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Characterization of lens crystallins and their mRNA from the carp lenses

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Crystallins from carp eye lenses have been isolated and characterized by gel permeation chromatography, SDS-gel electrophoresis, immunodiffusion and amino acid analysis. γ-Crystallin is the most abundant class of crystallins and constitutes over 55% of the total lens cytoplasmic proteins. It is immunologically distinct from the α- and β-crystallins isolated from the same lens and its antiserum shows a very weak cross-reaction to total pig lens antigens. Comparison of the amino acid compositions of carp γ-crystallin with those of bovine γ-II, haddock γ- and squid crystallins indicates that γ-crystallin from the carp is very closely related to that of the haddock, and probably also related to the invertebrate squid crystallin. In vitro translation of total mRNAs isolated from carp lenses confirms the predominant existence of γ-crystallin. The genomic characterization of carp crystallin genes should provide some insight into the mechanism of crystallin evolution in general.

Recent progress in recombinant DNA techniques has facilitated elucidation of the gene structures of crystallins from several different species [1–4]. We have been interested in the molecular evolution of crystallins since the discovery of the existence of a single crystallin in the squid of invertebrate as compared to the complexity of crystallin classes encountered in the vertebrate [5,6]. Before the successful gene analysis of the squid crystallin, we think it is worthwhile to characterize the lens crystallins from a more primitive species of vertebrate. In this communication we have isolated and characterized the lens crystallins from the carp (*Cyprinus carpio*). The carps were provided by the local fishery company under a special contract.

The decapsulated lenses were homogenized in 10–20 ml 0.05 M Tris/sodium bisulfite buffer (pH 7.5)/5 mM EDTA as described before [6,7]. The supernatant from $27000 \times g$ centrifugation was adjusted to give a concentration of about 50–100 mg/ml and a 5.0 ml aliquot was applied to Fractogel TSK HW-55 (superfine grade, E. Merck). It provides good and well-defined resolution similar to that usually only found in the gel matrix of high-performance liquid chromatography (HPLC). The column (2.5×115 cm) was calibrated using the following standard proteins: thyroglobulin (670 kDa), catalase (240 kDa), transferrin (80 kDa), ovalbumin (45 kDa) and soybean trypsin inhibitor (20 kDa).

SDS-polyacrylamide slab gel (14%) electrophoresis was as described before [8] with some modification from the original Laemmli buffer system [9]. Immunodiffusion was performed es-

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sentially according to Ouchterlony's method [10]. The antisera was used without dilution and the final concentration of each antigen was about 1–5 $\mu g/\mu l$. The amino acid contents of the purified crystallins were determined with an LKB-4150 amino acid analyzer using a single-column system.

Isolation and extraction of the total cellular RNA were according to the guanidinium/hot phenol method as described by Maniatis et al. [11]. About 500 fish eye lenses were used for each batch preparation of cellular RNA. Total cytoplasmic RNA isolated from the above protocol was then applied to a column of oligo(dT)-cellulose for the affinity chromatography of poly-(A)-containing mRNA. The isolated total mRNA was then used to carry out in vitro translation in the nuclease-treated lysates prepared from rabbit reticulocytes. The incorporation of [^{35}S]methionine into the lens crystallins was determined by immunoprecipitation using antisera against total fish crystallins after SDS-gel electrophoresis on the Laemmli system. The synthesis of complementary DNA (cDNA) with reverse transcriptase was done on the total mRNA prepared as described above.

Fig. 1 shows a typical elution pattern of carp lens crystallins, which is comparable to that obtained by Bindels et al. [12] on a high-resolution HPLC. The native molecular masses determined for the three peaks are 410 kDa, 60 kDa and 20 kDa, respectively, with peak 3 constituting 56% of the total lens proteins. SDS-gel electrophoresis (Fig. 1A) shows that γ-crystallin of carp lens is a monomeric protein of molecular weight of 20000, as opposed to the multimeric nature of the other two fractions, which is similar to those found for the mammalian pig lens crystallins (Fig. 1B). Peak 1 (Fig. 1A) is apparently an aggregated protein of peak 2, since it contains all the subunits of peak 2 together with another 20 kDa subunit, supposedly belonging to α-crystallin found in various mammalian species. This conclusion is supported by the results of immunodiffusion experiments (Fig. 2), which show two antigens being detected in peak 1 and one antigen in peak 2 (neither of them cross-reacts with peak 3). The antiserum against the total carp crystallins has a very weak reaction with the total pig lens antigens and no cross-reaction with that of squid crystallin at all (wells 5 and 6 in Fig. 2). This is consistent with the previous

