat company. The decapsulated lenses were homogenized in 10–20 ml of 0.05 M Tris-Na bisulfite buffer (pH 7.5) containing 5 mM EDTA as described [5,10]. The supernatant from a 27000 × *g* centrifugation was adjusted to give a concentration of about 50–100 mg/ml and a 5.0 ml aliquot was applied to a Fractogel TSK HW-55 column (superfine grade, Merck). This offers good and well-defined resolution similar to that usually found in HPLC. Native molecular masses of the eluted fractions were determined on the same column (2.5 × 115 cm) using the follow-

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ing standard proteins: thyroglobulin (670 kDa), catalase (240 kDa), transferrin (80 kDa), ovalbumin (45 kDa) and trypsin inhibitor (soybean, 20 kDa).

Disc tube gel (7.5%) and SDS-polyacrylamide slab gel (14%) electrophoresis was as described in [11,12] with some modifications.

Amino acid compositions were determined with an LKB-4150 amino acid analyzer using a single-column system.

Peptide mapping of the purified crystallins was done on an HPLC system using a reverse-phase (SynChropak RP-P, C-18, 10 μm bead) column with the solvent systems described in the figure legends. The proteins of about 1.0 mg/ml in 1% ammonium bicarbonate buffer (pH 8.2) were digested with TPCK-trypsin (Worthington) at an enzyme/substrate ratio of 1:100 at room temperature for 4 h and overnight with a second digestion of the same protein solutions. The digested solutions were lyophilized before analyses.

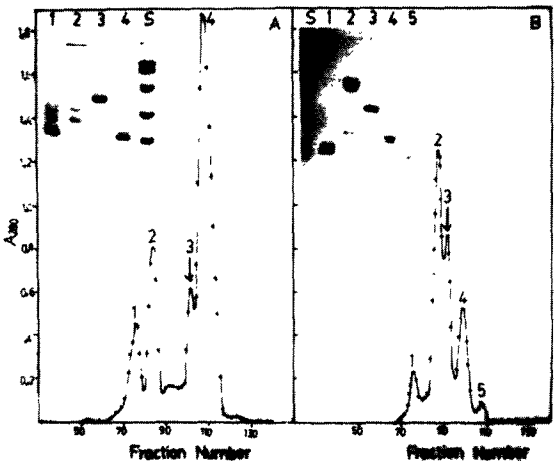

Fig.1. Comparative gel-permeation chromatography on Fractogel TSK HW-55(S) of lens extracts from frog (A) and duck (B) lenses. Conditions were as described in section 2. The column eluents (3.5 ml/tube per 4.1 min) were monitored for absorbance at 280 nm. The arrows in (A,B) indicate the peaks of the frog 39.5 -kDa and duck 37.5 -kDa crystallins purified for the characterization. Re-chromatography of the peaks on the same column to remove some cross-contaminating fractions is sufficient to obtain over 95% pure proteins as indicated in the SDS gels shown in the insets. Lanes S (insets) show the standard proteins used as molecular mass markers: transferrin (80 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa) and soybean trypsin inhibitor (20.1 kDa).

3. RESULTS AND DISCUSSION

During the course of comparative study of lens crystallins from the invertebrate and vertebrate lenses ([5,10] and unpublished) we found some structural variants. Especially interesting is the report [7] by Tomarev et al. who have determined the partial nucleotide sequence of the cloned gene coding for a 35-kDa polypeptide from the eye lenses of frog (*R. temporaria*). The protein was found in gel chromatography on Ultrogel AcA-34 as an oligomeric protein of 200 kDa, which is in sharp contrast with the report of Bindels et al. [9]. The latter group fractionated the lens extract from the same species of frog using HPLC of a TSK SW-type gel without detecting the protein fraction mentioned above. We have used a similar TSK HW-type gel of medium-performance permeation chromatography to fractionate the lens crystallins from *R. catesbeiana*. Fig.1 shows a typical elution

Fig.2. Gel electrophoresis of the duck 37.5 -kDa and frog 39.5 -kDa crystallins under native and denaturing conditions. (Right) SDS-PAGE of lens crystallins in the presence of 5 mM dithiothreitol [14]. Lanes: 1, duck 37.5 kDa (5 μg); 2, a mixture of duck 37.5 kDa (5 μg) and frog 39.5 kDa (10 μg); 3, frog 39.5 kDa (10 μg). (Left) Disc tube gel of duck 37.5 -kDa protein under nondenaturing conditions. The frog 39.5 -kDa protein (not shown) appeared as a similar smeared pattern moving further down to the anode (+).

pattern of our fractionation, which is comparable to that obtained by Bindels et al. [9] on high-resolution HPLC. A protein fraction of 39 kDa was found in peak 3 (fig.1) and no indication of any oligomeric protein with a subunit of 35 kDa present in the frog lens extract. It is also of interest to compare our separation pattern of the duck lens extract with that obtained by Stapel et al. [8] who could not detect the presence of γ-crystallin in their fractionation of Peking duck crystallins. As shown in the SDS-gel pattern of duck crystallins (inset to fig.1), γ-crystallin is clearly indicated in the last fraction of our chromatogram. This discrepancy could be ascribed to the different species of ducks used or the different chromatography gels employed in these studies. However, our superior and well-defined separation is comparable with that obtained by HPLC [9].

