MOLECULAR STRUCTURE OF A NOVEL TYPE OF RHODOPSIN

GENE OF THE COMMON CARP (*Cyprinus carpio*)

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The underwater environment limits both the intensity and the spectral bandwidth of ambient light for vision and aquatic survival (Lythgoe, 1980); yet retina anatomy and photoreceptor proteins (opsins) show structural similarities between land and aquatic vertebrates (Yokote, 1982; Nathans et al ., 1986). In order to further understand the comparative aquatic visual physiology we initiated a study on the opsins of the common carp (Cyprinus carpio), an important aquacultured species that occupies a more bottom habitat.

Total retinal RNA of carp was prepared by using the acid/phenol method described by Chomczynski and Sacchi (1987), with an additional gel filtration through a PD-10 column (Pharmacia) to remove melanin. The poly(A)$^+$ RNA was then isolated and the retinal cDNA library was constructed in a lambda gt10 (Promega) using *Escherichia coli* VC257 as the host cells. Plaque hybridization methods were used for screening recombinant phages containing the putative rhodopsin cDNA when goldfish rhodopsin cDNA (Johnson et al., 1993), kindly provided by Dr. Kathy Grant of the Department of Chemistry, Columbia University, was served as a probe. Results showed that a recombinant phage clone containing a 1,584 nucleotides (including 52 polyadenines) rhodopsin cDNA encoding a 354 amino acid polypeptide, was obtained (Tsai et el., 1994). We named it as the type I rhodopsin (Rh I) cDNA.

In this communication, we reported a recombinant phage clone containing a 1,660 nucleotides (including 30 polyadenines) insert encoding another type (type II) of carp rhodopsin (Rh II) cDNA. Polynucleotaides of Rh I and Rh II shared 97.2% identity after the nucleotide sequences of cloned rhodopsin cDNA was determined by following the dideoxynucleotide chain-termination method (Sanger et al., 1976) with a Sequenase kit (US Biochemical Corp., Cleveland). Similar to Rh I cDNA, Rh II cDNA consisted of a single open reading frame of 1,062 nucleotides at positions 72 to 1,133. However, Rh II cDNA had four polyadenylation signals (AATAAA or AATTAAA) at the 3' end untranslated region whereas Rh I cDNA had only one signal. Moreover, Rh II cDNA was 99 base pairs (bp) longer than that of Rh I. This DNA segment located at the end of 3' was specific for

Rh II cDNA of carp.

The deduced amino acid sequence of the carp Rh II differed from that observed for Rh I in only 5 out of 354 residues. The identity of deduced amino acid sequence between type I and type II of carp rhodopsin was 98.6%. Similar to Rh I, Rh II contained all the common residues that were shared with rhodopsin of other species, such as human (Nathans and Hogness, 1984), mouse (Al-Ubaidi et al., 1990), chicken (Takao et al., 1988), lamprey (Hisatomi et al., 1991), goldfish (Johnson et al., 1993) and sand goby (Archer et al., 1992). The palmitoylation site essential to membrane anchoring (Cys-322 and Cys-323), glycosylation (Asn-2 and Asn-15), disulfide bond formation (between Cys-110 and Cys-187), Schiff base counterion (between Glu-113 and Lys-296), interaction with the G-protein transducin (Glu-134 and Arg-135), control site of the equilibrium between photo-activated metarhodopsin I and metarhodopsin II (His-211) and phosphorylation sites (Ser and Thr at C-terminus) were all conserved. However, 5 amino acid residues of Rh II were different from Rh I. Two of them were non-homologous alternation: Val-169 and His-315 of Rh I were replaced by Glu-169 and Asn-315 of Rh II, respectively. Three of the 5 were homologous replacements for residues having similar properties: Val-19 - Ile-19; Ile-54 - Val-54 and Ile-108 - Val-108.

Rhodopsin is a GTP binding protein and belongs to the family of seven-membrane-span receptors (Ferretti et al., 1986; Khorana, 1992). The binding of rhodopsin with either retinaldehyde or the transducin is affected by the tertiary structures and the helix-helix interactions. Hydropathicity profiles of rhodopsin amino acid sequences from various species were analyzed according to Kyte and Doolittle (1982). The similarities observed in the hydropathy plots of carp and bovine rhodopsin suggest similarities in the transmembrane segmentation, i.e., repeating segment of 19-27 residues (Helix I-VII), as well as in high proline and aromatic amino acid contents. The secondary structure of rhodopsins among known species, the most common length of the fourth helix is 20-21 amino acids: 21 residues for human (Nathans and Hogness, 1984), mouse (Al-Ubaidi et al., 1990), bovine (Nathans and Hogness, 1983), chicken (Takao et al., 1988) and carp Rh I (Tsai et al . , 1994); and 20 residues for lamprey (Hisatomi et al., 1991) and fruit fly (Zuker et al., 1985). However, the length of the fourth helix of carp Rh II was 16 amino acids, which was five residues shorter than those of Rh I and most species' rhodopsins. The special formation of the 4th helix structure of carp Rh II may be because the Glu-169 of Rh II was a charged residue while the Val-169 of carp Rh I and other species' rhodopsins were a hydrophobic residue. A study to understand the physiological functions of this novel type of rhodopsin (carp Rh II) is underway.

Acknowledgement-- This work was supported by a grant from the National Science Council, Republic of China (NSC-85-2611-B-002-003).

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