The amino-terminal sequences of four major carp γ-crystallin polypeptides and their homology with frog and calf γ-crystallins

Shyh-Horng Chiou, Shun-Wen Chen and Tung-Bin Lo

Institute of Biochemical Sciences, National Taiwan University and Institute of Biological Chemistry, Academia Sinica, PO Box 23-106, Taipei, Taiwan, Republic of China

Received 7 October 1986

Four major γ-crystallin subfractions have been isolated from the carp *(Cyprinus carpio)* and their N-terminal sequences determined by Edman protein sequencing. Extensive homologies indicative of close relatedness in their primary structure were found in these four γ-crystallin polypeptides. Comparison of the carp N-terminal sequences with those of mammalian and amphibian γ-crystallins also showed a high degree of homology present in their N-terminal segments despite the dissimilarity of amino acid compositions of fish γ-crystallins to those of higher classes of vertebrates. The distinct yet closely-related partial sequences of carp γ-crystallins could account for the profound microheterogeneity detected in the characterization of carp crystallins, suggesting the presence of a multigene family for γ-crystallin in the lowest class of vertebrates, i.e. the fish.

Lens crystallin *Protein sequence* *Sequence homology* *Microheterogeneity* *Multigene family*

1. INTRODUCTION

Lens crystallins form a complex group of evolutionarily related polypeptides of vertebrate lenses and comprise several major classes of proteins with various extents of heterogeneity [1–3]. Recent progress in recombinant-DNA techniques has facilitated the elucidation of gene structures and their corresponding protein sequences from several different species [4–7]. We have successfully characterized mRNA from the carp lenses and established the complementary DNA (cDNA) library from the same species of fish [8]. In this communication we wish to report the N-terminal sequences of the four major γ-crystallin polypeptides isolated from the cation-exchange chromatography of carp γ-crystallins. The closely-related N-terminal sequences for these subfractions of γ-crystallins attest to the existence of a multigene family for γ-crystallins of the fish, similar to those found for the higher classes of vertebrates [9,10].

2. MATERIALS AND METHODS

Carp (*Cyprinus carpio*) lens crystallins were isolated and characterized as described before [11,12]. The γ-crystallin fraction obtained from gel-permeation chromatography of total carp soluble crystallins on Fractogel TSK HW-55 (S) was further separated into its subfractions on TSK CM-650 (S) cation-exchange column with a linear gradient of 0.05–0.25 M ammonium acetate in the presence of 0.1% 2-mercaptoethanol, pH 5.9.

Discontinuous native gel (3% stacking/8% resolving gel) electrophoresis was done according to Davis [13]. SDS-polyacrylamide slab gel (5% stacking/14% resolving gel) was as described [14] with some modifications.

N-terminal sequences of the four major frac-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50 © 1986 Federation of European Biochemical Societies

tions from the cation-exchange column were carried out by automated Edman degradation with a gas-phase protein sequencer (model 470A, Applied Biosystems). The lyophilized crystallin samples were dissolved in 200 μl of 0.1% SDS and 10 μl each containing about 5 nmol of γ-crystallins used for sequence determinations. Since the authentic phenylthiohydantoin (PTH) derivative of cysteine was not usually recovered in good yield to be detected with a reversed-phase HPLC system, the alkylation of the crystallin samples with iodoacetamide by the procedure of Crestfield et al. [15] was also performed for the determination of PTH derivative of *S*-carboxyamidomethylcysteine.

3. RESULTS AND DISCUSSION

The preliminary characterization of lens crystallins from two species of carp (*Cyprinus carpio* and *Ctenopharyngodon idellus*) has indicated a major predominance of γ-crystallin at both the level of cDNA and in the in vitro translation products [8]. Due to the unusually high content of methionine (14%) in their amino acid compositions, it is deemed necessary to determine the partial N-terminal protein sequences in order to locate the initiation codon of the open reading frame in one of the cloned cDNAs. It is of interest to find a profound heterogeneity present in the amino-terminal segments of each purified fraction and a homology of their sequences to those of frog and calf γ-crystallins reported previously [9,16].