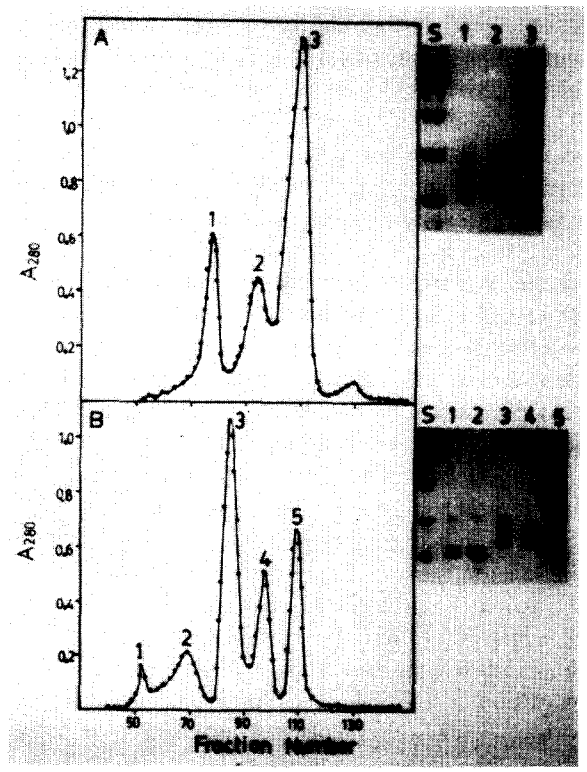

Fig. 1. Comparative gel permeation chromatography on Fractogel TSK HW-55(S) of lens extracts from the carp of *Cyprinus carpio* (A) and pig (B) lenses. The conditions are as described in the text. The column eluents (3.5 ml/tube per 4.1 min) were monitored for absorbance at 280 nm. The numbers in (A) and (B) indicate the peak fractions used for SDS-gel electrophoresis. Rechromatography 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 of the figure. S lanes in the insets show the standard proteins used as molecular weight markers; transferrin (80 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa) and soybean trypsin inhibition (20.1 kDa).

serological studies, which showed no cross-reactivity of lens homogenates between cephalopods and most vertebrate species [13–15].

It is of great interest to compare the amino acid compositions of various crystallins in order to gain some insight into the phylogenetic relatedness of different families of proteins in the absence of complete sequence data. Despite some distinct differences in several amino acids between carp γ-crystallin and squid crystallin [6,16], there

is some extent of similarity in their overall amino acid compositions (Table 1). The pair-wise statistical comparisons [17,18] of the amino acid content of carp γ-crystallin with those of haddock γ-, bovine γ-II and squid crystallins [6] clearly indicate that carp γ- and haddock γ-crystallins are almost indistinguishable, whereas the difference between carp γ- and bovine γ-II seems to be greater than that of carp γ- and squid crystallins. However, it has been shown by Croft [19] that the N-terminal sequence of the first seven residues is identical in the calf and haddock γ-crystallins. This points to the possibility of finding sequence homology even between crystallins of vertebrate and invertebrate. Attention is drawn to the fact that β- and γ-crystallins have been found to share some sequence homology and probably form a single superfamily of β/γ class [20]. Wistow et al. [21] recently reported that a bacterial protein with no apparent functional relatedness to crystallins shares some sequence homology and the four-fold repetition of a Greek key folding tertiary structure with β/γ crystallins of the vertebrate eye lens. These observations coupled with our comparisons of the amino acid compositions of the vertebrate and invertebrate crystallins may indicate that the evolution of crystallins is the result of divergent rather than convergent evolution which has been commonly assumed in the literature [16,22]. On the other hand, the sequence of the mentioned bacterial protein appeared to show more evidence of the homology in the tertiary structure with mammalian γ-crystallin [21] than that of partial sequences with the squid crystallin (Ref. 16 and unpublished data), which would indicate convergent evolution. The settlement of these two different views with regard to the evolution of invertebrate and vertebrate crystallins needs to await the complete sequencing of fish and squid lens proteins.

The preliminary isolation and characterization of mRNA from the two species of carp (*Cyprinus carpio* and *Ctenopharyngodon idellus*) have indicated a major predominance of γ-crystallin both at the levels of cDNA and in the in vitro translation products (Fig. 3). The major translated protein has an electrophoretic position the same as that of α- or γ-crystallin of molecular mass 20

TABLE I

AMINO ACID COMPOSITIONS (mol%) OF CARP, HADDOCK, SQUID AND BOVINE CRYSTALLINS

Amino acid composition data for the three carp crystallin fractions (Fig. 1A) represent the average of triplicate determinations. Data for the other three crystallins are taken from the previous papers [6,19] and the references therein. The values reported for half-cystine are determined from the cysteic acid obtained from the performic acid oxidation of protein samples before hydrolysis. n.d., not determined.

Amino acids	Carp-1	Carp-2	Carp-3	Haddock-γ	Squid	Bovine γ-II
1/2Cys	3.7	3.5	4.8	5.2	1.2	3.6
Asx	10.1	10.2	11.5	12.0	15.0	10.9
Thr	2.4	2.7	1.7	1.8	3.4	3.0
Ser	5.8	6.0	7.0	9.3	5.8	7.3
Glx	15.3	16.8	10.6	11.3	8.8	10.9
Pro	4.8	4.9	3.1	3.2	4.3	4.9
Gly	8.7	9.2	8.2	7.3	7.0	7.3
Ala	4.0	4.5	1.5	0	4.0	1.2
Val	6.4	6.2	2.7	1.8	1.4	3.6
Met	4.2	2.9	14.1	13.0	12.8	4.2
Ile	4.5	4.4	3.7	3.0	3.2	3.6
Leu	4.6	4.3	2.5	1.4	4.9	7.9
Tyr	4.9	5.2	7.1	8.8	6.4	9.1
Phe	5.6	5.4	6.0	5.7	6.4	4.9
His	3.4	3.2	3.2	2.4	2.0	3.0
Lys	4.6	4.4	1.4	1.9	5.6	1.2
Arg	7.1	6.6	10.8	11.2	7.8	11.5
Trp	n.d.	n.d.	n.d.	0.7	n.d.	1.8

