Biochimica et Biophysica Acta 910 (1987) 89–92
Elsevier

BBA 90104

BBA Report

Cloning and sequencing of a carp β_s-crystallin cDNA

Tschining Chang and Wen-Chang Chang

Institute of Biochemical Sciences, National Taiwan University and Institute of Biological Chemistry, Academia Sinica, Taipei (Taiwan, China)

(Received 9 February 1987)

Key words: cDNA cloning; Nucleotide sequence; β_s-Crystallin; (Carp lens)

The mRNAs were extracted from common carp (*Cyprinus carpio*) lenses, purified, reverse transcribed, dC tailed and cloned into *Escherichia coli* with pBR322 as vector. The cloning efficiency was around $1 \cdot 10^7$ colonies per μg of mRNA. A clone (pC20) was found by hybrid-arrested translation to contain the cDNA related to carp crystallins. However, comparison of the derived amino-acid sequence with bovine γ-II and β_s-crystallins indicates that this carp crystallin sequence resembles closely the bovine β_s-crystallin and should be better classified as such except that this fish sequence does not contain the N-terminal 'arm' of four amino-acid residues present in bovine β_s-crystallin.

There are four major families of structural proteins in the lens of vertebrate, known as α, β, γ and δ crystallins. The crystallins of each class are expressed at a precise stage of lens development [1]. In rat and newt, γ-crystallin is the latest expressed one in the embryonic lens epithelium but the most abundant in the fiber cells [2]. In various forms of cataract, γ-crystallin was found to be reduced in amounts [3]. Sequencing studies of γ-crystallin DNA from rat [4,5], mouse [6,7], frog [8], and man [9] have shown that the γ-crystallins are evolutionally well conserved. Recently another monomeric variant closely related to γ-crystallin has been reported as the β_s type [10]. Although considerable progress has been made as indicated above, little is known about the structure of crystallins of the fish, which is the most primitive class in the vertebrate. Here, we report the nucleotide sequence of a cDNA corresponding to a β_s-crystallin from the lens of the carp (*Cyprinus carpio*). As far as we know, this is the first reported crystallin cDNA sequence of the fish.

The mRNAs of carp (*C. carpio*) lenses were purified and identified as described previously [11]. The cDNA library was constructed following the method of Gubler and Hoffmann [12] with pBR322 as vector. Positive clones were selected by the method of hybrid-arrested translation [13].

One clone (hereafter named as pC20) was able to abolish the in vitro translation of carp crystallins almost completely when its plasmid DNA was denatured and allowed to hybridize with the mRNA of carp lenses. Therefore this pC20 must contain a cDNA corresponding to one of the carp crystallins. The cDNA contained in pC20 was then subjected to sequence determination [14] as shown in Fig. 1. The complete nucleotide sequence with those of other known γ-crystallins are shown in Fig. 2. Excluding the dG.dC homopolymer, which was introduced during cloning, the length of the cDNA is 700 nucleotide pairs. The longest open reading frame in the cDNA encodes a polypeptide with 174 amino acids. The size of

Correspondence: W.-C. Chang, Institute of Biological Chemistry, Academia Sinica, P.O. Box 23-106, Taipei, Taiwan, China.

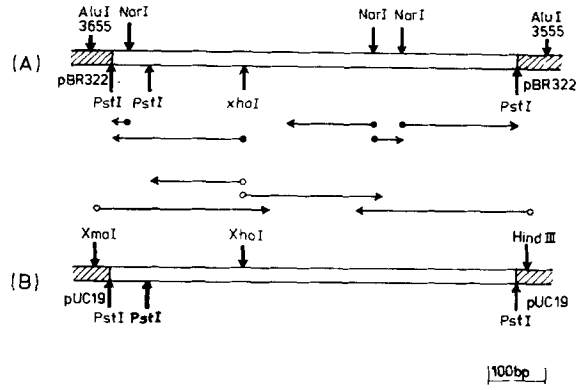

Fig. 1. The sequencing strategy and restriction map for pC20. (a) cDNA with poly dC tailing was inserted at the PstI site of pBR322. (b) The same cDNA was subcloned at PstI site of pUC 19. The solid (for the pBR system) and open circles (for the pUC system) are the sites for [α-^{32}P]nucleotide labelling by DNA polymerase I Klenow fragment.

this protein is similar to the known β_s and γ-crystallins [4–10]. The 5′-end noncoding region upstream from the initiator ATG codon of this open reading frame consists of 30 nucleotides. In 3′-end noncoding region, we found the polyadenylation signal, AATAAA, and a stretch of 36 A residues following this signal. Fig. 3 shows the comparison of the derived amino-acid sequence with bovine β_s and γ-crystallins. It reveals 68% and 56% overall homology when the carp sequence is compared with bovine β_s and γ-II, respectively. Even when the respective motif 3 sequences are compared, the homology is as high as 63% between carp and ox. The four-fold homology also shows that Gly-13 and Ser-34 are absolutely conserved at the topology equivalent positions in all motifs in the three proteins while an aromatic residue is always pre-