Fig.1 showed the elution pattern of the total γ-crystallin fraction isolated from the gel-permeation chromatography [8] on a cation-exchange column using a salt-gradient elution buffer. Efforts have been made to ensure a conservative collection of the 4 major peaks for sequence analyses, the shoulders and trailing edges of elution peaks being excluded. These fractions represented the 4 major bands shown in the gel electrophoresis (lane 3 of fig.2), indicative of the presence of at least 4 isoproteins (charge isomers) with a similar molecular mass of about 20 kDa as determined by SDS-PAGE (lane 2 of fig.2). Some faint minor bands could also be detected on the gel despite their relatively smaller amounts as compared to the 4 major subfractions. The complexity of γ-crystallin polypeptides is similar to those found for the mammalian γ-crystallins [10,17]. It is a prerequisite to obtain the detailed information in their primary sequences in order to establish the basis for the multiplicity of the crystallin polypeptides. Protein sequencing and molecular cloning of the cDNAs encoding the corresponding crystallin peptides were hence simultaneously started for the carp crystallins. Table 1 summarized the sequenced N-terminal segments for the 4 subfractions of γ-crystallins.

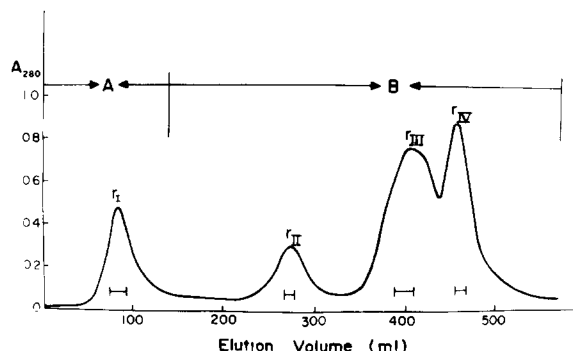

Fig.1. Cation-exchange chromatography on TSK CM-650(S) (2.5 × 15 cm column) of crude γ-crystallin fraction isolated from gel-permeation chromatography [12]. The column eluents (2.4 ml/tube per 2.7 min) were monitored for absorbance at 280 nm. About 50 mg of crude crystallins dissolved in the starting buffer of 0.05 M ammonium acetate with 0.1% 2-mercaptoethanol, pH 5.9, was applied to the column equilibrated in the same buffer. Elution was carried out in two steps: (A) elution with starting buffer and (B) elution with a linear gradient of 0.05–0.25 M ammonium acetate in 0.1% 2-mercaptoethanol, pH 5.9. Solid bars under the 4 major subfractions (γ-I to γ-IV) indicate pooled fractions used for N-terminal sequence analyses.

It is clear that the four subfractions of γ-crystallin are closely related to each other with almost identical N-terminal sequences except minor changes at some of the residues (table 1). In fact, several crystallin cDNA clones have been isolated and one of the clones was found to encode the N-terminal segment of γ-II (Chang et al., manuscript in preparation). There are 5 positions in γ-II at which two amino acids have been identified by Edman degradation in a gas-phase sequencer. The reasons for these micro-heterogeneities are still unknown. Although the existence of allelic variants (genetic polymorphism) from the pooled lenses of different individual

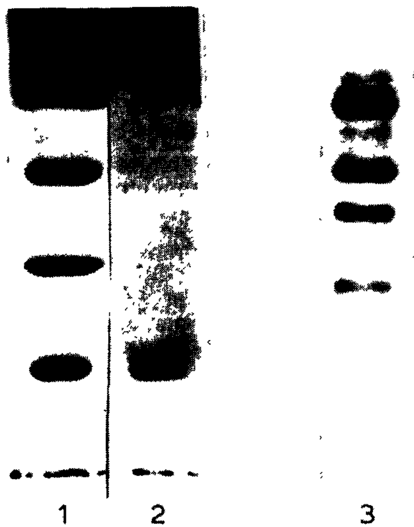

Fig.2. Gel electrophoresis of carp γ-crystallins under native and denaturing conditions. Lanes 1 and 2: SDS-PAGE in the presence of 5 mM dithiothreitol. (1) Standard protein markers (transferrin, 80 kDa; bovine serum albumin, 66 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 20 kDa) and (2) crude γ-crystallins with a molecular mass of about 20 kDa. Lane 3: electrophoresis of crude γ-crystallins under nondenaturing conditions. The fastest moving band corresponds to γ-I and the slowest one γ-IV.

carps may not be ruled out, it is more likely that these polypeptides are the products of various nonallelic genes similar to those found in γ-crystallins of mammalian lenses [17,18]. The multiple nonallelic genes encoding γ-crystallins are not uncommon among the higher classes of vertebrate lenses [4,9]. For instance, there are more than 5 γ-crystallin polypeptides encoded by total rat lens mRNAs [4]. Judging from the extensive heterogeneity present even in the short segments of these 4 γ-crystallin polypeptides, it is to be expected that the complexity of γ-crystallin genes in the fish may be more pronounced than those found in the higher classes of vertebrates.

