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Predicted Secondary and Tertiary Structures of Carp γ-Crystallins with High Methionine Content: Role of Methionine Residues in the Protein Stability[1]

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A systematic structural comparison of several carp γ-crystallins with high methionine contents was made by the secondary-structure prediction together with computer model-building based on the established X-ray structure of calf γ-II crystallin. The overall surface hydrophilicity profile and the distribution of helices, β-sheets, and β-turns along the polypeptide chains are very similar among these carp γ-crystallins. In addition, their general polypeptide packing is close to the characteristic 2 domain/4 motif Greek key three-dimensional conformation depicted for the calf γ-II crystallin. Interestingly, most hydrophobic methionine residues are located on the protein surface with only a few buried inside the protein surface or in the interface between two motifs of each domain. The exposed hydrophobic and polarizable methionine cluster on the protein surface may have a bearing on the crystallin stability and dense packing in the piscine species, and probably also provides a malleable nonpolar surface for the interaction with other crystallin components for the maintenance of a clear and transparent lens.

γ-Crystallin is one of three major classes of lens proteins present in all vertebrates except the bird class, which is absent or present in decreased amounts in crystalline lenses. Extensive protein-sequence homology between γ-crystallins from the evolutionarily lower piscine class and the more advanced mammalian class exists despite their dissimilarity in amino acid composition and weak immunological cross-reactivity (*1, 2*). The γ-crystallin comprises about 20% of the total water-soluble proteins of calf lens and is found in larger amounts in the nucleus than the cortex. The γ-crystallin, unlike other lens crystallins, is monomeric in solution, and it has the highest sulfhydryl content of all crystallins. X-ray crystallographic investigation by Blundell *et al.* (*3, 4*) has shown that the protein has a symmetrical structure of two globular domains packed together with a single connection. It is suggested that the stability of γ-crystallin may be due to the interaction of polarizable amino acid groups and sulfur-containing residues present in the core of each domain.

The characterization and comparison of γ-crystallins from different species belonging to all major classes of vertebrates has revealed amino acid compositions with unusually high methionine content (12-14%) in some aquatic animal species such as various teleostean fishes and squid (*5*). We have recently determined the primary sequences of carp crystallins (*6-8*), two γ-crystallins and one γ-like βs crystallin [recently classified and renamed γs crystallin (*9*)] by molecular cloning and cDNA sequencing. The three determined sequences lay a firm basis for complete analysis and comparison of their secondary structures and location of the hydrophobic residues involved in the core packing and distribution of various methionine residues. Since the high-resolution X-ray structure of an analogous γ-II crystallin from calf has been established (*3, 4*), this was used as a basis for computer-graphics model-building of carp γ-crystallins based on the coordinates of this three-dimensional structure. Some important insights into the location and distribution of the methionine residues in carp γ-crystallins have been revealed through the examination of these graphic models.

MATERIALS AND METHODS

Secondary Structure Prediction—A consensus prediction of two methods was produced for the three carp γ-crystallin sequences (*6-8*) using the MacVector sequence analysis software for Macintosh computers (International Biotechnologies, New Haven, CT). The prediction methods employed were those of Chou and Fasman (*10*) and Garnier *et al.* (*11*). The program sums the predictions at each residue in the sequence to produce a joint result.

Hydrophilicity Profile—This profile graphs the local hydrophilicity of a protein along its amino acid sequence based on the Kyte-Doolittle hydropathy scale (*12*). The signs of the values have been reversed in order to plot the hydrophilicity instead of hydrophobicity scale. A window of size $n=7$ is run along the length of the protein; for each window, the hydropathy values of the 7 amino acids are summed and divided by 7 to obtain the average hydrophilicity per residue for the window. Values above the axis

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The sequence data of carp γ-crystallins have been deposited in the EMBL/GenBank Data Banks under the accession numbers X12902 (γ-m1) and X12903 (γ-m2), respectively.
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TABLE I. **The sequences of calf γ-II (*3, 4, 15*), carp γ-m1 (*7*), γ-m2 (*7*), and γs crystallins (*6*) aligned for optimal homology.**

