Comparison of the γ-crystallins isolated from eye lenses of shark and carp

Unique secondary and tertiary structure of shark γ-crystallin

S.-H. Chiou[1], S.-W. Chen[1], T. Itoh[2], H. Kaji[2] and T. Samejima[2]

[1]*Laboratory of Crystallin Research, Institute of Biochemical Sciences, National Taiwan University and Institute of Biological Chemistry, Academia Sinica, PO Box 23-106, Taipei, Taiwan ROC* and [2]*Department of Chemistry, College of Science and Engineering, Aoyama Gakuin University, Setagaya-ku, Tokyo, Japan*

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γ-Crystallin isolated from the shark of cartilaginous fishes was compared with the cognate γ-crystallin from the carp of bony fishes. Distinct differences in amino acid compositions, primary, secondary and tertiary structures were found. The most salient features of shark γ-crystallin lie in the fact that this crystallin possessed a significant α-helical structure in the peptide backbone as revealed by circular dichroism study, in contrast to those orthologous γ-crystallins from other vertebrate species including bony fishes which all show a predominant β-sheet secondary structure. The tertiary structure as reflected in the intrinsic microenvironments of various aromatic amino acids in the native crystallins also shows unambiguous differences between these two classes of γ-crystallins. N-Terminal sequence analysis corroborates the structural differences between shark and carp γ-crystallins. γ-Crystallin from the more primitive shark seems to be more in line with the main evolutionary phylogeny leading to the modern mammalian γ-crystallin.

Lens crystallin; Chondrichthye; Osteichthye; Circular dichroism; Sequence comparison; Phylogeny; Shark; Carp

1. INTRODUCTION

Fish represents the oldest, largest and most diverse group of vertebrates. They evolved about 500 million years ago and today constitute more than half of vertebrate species, i.e. there are more fish species than all other vertebrates combined [1]. The modern fishes are composed of two major classes: (i) Chondrichthyes or cartilaginous fishes and (ii) Osteichthyes or teleostean (bony) fishes.

The mechanism governing the presence and distribution of various common and specific classes of structural proteins, i.e. lens crystallins, in evolutionarily distant classes of vertebrates is intriguing regarding some aspects of protein evolution in these supposedly structurally homologous and functionally degenerate proteins [2–4]. We have recently characterized crystallins from the shark lenses of cartilaginous fishes [5]. Preliminary characterization has revealed that a distinct amino acid composition with low methionine content is associated with the γ-crystallin class of shark crystallins in contrast to those γ-crystallins found for other teleostean fishes such as carp [6]. In the present investigation a special effort is directed to study the conformational differences between the purified γ-crystallins of these two piscine classes by circular dichroism spectropolarimetry.

Correspondence address: S.-H. Chiou, Institute of Biochemical Sciences, National Taiwan University and Institute of Biological Chemistry, Academia Sinica, PO Box 23-106, Taipei, Taiwan ROC

2. MATERIALS AND METHODS

2.1. *Isolation and purification of γ-crystallins*

The sharks (*Scoliodon walbeemii*) and carps (*Cyprinus carpio*) were provided by the local fishery company under a special contract for scientific research. The isolation and purification of γ-crystallins employing gel-permeation and cation-exchange chromatography were essentially according to the previous reports [6–7]. Cation-exchange chromatography using TSK CM-650 (Merck, Darmstadt, FRG) resolved carp and shark γ-crystallins into 4 and 6 subfractions, respectively, and the major peaks (carp γ-IV and shark γ-II) with the highest optical density were subjected to amino acid and N-terminal sequence analysis. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was used to check the purity. The separated subfractions were also rechromatographed on reversed-phase HPLC using Waters μBondapak C_{18} column (3.9 × 300 mm) in 0.1% trifluoroacetic acid (TCA) and acetonitrile gradient to remove the salts before amino acid and sequence analyses.

2.2. *Amino acid analysis*

Amino acid compositions were determined with the Beckman High-Performance Amino Acid Analyzer (Model 6300) with dual-channel data system using a single-column based on ion-exchange chromatography. The half-cystine content and tryptophan could be determined with accuracy by hydrolysis with 4 M methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole (Pierce, Rockford, IL, USA) as described previously [8].

2.3. *N-Terminal sequence analysis*

The N-terminal sequences of major fractions from HPLC column were carried out by automated Edman degradation with a pulsed-liquid phase sequencer (Model 477A, Applied Biosystems, Foster City, CA, USA). The vacuum-dried samples each containing about 0.1–0.5 nmol of purified peptides were dissolved in 50 μl of 0.1% trifluoroacetic acid (TFA) and 10 μl each for sequence determinations.

