Molecular Cloning of Silver Carp and Bighead Carp Prolactin

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Accepted December 24, 1991

The cDNAs encoding the prolactin of silver carp (scPRL) and bighead carp (bcPRL) have been cloned. Deduced from the nucleotide sequences, both scPRL and bcPRL are composed of 187 amino acid residues. Only one residue is different between scPRL and bcPRL. Homology analysis indicates that scPRL and bcPRL are highly homologous to carp PRL (97%), relatively conserved in relation to PRLs of salmon, trout, and tilapia (64–69%), and diversified from avian and mammalian PRL (30–35%). Similar to PRLs of other species of fish, scPRL and bcPRL lack the first 12 N-terminal residues of avian and mammalian PRLs. © 1992 Academic Press, Inc.

Prolactin (PRL) is a polypeptide hormone secreted from the pituitary gland. Together with growth hormone and placental somatomammotropin, it belongs to a family of hormones evolved from a common ancestral gene (Miller and Eberhardt, 1983; Nicoll *et al.*, 1986). This family has been extended due to the discovery of mouse proliferin (Linzer and Nathans, 1984; Linzer *et al.*, 1985), bovine PRL-related cDNA I (Schuler and Hurley, 1987), rat PRL-like protein A (Deb *et al.*, 1989), and somatolactins of flounder, Atlantic cod, and chum salmon (Ono *et al.*, 1990; Rand-Weaver *et al.*, 1991; Takayama *et al.*, 1991). In fish, PRL is involved in many biological functions such as osmoregulation, reproduction, behavior, and metabolism (Clarke and Bern, 1980).

The amino acid sequence of PRL had been determined chemically or deduced from the nucleotide sequence of cDNA in many species of vertebrates including human (Cooke *et al.*, 1981), cattle (Miller *et al.*, 1981), sheep (Li *et al.*, 1967; Varma *et al.*, 1989), pig (Li, 1976), rat (Cooke *et al.*, 1980), mouse (Linzer and Talamantes, 1985), whale (Tsubokawa *et al.*, 1985),

chicken (Hanks *et al.*, 1989), carp (Yasuda *et al.*, 1987; Chao *et al.*, 1988), salmon (Yasuda *et al.*, 1986; Song *et al.*, 1988), rainbow trout (Mercier *et al.*, 1989), and tilapia (Yamaguchi *et al.*, 1988; Rentier-Delrue *et al.*, 1989). In order to obtain more information about the evolution of PRL, we determined the primary structures of PRL of silver carp (scPRL) and bighead carp (bcPRL) by using cDNA cloning technique. In this paper, the cDNA nucleotide sequences and the deduced amino acid sequences of scPRL and bcPRL are presented and their sequences are compared to those of PRLs from other fish and vertebrates.

MATERIALS AND METHODS

Construction of cDNA library and screening of PRL cDNA. The method used to construct cDNA library was essentially the same as previously described (Chang *et al.*, 1990). Briefly, the pituitary polyadenylated RNAs of silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*H. nobilis*) were prepared from liquid nitrogen frozen tissues by guanidinium/CsCl method (Ullrich *et al.*, 1977) followed by oligo(dT)-cellulose column chromatography. The double-stranded cDNA, synthesized by the method of Gubler and Hoffman (1983), was ligated with *EcoRI* linker and subsequently inserted into *EcoRI* site of λ gt10. To

screen the recombinant DNA clones containing the PRL cDNA from the cDNA libraries, the cDNA coding for carp PRL (cPRL) previously cloned in our laboratory was ^{32}P -labeled by nick-translation and used as a hybridization probe. Plaque hybridization was performed according to the method of Benton and Davis (1977).

Nucleotide sequencing of cDNAs encoding scPRL and bcPRL. The cDNAs encoding scPRL and bcPRL were cleaved with *Ava*II for subcloning. The nucleotide sequences were determined by the dideoxy chain termination method (Sanger *et al.*, 1977) using supercoiled plasmid DNA as template (Chen and Seeburg, 1985).