Comparison of the amino-terminal sequences of γ-crystallins from the 4 different species (fig.3) with distant evolutionary relationships showed a high degree of sequence homology. The variations at some of the residues are also conservative in nature, i.e. Lys/Arg at residue no.9 and Phe/Leu at residue no.25. The percentage of sequence identity for the 25 N-terminal residues is 64% (16 out of 25) between the fish and mammalian species. It is noteworthy that the amino acid composition of carp γ-crystallin is quite different from those of amphibian and mammalian crystallins [8,12], with a characteristically high methionine content (14%) similar only to that of invertebrate squid crystallin [19]. However there is no homology found between the N-terminal segments of carp and squid crystallins ([20] and unpublished data).

It is estimated that there are about 25 methionine residues in the carp γ-crystallin on the basis of its methionine content (14%) and the revised sequence [21] of calf γ-II [16] with 174 amino acid residues. We have so far detected only

Table 1

The amino-terminal sequences of γ-crystallin polypeptides from the carp lenses

Residue no.	γ-I	γ-II	γ-III	γ-IV
1	Gly	Gly	Gly	Gly
2	Lys	Lys	Lys	Lys
3	Ilu	Ilu/Val	Ilu	Val/Ilu
4	Val/Thr	Ilu	Thr	Thr
5	Phe	Phe	Phe	Phe
6	Tyr	Tyr	Tyr	Tyr
7	Glu	Glu	Glu	Glu
8	Asp	Asp	Asp	Asp
9	Lys	Lys	Lys	Lys
10	Asn	Asn	Asn	Asn
11	Phe	Phe	Phe	Phe
12	Gln	Gln	Gln	Gln
13	Gly	Gly	Gly	Gly
14	Arg	Leu	Leu	Leu
15	His/Ser	Ser/His	Asn/His	Ser/Asn
16	Tyr	Tyr	Tyr	Tyr
17	Glu	Glu/Asp	Glu/Asp	Glu/Asp
18	Cys	Cys	Thr/Cys	Thr/Cys
19	Asp	Asp/Met	Met	Met
20	Ser/His	Ser/His	His/Ser	Ser/His
21	Asp	Asp	Asp	Asp
22	Cys	Cys	Cys	Cys
23	Ser/Ala	Ala	–	Ala
24	Asp	Asp	–	–
25	Phe/Met	Phe	–	–

The residues with two amino acids denote those positions where two PTH derivatives were detected by gas-phase sequencing. Dash lines indicate the unidentified amino acids

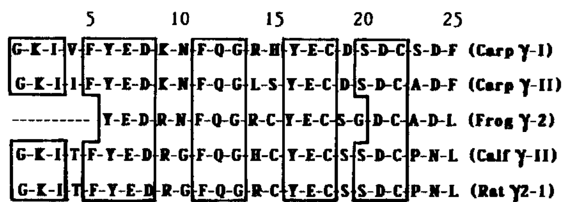

Fig.3. Comparison of N-terminal sequences of carp γ-crystallin polypeptides with those of frog, calf and rat crystallins. The sequences listed for carp γ-crystallins were taken from this study (table 1) and those for other species from [9,21]. The sequence of the frog γ-2 reported in [9] lacks the first 5 N-terminal amino acids. Identical residues in 5 sequences are boxed. Amino acid residues are represented by one-letter symbols.

one methionine residue in the N-terminal segment of 25 amino acids. The uneven distribution of these methionine residues may probably contribute more sequence variation between carp and other mammalian γ-crystallins in their middle and C-terminal regions of the sequences [22]. This needs to await the nucleotide sequence analysis of several cDNA clones for carp γ-crystallins, which is currently in progress.