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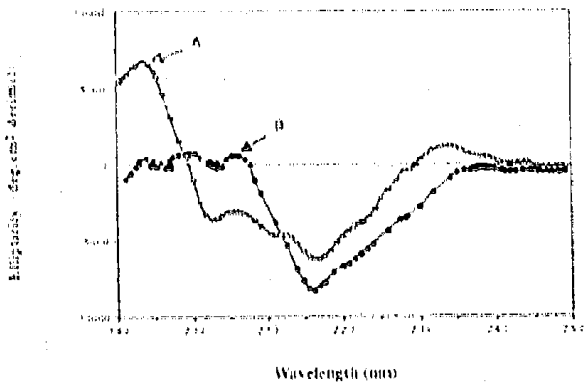


Fig. 1. CD spectra of shark (A) and carp (B) γ-crystallins in the far UV region. Conditions are as described in section 2. The ellipticity in degrees·cm²/decimole is calculated on the basis of a mean residue weight of 115 for these crystallins. The protein concentration was 0.0648 and 0.0729 mg/ml for shark (A) and carp (B) crystallins, respectively. The ellipticity values were taken from the average of 10 scans at a time constant of 2 s. Note the presence of a characteristic α-helical feature with two negative ellipticity peaks located at about 203 and 218 nm in (A) and a β-sheet structure with a peak at 217 nm in (B).

2.4. *Circular dichroism (CD) studies*

The circular dichroic spectra for γ-crystallins were obtained on a Jasco J-600 automatic recording dichrograph at room temperature (20°C). The instrument was calibrated with an aqueous solution of (+)-10-camphorsulfonic acid. The crystallins were dissolved in 0.05 M Tris buffer, pH 7.8 at a concentration of 0.050–2.25 mg/ml. All protein solutions were centrifuged and the clear supernatant fractions used for measurement of CD spectra. The protein concentrations of γ-crystallin solutions for the CD study were estimated using an absorption coefficient of 2.14 and 2.44 (1 mg/ml, at 280 nm), respectively, for carp and shark γ-crystallins, determined from the protein dye-binding method [9] using bovine serum albumin as standard. The ellipticity data were converted to mean-residue-weight ellipticity using a mean-residue weight of 115 for all γ-crystallins. Analysis of CD spectrum in the far UV region in terms of the fractions of the structural elements, i.e. helix, β-sheet, β-turn and unordered form, was carried out according to the procedure of Chang et al. [10]. A non-linear least-squares curve fitting in the 190–250 nm region at 1-nm intervals of CD spectra was used to find the best estimate for the percent contribution of each structural element in the studied crystallins.

3. RESULTS AND DISCUSSION

The modern Chondrichthyes class of fishes (for example, sharks, electric rays and their relatives) are distinguished by their cartilaginous skeletons in contrast to the bony skeletons of Osteichthyes (bony fishes). Sharks diverged from the Placodermi long before the appearance of modern bony fishes and amphibians. They constituted the early forms of fishes and are thought to have been ancestral to the land vertebrates [11]. We have previously studied the crystallins from the carp of Osteichthyes on the protein structure and sequences [12,13]. The salient feature of carp γ-crystallin lies in the fact that it possesses a high content of methionine (12–15 mole %) in amino acid composition as compared to that of mammalian γ-crystallins with low methionine content (3–5 mole %). Unexpectedly the preliminary structural study of crystallins from shark lenses [5] indicated that γ-crystallin isolated from this cartilaginous fish possesses an amino acid composition similar to that of mammalian γ-crystallin. In this report we have investigated the conformational differences between these two classes of piscine γ-crystallins by comparison of their CD spectra in the peptide backbone and aromatic regions.

Fig. 1 shows the CD spectra in the far UV region (190–250 nm) for the purified shark and carp γ-crystallin fractions isolated from cation-exchange chromatography [7]. It is noteworthy that shark γ-crystallin shows the characteristic α-helical feature with two negative ellipticity peaks of maxima located at about 203 and 218 nm. In contrast γ-crystallins of bovine and carp lens show the predominance of a β-sheet structure with the maximum of negative ellipticity located at about 217 nm [6,14]. The estimations of the fractions of four basic structural elements (Table I) indicated about 18% α-helical structure for shark γ-crystallin in contrast to 9% and 10% helices found for carp and bovine γ-crystallins respectively [15–17]. The existence of a significant α-helical conformation in the peptide backbone of γ-crystallins in the primitive fishes such as shark is striking since most mammalian crystallins such as α-, β- and γ-crystallins all exhibit a common β-sheet structure, which is supposedly to be related to the close-packing of crystallins inside the lens fiber cells [18].