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Bovine γ-II  *1  *GKITFYEDRG*11 *FQGHCYECSS*21 *DCPNLQPYFS*31 *RCNSIRVDSG*41 *CWMLYERPNY
Carp   γ-m1  *1  *GKIIFYEDRN*11 *FQGRSYDCMS*21 *DCSDISSYLS*31 *RVGSIRVESG*41 *CFMVYERNSY
Carp   γ-m2  *1  * KVTFYEDRN*10 *FQGRSYDCMS*20 *DCADFSSYMS*30 *RCHSCRVSHG*40 *CWMMYDQPNY
Carp   γs    *1  *GRIIFYEDKN*11 *FQGRRYECDS*21 *DCSDFHAFLN*31 *RCNSIRVESG*41 *AWVIYERPNF

Bovine γ-II  *51 *QGHQYFLRRG*61 *DYPDYQQWMG*71 *FN   DSIRS*78 *CRLIPQHTGT*88 * FRMRIYERD
Carp   γ-m1  *51 *MGNQFFLRRG*61 *EYHDMQRMMS*71 *MGMMFDTIRS*81 *CRMIPPYRGS*91 * YRMRIYERD
Carp   γ-m2  *50 *MGNQYFFRRG*60 *EYADYMSMFG*70 *MS   NCIRS*77 *CRMIPMHRGS*87 * YRMRIYERE
Carp   γs    *51 *MGYQYVLTRG*61 *EYPDYQRWMG*71 *LN   DRLCS*78 *CKMIHFVSGS*88 *EYKIQLYDKG

Bovine γ-II  *97 *DFRGQMSEIT*107*DDCPSLQDRF*117*HLSEVHSLNV*127*LEGSWVLYEM*137*PSYRGRQ YL
Carp   γ-m1  *100*NFGGQMHEVM*110*DDCDNIMERY*120*RMSDWQSCHV*130*MDGHWLFYEQ*140*PHYRGRMWYF
Carp   γ-m2  *96 *NFMGQMYEMA*106*DDCDSIMDRY*116*RMPHCQSCHV*126*MDGHWLMYEQ*136*PHYRGRMWYF
Carp   γs    *98 *DFTGQVYEST*108*EDCPSVVDRF*118*RTREVHSCKV*128*LDGIWIFYEH*138*PNYRGRQ YL