GGCAAGAGAATAAAGGAACCAGTTAAATAATGGCTGAAGGATCCAGACTATACTTTGCA	60
M A E G S R L Y F A	
-23 -20	
GTGACTGTCCTGATGTGTGCGTTTGTCTCAATCAACGGTGTGCGTCTGAATGATTTACTG	120
V T V L M C A F V S I N G V G L N D L L	
-10 -1 1	
GAAAGAGCCTCTCAACTTTCAGACAAACTTCACTCCCTCAGCACCTCTCTACCAATGAC	180
E R A S Q L S D K L H S L S T S L T N D	
10 20	
CTGGATTCTCACTTTCCTCTGTGGGAGGGTAATGATGCCCGTCCGTCGATGTGCCAC	240
L D S H F P P V G R V M M P R P S M C H	
30 40	
ACATCCTCCCTTCAAATTCCTCAATGACAAAGACCAAGCCCTGAAAGTGCCGGAGGATGAG	300
T S S L Q I P N D K D Q A L K V P E D E	
50 60	
TTACTTTCTTTGGCTCGGTCTCTGCTGCTGGCGTGGTCCGATCCCTCGGCCCTCTCTCC	360
L L S L A R S L L L A W S D P L A L L S	
70 80	
TCTGAGGCGTCCAGCCTGGCAGATCCAGAACGCAACACCATTAACAGCAAGACCAAAGAA	420
S E A S S L A H P E R N T I N S K T K E	
90 100	
CTGCAAGACAACATCAACAGCCTGGGTGCAGGTCTGGAGCGTGTCTTCACAAGATGGGC	480
L Q D N I N S L G A G L E R V V H K M G	
110 120	
TCATCCTCAGACAACCTGTCTCTCTCCCTTTTACAGCAACAGCCTTGGCCAGGATAAA	540
S S S D N L S S L P F Y S N S L G Q D K	
130 140	
ACCTCTCGACTTGTCATTTCCATTTTCTGTTGTCCTGCTTCCGCAGGGACTCCCACAAA	600
T S R L V N F H F L L S C F R R D S H K	
150 160	
ATTGACAGTTTCTCAAAGTTCTGCGGTGCCGGGCAAGAAAGAGACCTGAGATGTGC	660
I D S F L K V L R C R A A K K R P E M C	
170 180 187	
TAAAGTGAAAATGCTACTCTGCTTCTCTCATTGTGGATGTTAGGTAAAAATGGCAGAGCA	720
GTGAGTGATTTGAAATGTTTCTTTATAATACCGCATGGCAAAACATGCCCTTTATTGTTT	780
CAAAGATGTAGATATTTGATTCACTTTCTTATATACTTGAATAAAATACTGGACCAACGG	840
GCATATTATTCTGTCCCCAGAGATAAAATACAACAAAAAGTAAACAAAAGTATTTCAATT	900
AAATTTTAAAAATGAAGGTCATATAAAAGGTCGCATGCAGTCATACTGTTTGATGAATA	960
AATGTATATTGAAATGTTTGACCATGCTCAGTTTAATAAGACATTTGTATAGTAAGTATT	1020
TATTAAGAAATGATGTTGGATCTTTAACACGTTTATTTTAACATTATCTTCTGAAATGCA	1080
ATATTTAAAGCTTTGCACCAATGTCAATAAATTTCTATCATGC-PolyA	1170

FIG. 1. The nucleotide sequence and deduced amino acid sequence of bcPRL cDNA.

RESULTS AND DISCUSSION

When cPRL cDNA was used as a probe, 8 and 9 positive clones were obtained from 5000 and 6000 clones from silver carp and bighead carp pituitary cDNA library, respectively. In this investigation, only the clone with the largest insert of each library was chosen for further study.