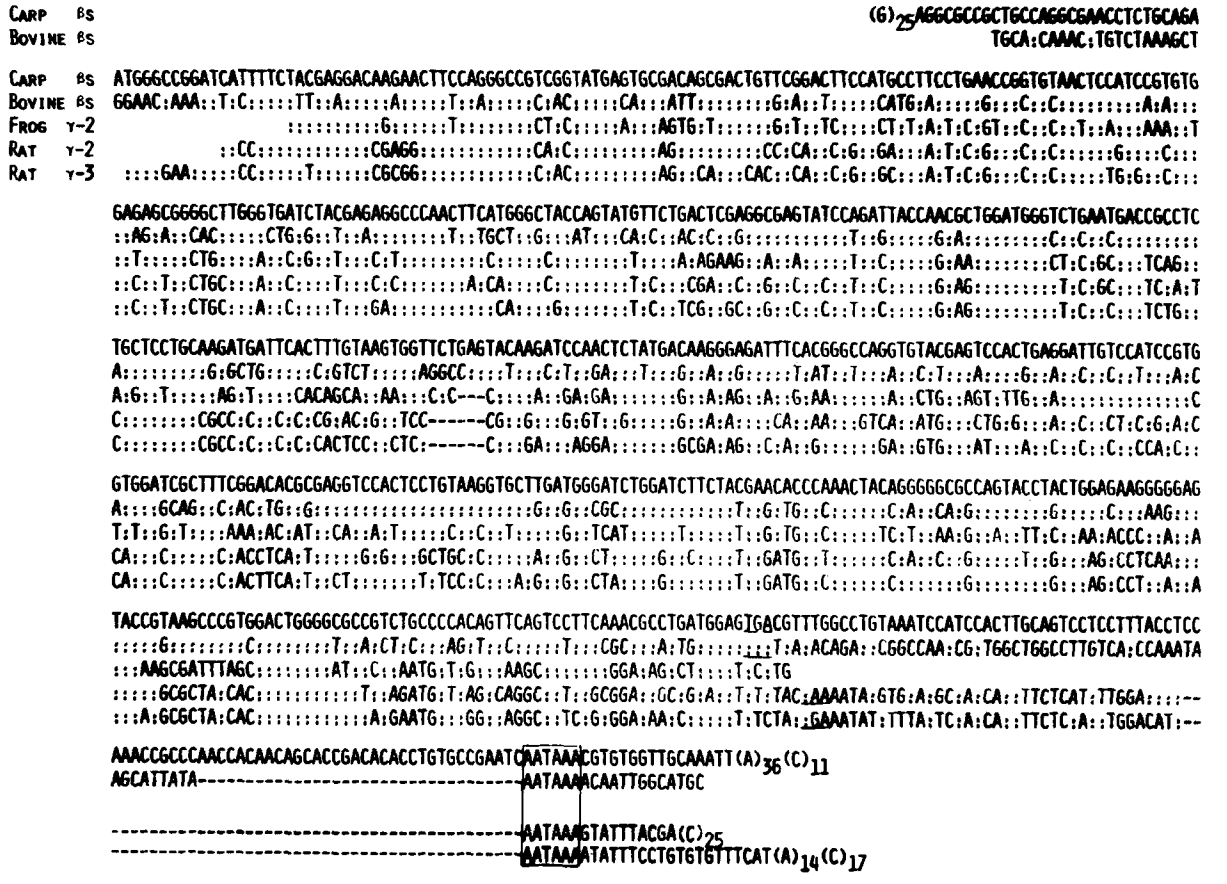

Fig. 2. Comparison of cloned cDNA sequences coding for β_s and γ-crystallins. The sequences are aligned according to their corresponding amino-acid sequence. The stop codons for the longest open reading frame are underlined. Polyadenylation signals AATAA are blocked.