Bovine γ-II  *146*LRPGEYRRYL*156*DWG  AMNAK*164*VGSLRRVMDF*174*Y
Carp   γ-m1  *150* RPGEYRSFR*159*DMGYSNM  R*167*FMSMRRITDM*177*C
Carp   γ-m2  *146* RPGEYRSFS*155*NMG  GM  R*161*FMSMRRIMDS*171*WY
Carp   γs    *147*LEKGEYRKPV*157*DWG  AVCPT*165*VQSFKRLME
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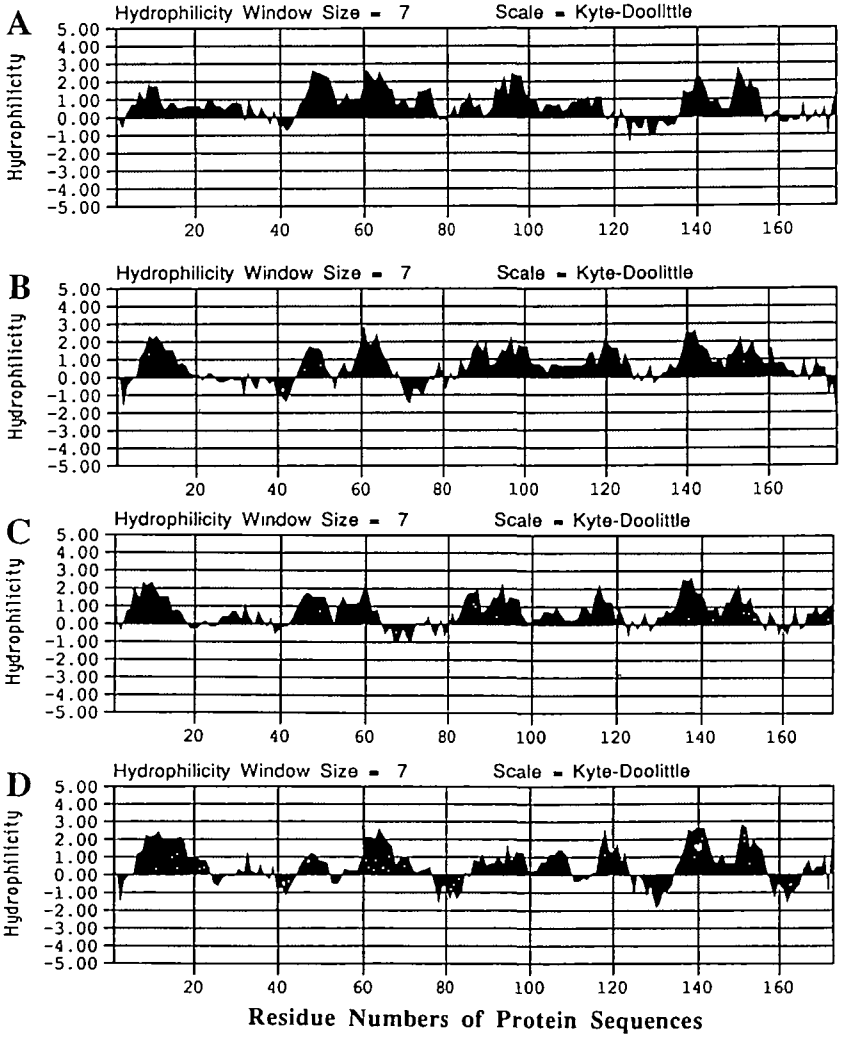

Fig. 1. **Hydropathy profiles for bovine γ-II (A), carp γ-m1 (B), carp γ-m2 (C), and carp γs (D) crystallins.** The method for the analysis of the local hydrophilicity of each amino acid along the crystallin sequences based on the modified Kyte-Doolittle scale (*12*) is described in "MATERIALS AND METHODS."