We also compared the biochemical characteristics of this frog crystallin (39.5 kDa as shown in fig.1) with that of the 120 kDa trimeric duck ϵ-crystallin characterized by Stapel et al. [8]. These two crystallins possess a similar subunit of approx. 38 kDa as shown by SDS gel electrophoresis. However, they can be separated on SDS gels by means of their slight difference in electrophoretic mobilities (fig.2), with subunit molecular masses of 39.5 and 37.5 kDa estimated for frog and duck crystallins, respectively. The charge heterogeneity for both crystallins can also be detected under non-denaturing conditions (fig.2). This is consistent with the result of isoelectric focusing for the duck ϵ-crystallin [8], which indicated the charge heterogeneity between p*I* 6 and 7 for the isolated native ϵ-crystallin. The amino acid compositions of these

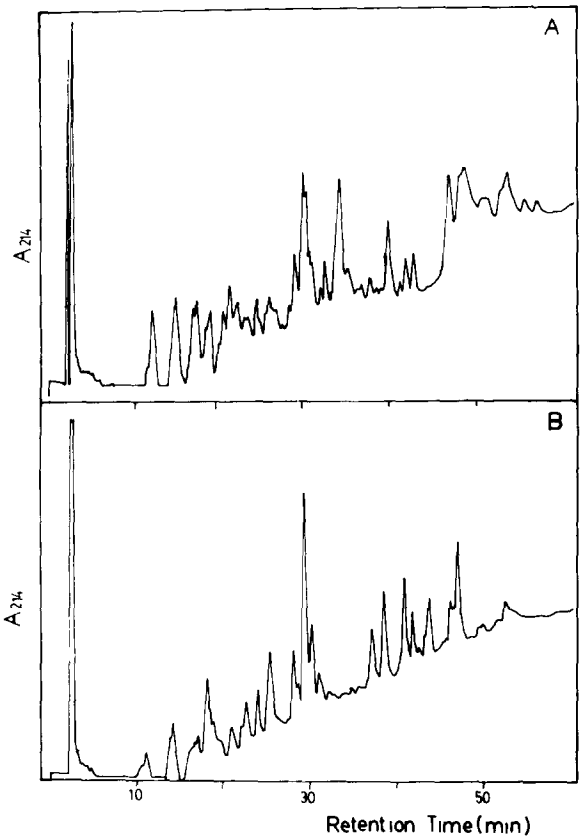

Fig.3. Tryptic peptide maps of frog 39.5 -kDa (A) and duck 37.5 -kDa (B) proteins in HPLC. Solvent A: 0.1% trifluoroacetic acid (TFA) in water. Solvent B: 0.1% TFA in acetonitrile. A linear gradient from 0 to 70% solvent B at a flow rate of 1 ml/min was run for the entire 60 min. Peptide peaks were monitored at 214 nm.

Table 1

Amino acid compositions of frog 39.5 -kDa and duck 37.5 -kDa crystallins

Amino acids	Amino acid content (mol%)	
	Frog 39.5 -kDa crystallin	Duck 37.5 -kDa crystallin
1/2Cys	2.5	2.1
Asx	12.8	10.0
Thr	2.6	4.3
Ser	4.2	7.2
Glx	10.7	10.1
Pro	5.0	3.9
Gly	7.4	7.6
Ala	6.1	7.3
Val	5.8	12.5
Met	1.0	1.6
Ile	5.4	5.6
Leu	10.1	11.3
Tyr	3.7	1.1
Phe	5.6	2.0
His	3.1	2.6
Lys	8.2	8.4
Arg	6.2	2.9
Trp	n.d.	n.d.

Data represent the average of triplicate determinations. Values reported for half-cystine were determined from the cysteic acid obtained from performic acid oxidation of protein samples before hydrolysis. n.d., not determined

two crystallins are shown in table 1. There are distinct differences in their contents of serine, valine, tyrosine, phenylalanine and arginine despite some degree of similarity between other amino acids. Peptide mapping (fig.3) of the tryptic digests of the two crystallins by HPLC also shows distinct differences in the peptide maps for these two proteins, with the frog crystallin showing a more complex peptide pattern than that of the duck. This is reflected in the higher arginine content of frog protein as compared to the duck ϵ-crystallin.

In conclusion, we have found differences in the protein structures of these two crystallins in their electrophoretic mobilities under native and denaturing conditions together with distinct differences in their amino acid contents and tryptic peptide maps. It is of great interest to note that some degree of similarity in their overall amino acid compositions seems to point to the possibility of sequence homology in their primary structures [13]. This should await the detailed analysis of the protein or gene sequence in the future.

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