Phylogenetic comparison of lens crystallins from the vertebrate and invertebrate – convergent or divergent evolution?

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A systematic biochemical comparison has been made of the crystallins isolated from the lenses of five different species belonging to the five major classes of vertebrates. Gel-permeation chromatography of the lens homogenates on Fractogel TSK HW-55(S) revealed well-defined elution patterns with a characteristic distribution of different classes of crystallins from each species. SDS gel electrophoresis and statistical comparison of the amino acid contents indicated that all crystallin groups from different classes share some common subunits and similarity in their amino acid compositions. The results coupled with the relatedness shown in the amino acid compositions of fish γ-crystallin with those of mammalian γ-crystallin and the squid crystallin from the invertebrate pointed to the possibility of the existence of a common ancestral protein for all crystallins. This is in favor of the divergent rather than convergent evolution of lens crystallins as commonly assumed in the literature.

Crystallin (Vertebrate lens) Amino acid composition Sequence homology Evolution

1. INTRODUCTION

The lens crystallins of vertebrates form a complex group of highly conserved structural proteins with distant evolutionary relationships [1]. The morphological similarity and immunological non-cross-reactivity of the invertebrate and vertebrate lenses led to the prevalent view of convergent evolution of lens crystallins in the animal kingdom [2]. However in such structural proteins endowed with the same physiological function, it seems very likely that these functionally related proteins might share some sequence homology or would have evolved from a common ancestral precursor as exemplified in the widely acknowledged cytochrome *c* and histone families of proteins. Attention has been drawn to the enlightening finding that β- and γ-crystallins share some sequence homology and probably form a single superfamily of the β/γ class [3]. In addition, Wistow et al. [4] recently reported that a bacterial protein with no apparent functional relatedness to crystallins also shares some sequence homology and the 4-fold repetition of a 'Greek Key' folding tertiary structure with β/γ-crystallins of the vertebrate eye lens. These observations, coupled with the results of the close relatedness between the squid crystallin [5] of a subvertebrate species and carp γ-crystallin [6] as judged by their similarity in amino acid compositions, have reinforced our speculation that all crystallins probably originated from a common ancestral protein and evolved divergently to the present-day complexity of the mammalian crystallin family.

2. MATERIALS AND METHODS

Carp (*Cyprinus carpio*), bull frog (*Rana catesbeiana*), river turtle (*Amyda sinensis*), duck (mule duck, a hybrid between *Cairina moschata*

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and *Anas platyrhynchos* var. *domestica*) and pig (*Sus scrofa* 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 in [5,7]. The supernatant from a 27000 × *g* centrifugation was adjusted to give a concentration of about 20–30 mg/ml and a 5.0 ml aliquot was applied to Fractogel TSK HW-55 (superfine grade, Merck). This offers good and well-defined resolution similar to that usually found in high-pressure liquid chromatography (HPLC). Native molecular masses of the eluted fractions were determined on the same column (2.5 × 115 cm) using the following standard proteins: thyroglobulin (670 kDa), catalase (240 kDa), transferrin (80 kDa), ovalbumin (45 kDa) and trypsin inhibitor (soybean, 20 kDa).

SDS-polyacrylamide slab gel (5% stacking and 14% resolving gels) electrophoresis was as described [8] with some modifications.

The amino acid compositions were determined with an LKB-4150 amino acid analyzer using a single-column system. The procedure of Marchalonis and Weltman [9] was used to analyze and compare the relatedness of amino acid compositions using the equation $S\Delta Q = \Sigma(X_{ij} - X_{kj})^2$, where the subscripts i and k identify the particular protein pairs being compared and X_j is the mole content of a given amino acid of type j. The summation is carried out over the 17 types of amino acids typically determined on 6 N HCl hydrolysates of crystallin samples.

3. RESULTS AND DISCUSSION

In contrast to the previous claim [10] that the evolutionarily conservative crystallins of the eye lens offer a unique opportunity to study convergent evolution of vertebrates and invertebrates at the molecular level, a biochemical comparison is given with regard to lens crystallins from vertebrate and invertebrate species to draw some support for the divergently evolutionary aspect of lens crystallins.

Fig.1 shows general elution patterns of our fractionation for the 5 major classes of vertebrates, which are comparable to that obtained by Bindels et al. [11] on high-resolution HPLC. It is also of interest to compare out separation pattern of the duck lens extract with that obtained by Stapel et al. [12] who could not detect the presence of γ-crystallin in fractionation of Peking duck crystallins. In addition, the elution pattern of a reptile species, river turtle (*A. sinensis*), is to our knowledge the first report of a systematic study of this species. It reveals a novel crystallin with a