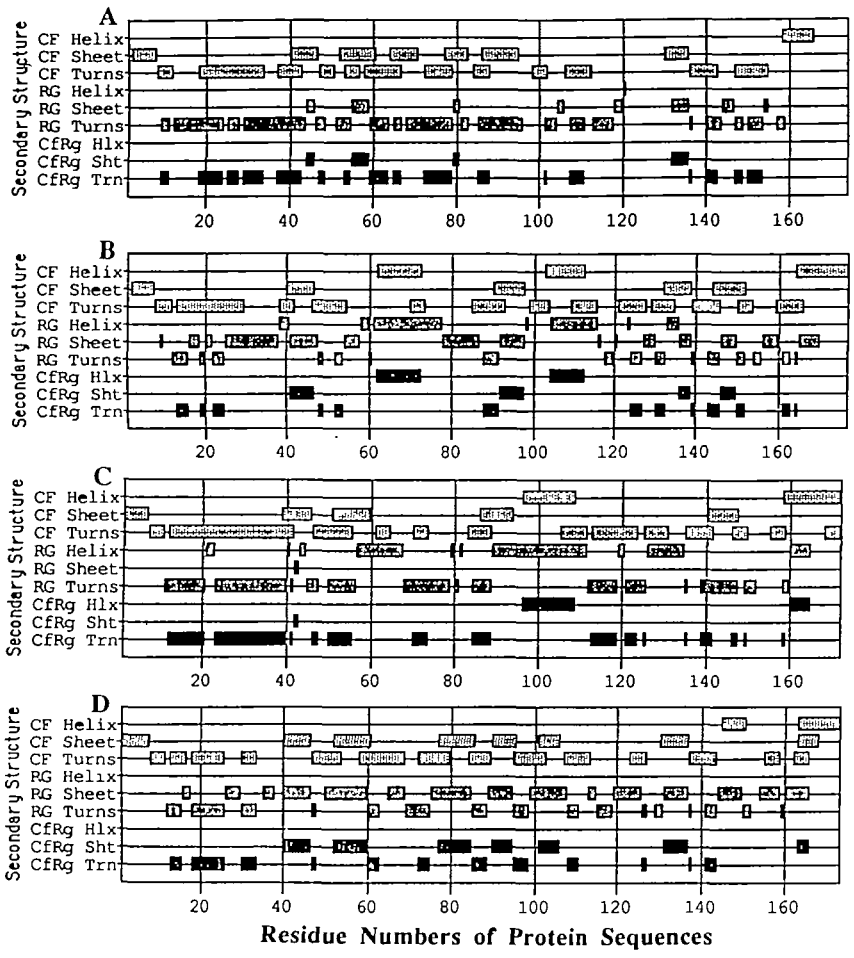

Fig. 2. **Secondary structure predictions of bovine γ-II (A), carp γ-m1 (B), carp γ-m2 (C), and carp γs (D) crystallins.** The analysis of each structural segment (α-helix, β-sheet, and β-turns) along the protein sequences was based on the methods of Chou and Fasman (CF) (*10*) and Garnier *et al.* (RG) (*11*) and a consensus joint result (CfRg).

denote hydrophilic regions which may be exposed on the outside of the protein molecule, and those values below the axis indicate hydrophobic regions which tend to be buried inside the protein.

Molecular Model Building—The sequences of the three carp γ-crystallins were aligned with the sequences of bovine γ-II crystallins from the database of GenBank to locate sequence-conserved regions (Table I). The coordinates of bovine γ-II crystallin which were determined by X-ray diffraction analysis and refined to 1.6 Å (*3, 4*) were used as a template for model-building of the carp γ-m1 and γ-m2 crystallins by employing the graphics program QUANTA (Polygen, 1989) on a Silicon Graphics IRIS 4D/25 workstation.

Under the QUANTA environment, a multiple-sequence alignment algorithm (*13*) was used to align two carp γ-crystallin sequences to the sequence of bovine γ-II crystallin. The main chain coordinates of the carp γ-crystallins were copied from the known bovine γ-crystallin structure, and the region of residues 73–75 (Met-Met-Phe) of carp γ-m1 crystallins (*7, 8*) was determined by a fragment-search process. The coordinates of the side chain and the undefined terminal residues were built and relaxed by using the "regularization modeling" technique in QUANTA. The gap between residues 157 and 158 of carp γ-m2 crystallin was annealed by the CHARM energy minimization process. The complete structures were obtained by 200 cycles of the "region regularization" tool using the "adopted basis set NR" protocol in both minimization stages in order to remove close contacts and reduce strains. The structures were further minimized by heating up to 300 K, equilibration for 0.6 ps (600 steps), and subjection to 200 cycles of Powell conjugate gradient minimization and 300 cycles of conjugate gradient minimization to optimize the hydrogen bonds, ion pair, and hydrophobic interactions. The final models are illustrated in Figs. 3 and 4.

RESULTS AND DISCUSSION

The previous N-terminal sequence analyses of γ-crystallin subfractions from several different species have revealed extensive homology in the amino-terminal segments of fish and mammalian γ-crystallins (*1, 2*), which suggested a common ancestry for this class of vertebrate crystallins. Therefore it is of interest to compare the secondary and tertiary structures of the γ-crystallins from the lowest piscine class with that of the mammalian class to shed some light on the structural evolution of γ-crystallins.