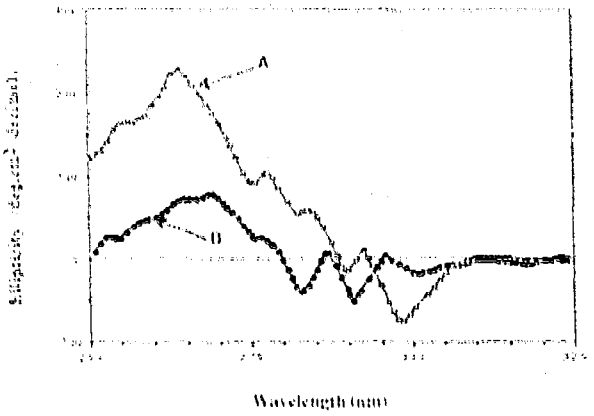
Fig. 2 shows the CD spectra in the near UV region (250–325 nm). The well-defined dichroic peaks were found for γ-crystallins of both species, which may indicate a rigid defined environment for the aromatic residues of these piscine crystallins. It is of special interest to see the double negative-ellipticity peaks at 288/298 nm and 283/291 nm for shark and carp γ-

Table I

Estimation of secondary structures for shark and carp γ-crystallins

(%)	α-Helix	β-Sheet	β-Turn	Unordered form
Shark γ	18 ± 5	40 ± 12	32 ± 9	10 ± 7
Carp γ	9 ± 5	49 ± 8	14 ± 6	32 ± 6
Bovine γ	10 ± 5	30 ± 11	25 ± 10	35 ± 3

The method used for determination of these values is described in section 2.4. The values shown for % secondary structures represent the estimates from non-linear least-squares curve fitting of CD spectra ± (estimated uncertainty in the fitting procedure). Data for bovine γ-crystallin are taken from [15].



Wavelength (nm)

Fig. 2. CD spectra of shark (A) and carp (B) γ-crystallins in the near UV region. Conditions are as described in section 2. The ellipticity in degrees·cm²/decimole is calculated on the basis of a mean residue weight of 115 for these crystallins. The protein concentration was 1.012 and 1.055 mg/ml for shark (A) and carp (B) crystallins, respectively. The ellipticity values were taken from the average of 10 scans at a time constant of 2 s.

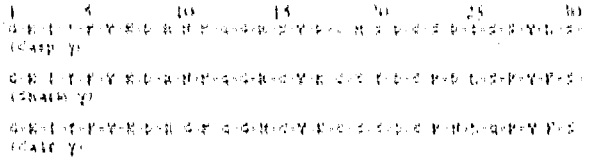


Fig. 3. Comparison of N-terminal sequences of γ-crystallins from carp, shark and bovine lenses. The sequences listed for carp and bovine crystallins were taken from [13,20]. Note that there are 9 and 6 amino acid substitutions between carp/shark and shark/bovine, respectively, for the listed N-terminal sequences of 30 residues. Amino acid residues are denoted by one-letter symbols.

crystallins, respectively, which may be reflective of the microenvironments of phenylalanine and tyrosine residues in these crystallins [19]. However, these peaks showing negative ellipticity maxima at the different wavelengths would certainly indicate different tertiary structures present in these two crystallins.

N-Terminal sequence analyses of the major shark and carp γ-crystallin subfractions isolated from TSK CM-650 by Edman degradation up to the thirty residues are shown in Fig. 3. The sequence of the corresponding segment in bovine γII-crystallin [20] is also shown for pair-wise comparison. Unexpectedly 9 differences in the first N-terminal 30 residues between the sequences of shark and carp γ-crystallins were found. Among these substitutions between carp and shark crystallins there are several conservative changes of amino acids such as Asp/Glu at residue no. 17, Ser/Thr at residue no. 20 and Ile/Leu at residue no. 25. However nonconservative changes of amino acids were also identified at residue no. 4 (Ile/Thr), 15 (Ser/Cys), 19 (Met/Ser), 23 and 27 (Ser/Pro). It is of interest to note that there are only 6 differences among the 30 N-terminal residues between shark and bovine γ-crystallins. In addition the five non-conservative sequence substitutions were not observed when comparing the sequences of bovine and shark proteins. It would seem to indicate that γ-crystallins of bovine and shark are more closely related than those between carp and shark. The detailed sequence comparison may need to await the complete sequence information of shark γ-crystallin which is currently under investigation by cDNA cloning and sequencing.

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