kDa. However, from the gel permeation chromatography of lens homogenate, it seems very likely that the major in vitro translated protein corresponds to the product of mRNA coding for γ-crystallin. This is corroborated by the size determination of cDNAs made from the total mRNA, which indicated a major species of 650 nucleotides in length (data not shown). This is also consistent with the results of Tomarev et al. [23], which showed that mRNA species coding for all γ-crystallins of the frog lens are about 650 nucleotides and much smaller than those coding for α- or β-crystallins [24].

In conclusion, we have characterized the lens crystallins from the fish lens and they have been compared with the mammalian and cephalopod crystallins with regard to amino acid composition and serological properties. It is noteworthy that there seems to be some extent of similarity present in the overall amino acid contents between the predominant γ-crystallin of fish lens and that of cephalopod squid lens. This is also found in the seemingly unrelated duck 37.5 kDa and frog 39.5 kDa crystallins [25,26]. The simplicity of the mRNA species in the carp lenses will enable us to characterize the gene sequence from the cDNA coding for the major γ-crystallin in the near future, which should shed some light on the evolution of lens crystallins in the animal kingdom.

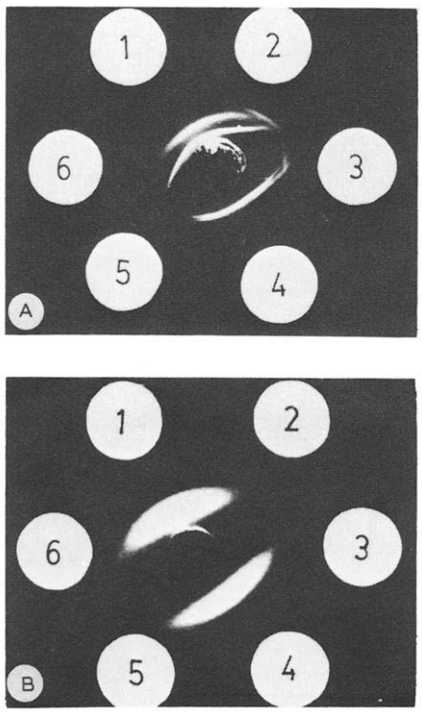

Fig. 2. Immunodiffusion analyses of the antisera against total carp crystallins (A) and purified carp-γ crystallin (B) from peak-3 of Fig. 1A to various antigens. The antisera are placed in the center well and the antigens of the surrounding wells (numbered 1 to 6) are as follows: 1, total carp lens crystallins; 2, purified carp-1 crystallin of the peak-1 of Fig. 1A; 3, purified carp-2; 4, purified carp-3 (γ-crystallin); 5, total porcine crystallins; 6, purified squid crystallin (Ref. 5 and 6). Note two precipitating lines showing for the carp-3 (well 4) at a low antigen concentration (A) and a fused broad line at a higher concentration (B).

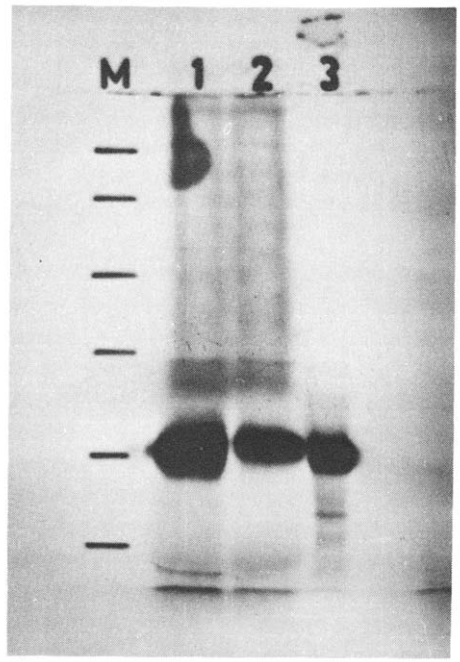

Fig. 3. Autoradiography of the SDS-slab gel of the in vitro translations in the presence of [^{35}S]methionine using total mRNAs isolated from the carp lenses of two different species. Lane 1, total translated products of mRNA from the grass carp (*Ctenopharyngodon idellus*); lane 2, total translated products of mRNA from the common carp (*Cyprinus carpio*); lane 3, total translated products of lane 2 pretreated with antiserum against total carp crystallins of *C. carpio* and boiled in the SDS solution (to dissociate the antigen-antibody complexes). Lane M indicates the electrophoretic positions of the marker proteins: phosphorylase *b* (94 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa).

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