Table I shows the sequences of three carp γ-crystallins and bovine γ-II crystalllin aligned together for comparison. Several gaps were created in these γ-crystallin sequences in order to obtain optimal alignment of these homologous sequences. Figure 1 shows the hydropathy profiles for these 4 γ-crystallins. It is noteworthy that the overall profiles show close similarity in the distribution of hydrophilic

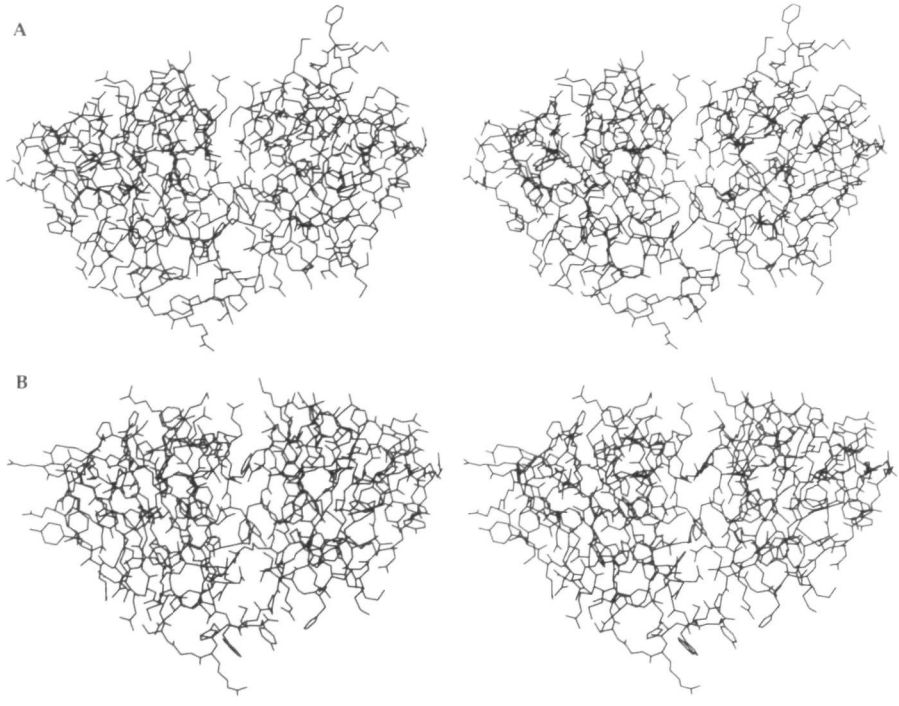

Fig. 3. **Stereo views of the graphic models of carp γ-m1 (A) and γ-m2 (B) crystallins.** The three-dimensional organization of crystallin molecules consisted of N-terminal (right side) and C-terminal (left side) domains, each composed of 2 Greek-key motifs.

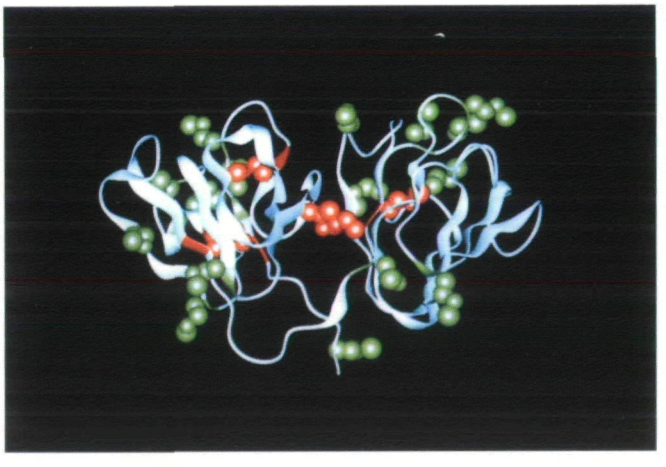

Fig. 4. **Computer graphics of the ribbon drawing of carp γ-m1 crystallin.** All the side-chain atoms of methionine residues are shown by van der Waals ball representations, with those residues exposed on the surface shown in green and those buried inside the proteins or in the interfacial region of two domains shown in red. Carp γ-m1 crystallin comprises 22 methionine residues (12.4%) with 6 buried inside (Met-43, 68, 93, 146, 160, and 170) and 16 exposed on the surface (Met-19, 51, 65, 69, 71, 73, 74, 83, 105, 109, 116, 121, 130, 165, 168, and 176). Carp γ-m2 crystallin with 24 methionine residues (14%) showed an essentially similar distribution pattern of methionine residues on the protein surface.