In conclusion, the partial sequence analysis of 4 major γ-crystallin polypeptides has for the first time revealed the presence of microheterogeneity at their N-terminal segments in the lowest class of vertebrates, the fish. The sequence comparison of the γ-crystallin polypeptides from 3 major classes of vertebrates has provided some support for the hypothesis of divergent evolution in the vertebrate γ-crystallin family [23].

ACKNOWLEDGEMENTS

We thank Dr T.-Y. Liu of the Division of Biochemistry and Biophysics, Food and Drug Administration, Bethesda, MD, USA for providing the gas-phase sequencer in the initial stage of sequence analysis. This work was supported in part by Academia Sinica and the National Science Council, Taipei, Taiwan, Republic of China.

REFERENCES

[1] Bloemendal, H. (1982) CRC Crit. Rev. Biochem. 12, 1–38.
[2] De Jong, W.W. (1981) in: Molecular and Cellular Biology of the Eye Lens (Bloemendal, H. ed.) pp.221–278, Wiley, New York.
[3] Harding, J.J. and Crabbe, M.J.C. (1984) in: The Eye (Davson, H. ed.) 3rd edn, pp.207–492, Academic Press, New York.
[4] Dodemont, H.J., Andreoli, P.M., Moormann, R.J.M., Ramaekers, F.C.S., Schoenmakers, J.G.G. and Bloemendal, H. (1981) Proc. Natl. Acad. Sci. USA 78, 5320–5324.
[5] King, C.R., Shinohara, T. and Piatigorsky, J. (1982) Science 215, 985–987.
[6] Tomarev, S.I., Dolgilevich, S.M., Kozlov, K.A., Zinovieva, R.D., Dzhumagaliev, E.B., Kogan, G.L., Skobeleva, N.A., Mikhailov, A.T., Frolova, L.Yu. and Gause, G.G. jr (1982) Gene 17, 131–138.
[7] Yasuda, K., Kondoh, H., Okada, T.S., Nakajima, N. and Shimura, Y. (1982) Nucleic Acids Res. 10, 2879–2891.
[8] Chiou, S.-H., Chang, T., Chang, W.-C., Kuo, J. and Lo, T.-B. (1986) Biochim. Biophys. Acta 871, 324–328.
[9] Tomarev, S.I., Zinovieva, R.D., Chalovka, P., Krayev, A.S., Skryabin, K.G. and Gause, G.G. jr (1984) Gene 27, 301–308.
[10] Den Dunnen, J.T., Moormann, R.J.M., Lubsen, N.H. and Schoenmakers, J.G.G. (1986) J. Mol. Biol. 189, 37–46.
[11] Chiou, S.-H., Chang, W.-C., Kuo, J., Pan, F.-M. and Lo, T.-B. (1986) FEBS Lett. 196, 219–222.
[12] Chiou, S.-H. (1986) FEBS Lett. 201, 69–73.
[13] Davis, B.J. (1964) Ann. NY Acad. Sci. 121, 404–427.
[14] Laemmli, U.K. (1970) Nature 227, 680–685.
[15] Crestfield, A.M., Moore, S. and Stein, W.H. (1963) J. Biol. Chem. 238, 622–627.
[16] Croft, L.R. (1972) Biochem. J. 128, 961–970.
[17] Shinohara, T., Robinson, E.A., Appella, E. and Piatigorsky, J. (1982) Proc. Natl. Acad. Sci. USA 79, 2783–2787.
[18] Breitman, M.L., Lok, S., Wistow, G., Piatigorsky, J., Treton, J.A., Gold, R.J.M. and Tsui, L.-C. (1984) Proc. Natl. Acad. Sci. USA 81, 7762–7766.
[19] Chiou, S.-H. (1984) J. Biochem. (Tokyo) 95, 75–82.
[20] Siezen, R.J. and Shaw, D.C. (1982) Biochim. Biophys. Acta 704, 304–320.
[21] Summers, L.J., Slingsby, C., Blundell, T.L., Den Dunnen, J.T., Moormann, R.J.M. and Schoenmakers, J.G.G. (1986) Exp. Eye Res. 43, 77–92.
[22] Croft, L.R. (1973) Biochim. Biophys. Acta 295, 174–177.
[23] Wistow, G., Slingsby, C., Blundell, T., Driessen, H., De Jong, W.W. and Bloemendal, H. (1981) FEBS Lett. 133, 9–16.