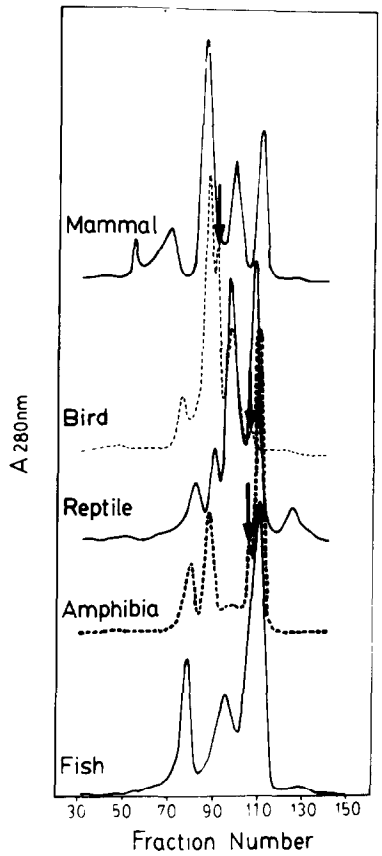

Fig.1. Comparative gel-permeation chromatography on Fractogel TSK HW-55(S) of lens extracts from the lenses of 5 species from vertebrates. Conditions were as described in section 2. The column eluents (3.5 ml/tube per 4.1 min) were monitored for absorbance at 280 nm. The arrows indicate the shoulder peaks of the frog 39.5-kDa, duck 37.5-kDa and γ-crystallins [20]. Rechromatography of the peaks on the same column to remove some cross-contaminating fractions is sufficient for the amino acid analyses and SDS gel electrophoresis. The absorbances at 280 nm are relative concentrations in arbitrary units. The small broad peaks after the γ-crystallin peak in the chromatograms of fish and reptile species are non-protein components of low molecular masses.

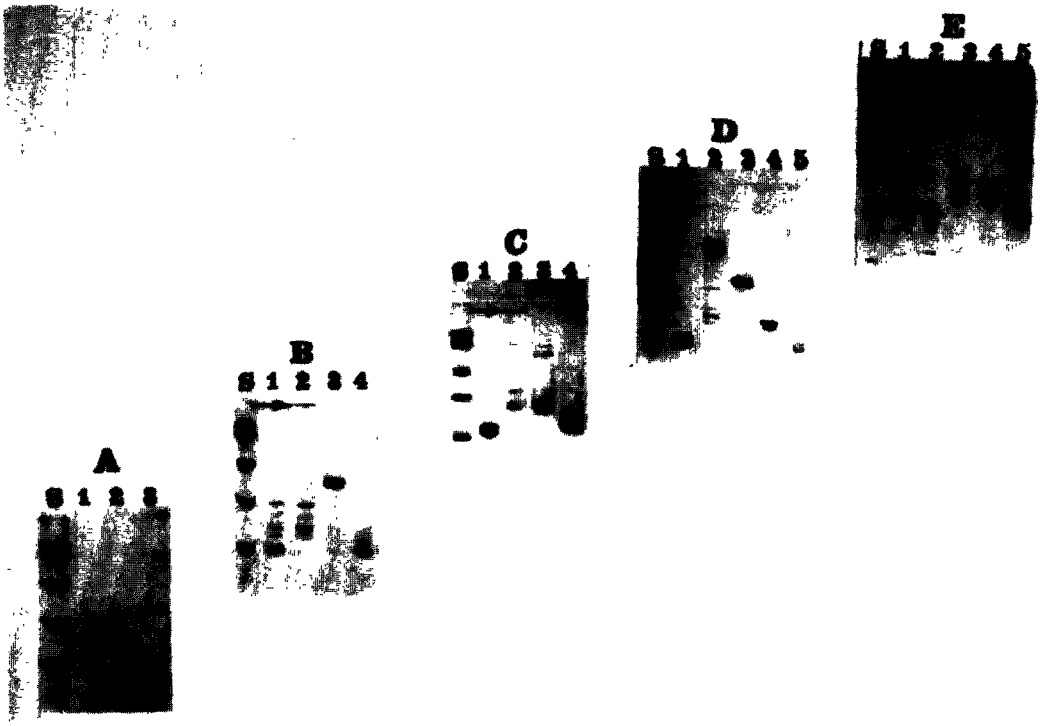

Fig.2. Gel electrophoresis of the isolated crystallins (fig.1) under denaturing conditions in the presence of 5 mM dithiothreitol. Insets A–E, SDS-PAGE of the crystallins from the species of fish to mammal, respectively. Lanes S (insets), standard proteins used as molecular mass markers (in kDa): transferrin (80), bovine serum albumin (66), ovalbumin (45), carbonic anhydrase (30) and soybean trypsin inhibitor (20.1). The gels were stained with Coomassie blue. The arrows in (B,C) show a novel crystallin with a molecular mass of 110–120 kDa, present only in the β-crystallin fraction of amphibia and reptile.

molecular mass of about 110–120 kDa, which is also present in the lens of frog (arrows in fig.2) and not detected in the lenses of other species from the other classes. The detailed characterization of this crystallin will be reported elsewhere. Our superior and reproducible resolution of the crystallin fractionation from the 5 major classes of vertebrates allowed us to make a detailed characterization and comparison of these vertebrate crystallins with that of the invertebrate squid species [5,10]. Fig.2 compares the subunit structures of various crystallins from these 5 classes of vertebrates. It shows the well-defined subunit patterns present in each eluted fraction of gel-permeation column and justifies the use of size-exclusion gel [13,14] in place of ion-exchange chromatography [15] in the general characterization and classification of lens proteins.