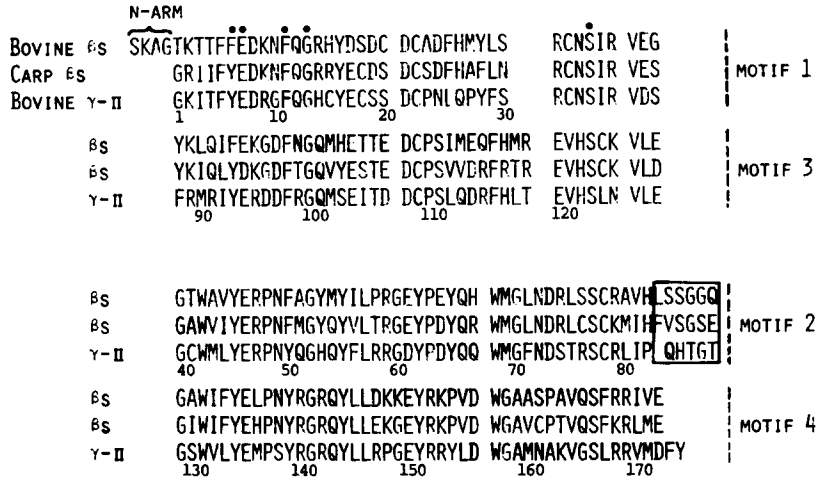

Fig. 3. Comparison of the carp crystallin sequence with that of bovine γ-II and β_s crystallins. Protein sequences are shown in the single-letter amino-acid code. The sequences are displayed by placing motif 1 on top of motif 3 and motif 2 on top of motif 4 so that topologically equivalent amino-acid residues can be easily compared. Solid circles indicate the conserved residues (see text). Connector peptides are blocked.

sent at positions topologically equivalent to Tyr-6 and Phe-11 of γ-II crystallin. However, the carp addition as well as one human γ-crystallin [15] sequence breaks the rule of having a Glu at positions topologically equivalent to Glu-7 of γ-II by substitution with an Asp at position 94 (Fig. 3). This substitution actually is a conservative one, since it will not change the charge of the protein. When the connector peptide and the C-terminus are considered, the carp sequence again resembles more closely the bovine β_s than the γ-II: the carp sequence and bovine β_s both have a hexapeptide connector in contrast to the pentapeptide in γ-II and the β_s and carp sequence lack the C-terminal tail (Phe-Tyr) of the γ-II. Based on these homology and structural comparisons it seems reasonable to classify this carp sequence as a β_s-crystallin, although β_s and γ-II are very homologous to each other and may have diverged from the same βγ-superfamily [10]. It has to be pointed out that the carp sequence lacks the N-terminal arm of four residues of the bovine β_s and in this respect it is more like bovine γ-II.

The crystal structure of bovine γ-II crystallin has been determined with a resolution of 1.9 Å [16]. Recently, the tertiary structure of a bovine β_s crystallin has also been modelled [10] by interactive computer graphics on the coordinates of γ-II crystallin. All the structurally important residues in the bovine γ-II and β_s-crystallins are completely conserved in the carp β_s-crystallin, such as Gly-13, -52, -100 and -141 (based on the numbering of γ-II), Ser-34, -77, -123 and -166 and the Trp-42, -68, -131 and -157. Therefore, the carp β_s must assume the tertiary structure of the γ-crystallins.

In conclusion, a crystallin sequence has been deduced from a cDNA library of carp lens which belongs to the β_s type on the basis of sequence homology and structural comparisons. The existence of β_s crystallin in fishes has been predicted from the phylogenetic studies [10] and seems to be confirmed by the present study.

References

1 Piatigorsky, J. (1981) Differentiation 19, 134–153
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 Garber, A.T., Stirk, L. and Gold, R.J.M. (1982) Exp. Eye Res. 36, 165–169
4 Moormann, R.J.M., Den Dunnen, J.T., Bloemendal, H. and Schoenmakers, J.G.G. (1982) Proc. Natl. Acad. Sci. USA 79, 6876–6880
5 Den Dunnen, J.T., Moormann, R.J.M., Lubsen, N.H. and Schoenmakers, J.G.G. (1986) J. Mol. Biol. 189, 37–46

6 Lok, S., Tsui, L-C., Shinohara, T., Piatigorsky, J., Gold, R. and Breitman, M. (1984) Nucleic Acids Res. 12, 4517–4529
7 Lok, S., Breitman, M.L., Chepelinsky, B., Piatigorsky, J., Gold, R.J.M. and Tsui, L-C. (1985) Mol. Cell. Biol. 5, 2221–2230
8 Tomarev, S.I., Zinovieva, R.D., Chalovka, P., Krayev, A.S., Skryabin, K.G. and Gause, G.G., Jr. (1984) Gene 27, 301–308
9 Meakin, S.O., Breitman, M.L. and Tsui, L-C. (1985) Mol. Cell. Biol. 5, 1408–1414
10 Quax-Jeuken, Y., Driessen, H., Leunissen, J., Quax, W., De Jong, W. and Bloemendal, H. (1985) EMBO J. 4, 2597–2602
11 Chiou, S-H., Chang, T., Chang, W.C., Kuo, J. and Lo, T.B. (1986) Biochim. Biophys. Acta 871, 324–328
12 Gubler, U. and Hoffmann, B.J. (1983) Gene 25, 263–269
13 Paterson, B.M., Toverts, B.E. and Kuff, E.L. (1977) Proc. Natl. Acad. Sci. USA 74, 4370–4374
14 Maxam, A.M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560–564
15 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
16 Wistow, G., Turnell, B., Summers, L., Slingsby, C., Moss, D., Miller, F., Lindley, P. and Blundell, T. (1983) J. Mol. Biol. 170, 175–202