amino acids along the polypeptide chains despite the differences in amino acid compositions and medial sequence homology between carp and bovine crystallins (only 50–55%). Figure 2 shows the secondary structure prediction based on the methods of Chou and Fasman (*10*) and Garnier *et al.* (*11*). A consensus joint prediction of two methods was produced for these 4 γ-crystallin sequences. Circular dichroism studies of carp (*2*) and bovine (*14*) γ-crystallins have indicated a predominantly β-pleated sheet structure for these crystallins, which is by and large in agreement with the consensus prediction of these two methods. However, either method alone gives a large amount of α-helical structures in the secondary structures of carp γ-m1 and γ-m2, especially the method of Garnier *et al.* (*11*). The secondary-structure prediction showed no great similarity between these four γ-crystallins, even though this was distinctly indicated by the hydropathy profiles in Fig. 1.

Figure 3 shows a stereo view of the complete models of carp γ-m1 and γ-m2. It is evident that the overall 2 domain/4 motif packing similar to that of bovine γ-II crystallin is conserved in each stereo pair for these two carp crystallins. They were also shown to be similar to those of the amphibian *Rana* γ-2 and γ-1 crystallins by molecular modeling reported previously (*15*). The whole γ-crystallin molecule consists of N- and C-terminal domains, each forming a pair of 4-stranded β-sheets (*i.e.* two motifs). Each sheet is also found to be composed of 3 strands from one motif and one strand from the neighboring motif. The two sheets in the center are formed from motifs 2 and 4, while motifs 1 and 3 comprise the outer, solvent-facing sheets. Thus both carp γ-crystallins are structurally similar to bovine γ-II crystallin (*3, 4*), despite considerable sequence divergence and variation amounting to more than 45% between these two evolutionarily remote classes (*6–8*). All the structurally important residues in the bovine γ-II and carp γ-crystallins are to some extent conserved. Therefore, it is expected that carp γ-crystallins must assume a similar tertiary structure to bovine γ-II crystallin.

Figure 4 illustrates the distribution and arrangement of methionine residues on the molecular surfaces and inside the carp γ-m1 crystallin. The most unusual feature of both carp γ-crystallins is the distribution of these hydrophobic

methionine residues on the surface and in the core structure. Inside the crystallin core region, the N-terminal domain is more conserved. But around the variable residues in the C-terminal domain are many sulfur-containing amino acids: Met-109, Met-116, Cys-127, Met-160, Met-168, Met-170, and Met 176 (in carp γ-m1). Moreover, more than 30% of the surface hydrophobic groups are composed of methionine: Met-19, -51, -65, -69, -71, -73, -74, -83 (N-terminal domain of γ-m1); Met-105, -109, -116, -121, -130, -165, -168, -176 (C-terminal domain of γ-m1). Because the core region is usually composed of hydrophobic residues and aromatic groups, interaction of the polarizable moieties between aromatic groups and methionine might contribute significantly to the protein stability (*16*). Recently there has been speculation about the role of methionine in the regulation of the surface polarity of proteins as judged by the unique properties of the thioether sulfur atom in this generally hydrophobic amino acid (*17*). Incorporation of multiple methionine residues into carp γ-crystallins may, therefore, represent one efficient way of optimizing various crystallin interactions and recognitions in the intact lens.

In conclusion, molecular modeling coupled with the prediction of hydropathy profiles for carp γ-crystallins had indicated that these ancient γ-crystallins from the lowest class of vertebrates can form characteristic Greek-key conformations in each of the four motifs similar to those described for bovine γ-II crystallin. It also sheds light on the relative distribution of multiple methionine residues on the surface and in the interior of the protein. The existence of unusually high levels of methionine residues on the crystallin surface suggests that these hydrophobic amino acids with polarizable sulfur atoms might play a role in the recognition and interaction of crystallin molecules in the piscine lenses.

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