The amino acid compositions of the 9 reported crystallins ranging from the invertebrate to all 5 vertebrate classes are shown in table 1. The pairwise comparisons of amino acid contents between these crystallins based on statistical analyses [9,16] gave a crude measure of the relatedness of different families of proteins in the absence of complete sequence data (values of $S\Delta Q$ in table 1). It is noteworthy that the similarity in amino acid contents is evident between the pair with $S\Delta Q < 100$ despite the fact that there is little or no immunological cross-reactivity ([6] and unpublished). The pairs of proteins with a large value of $S\Delta Q$ are sometimes also related at the primary sequence level. A prominent example is the comparison of βBp and γ-II crystallin polypeptides which showed a high degree of sequence homology in their basic two-domain structures [3] despite the difference in

Table 1

Pair-wise comparisons of amino acid compositions between crystallins

Amino acids	Squid	Carp γ	Frog γ	Cow γ	βBp	Lamprey	Frog 39.5 kDa	Duck 37.5 kDa	δ
1/2Cys	1.2	4.8	2.3	3.6	1.0	1.6	2.5	2.1	0.3
Asx	15.0	11.5	11.3	10.9	8.8	11.1	12.8	10.0	7.1
Thr	3.4	1.7	3.0	3.0	3.4	3.9	2.6	4.3	7.6
Ser	5.8	7.0	5.0	7.3	8.8	5.4	4.2	7.2	9.6
Glx	8.8	10.6	14.1	10.9	15.7	10.5	10.7	10.1	13.0
Pro	4.3	3.1	5.9	4.9	6.9	3.8	5.0	3.9	2.3
Gly	7.0	8.2	8.3	7.3	9.3	9.1	7.4	7.6	5.6
Ala	4.0	1.5	1.1	1.2	3.9	9.9	6.1	7.3	8.0
Val	1.4	2.7	4.1	3.6	6.9	6.9	5.8	12.5	8.0
Met	12.8	14.1	2.8	4.2	1.0	2.0	1.0	1.6	0.8
Ile	3.2	3.7	5.1	3.6	3.4	6.5	5.4	5.6	7.4
Leu	4.9	2.5	5.9	7.9	4.9	7.6	10.1	11.3	15.1
Tyr	6.4	7.1	8.8	9.1	4.4	3.1	3.7	1.1	0.8
Phe	6.4	6.0	5.5	4.9	3.9	3.7	5.6	2.0	2.2
His	2.0	3.2	4.1	3.0	3.9	2.4	3.1	2.6	1.2
Lys	5.6	1.4	2.3	1.2	6.4	9.3	8.2	8.4	7.2
Arg	7.8	10.8	10.8	11.5	4.9	4.5	6.2	2.9	3.9
Trp	n.d.	n.d.	n.d.	1.8	2.5	n.d.	n.d.	n.d.	n.d.
$S\Delta Q$	80	180	34	177	127	44	100	87	

Data are taken from this study and [3,5,6,12,18,20,21]. n.d., not determined. $S\Delta Q$ represents the pair-wise comparison of amino acid contents of the adjacent crystallins as described in section 2

their amino acid compositions ($S\Delta Q > 150$). It has also been shown by Croft [17] that the N-terminal sequence of the first 7 residues is identical in calf and haddock γ-crystallins ($S\Delta Q = 145$). This points to the possibility of finding sequence homology even between crystallins of squid and carp γ-crystallins since they have very similar amino acid contents ($S\Delta Q = 80$). As regards the pair-wise comparisons of amino acid contents, it is even more unexpected that the recently described novel crystallins from lamprey [18], frog [19,20] and duck [12,20] are all related to each other and to the well-characterized δ-crystallin [21] of the bird (table 1). The mutual comparisons of different crystallins here seemed to be in favor of the hypothesis of divergent rather than convergent evolution which has been commonly assumed in the literature [2,10]. On the other hand, despite the similarity in overall amino acid compositions, the homology between the secondary [7,22] and tertiary structures [23] of different classes of crystallins appeared to outweigh that between their primary sequences, especially in the comparison of α- and β- or γ-crystallins. The sequence homology generally implies that the protein pairs being compared probably originate from a common ancestral precursor while the analogous tertiary structures with no apparent sequence homology would suggest otherwise. The settlement of these two different views with regard to the evolution of invertebrate and vertebrate crystallins needs to await the determination of the complete sequences of fish and squid lens proteins.

In conclusion, a systematic and general approach has been used to isolate and characterize crystallins of 5 different species representing the major classes of vertebrates in order to shed some light on the development and evolution of complex crystallin classes in mammals. The comparisons were made with regard to the chromatographic and electrophoretic behavior of the isolated native crystallins and their subunits. It is of great interest

to note that the seemingly unrelated crystallins characterized here are mutually interrelated with regard to their amino acid compositions. This is also found in the crystallins of squid and fish species. The detailed characterization of these crystallins at both the gene and protein levels should provide a good testing ground for the two opposing hypotheses of protein evolution.

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