The cDNA encoding bcPRL investigated in this study is 1170 bp in length, consisting of a 5'-untranslated region of 30 bp, an open reading frame (ORF) of 630 bp, and a 3'-untranslated region of 510 bp. The ORF encodes a signal peptide of 23 amino acid residues and a putative mature PRL of 187 amino acid residues. Its nucleotide sequence and deduced amino acid sequence are presented in Fig. 1.

The cDNA encoding scPRL investigated in this study is 1060 bp in length. The nucleotide sequence of cDNA encoding scPRL is highly homologous to that of cDNA encoding bcPRL (data not presented). It contains an ORF of 621 bp and a 3'-untranslated region of 436 bp. The scPRL cDNA thus obtained is not full in length, lacking the translation initiation codon, AUG, and the poly(A) tract. Among the 207 amino acid residues coded by ORF, a putative mature PRL of 187 residues and an incomplete signal peptide of 20 residues are present.

The scPRL and bcPRL are similar to each other. Only one of 187 amino acid residues is changed (Fig. 2). When scPRL and bcPRL were compared to cPRL, from another species of Cyprinidae, a high extent of homology (97%) was found (Yasuda *et al.*, 1987; Chao *et al.*, 1988). A similar situation was also found among the PRLs of

three species of Salmonidae: chum salmon, chinook salmon, and rainbow trout where the homology of PRL is 98% or more (Yasuda *et al.*, 1986; Song *et al.*, 1988; Mercier *et al.*, 1989). On the contrary, when PRLs of different fish families were compared (Fig. 2), homology is decreased to the range of 64 to 74% (Cyprinidae vs Salmonidae, 69%; Cyprinidae vs tilapia, 64%; Salmonidae vs tilapia, 74%) (Yasuda *et al.*, 1986, 1987; Song *et al.*, 1988; Yamaguchi *et al.*, 1988; Mercier *et al.*, 1989; Rentier-Delrue *et al.*, 1989).

Most fish PRLs thus far investigated are composed of 187 to 189 amino acid residues except 177 residues are found in one form of two tilapia PRLs (Yamaguchi *et al.*, 1988; Rentier-Delrue *et al.*, 1989). In comparison, chicken PRL has 198 residues while ovine and human PRL have 199 residues. Such difference arises from the absence in fish PRLs of the first 12 N-terminal residues of higher vertebrates. Homology between fish and higher vertebrate PRL is low, about 30 to 35% (Li *et al.*, 1967; Li, 1976; Cooke *et al.*, 1980, 1981; Miller *et al.*, 1981; Linzer and Talamantes, 1985; Tsubokawa *et al.*, 1985; Hanks *et al.*, 1989; Varma *et al.*, 1989). Although the structure of PRLs of fish and higher vertebrates are diversified, two conserved segments of almost identical or chemically similar sequence, residues 46 to 51 and 160 to 180, were found in all PRLs thus investigated. These conserved sequences may be important for the biological activity of PRL. As shown in Fig. 2, PRLs of fish contain four cysteine residues while those of higher vertebrates contain six cysteine residues except for equine PRL (Li and Chung, 1983). The first two cysteine residues of higher

FIG. 2. Comparison of the primary structure of scPRL and bcPRL with PRLs of other fish and higher vertebrates. Residues identical to scPRL are indicated by dashes. Gaps, marked by X, are inserted to maximize structural alignment. References used: carp, Yasuda *et al.* (1987); chum salmon, Yasuda *et al.* (1986); chinook salmon, Song *et al.* (1988); rainbow trout, Mercier *et al.* (1989); *O. mossambicus* I and II, Yamaguchi *et al.* (1988); *O. nilotica* I and II, Rentier-Delrue *et al.* (1989); chicken, Hanks *et al.* (1989); sheep, Varma *et al.* (1989); human, Cooke *et al.* (1981).

	1	10	20	30	40
Silver carp (1)	VGLNDLLERASQLSDKHLSLSTSLTNDLDSHFPPVGRVMM				
Bighead carp (2)	-----				
Carp (3)	-----E-----				
Chum salmon (4)	I--S--M-----R-----K-----M-----				
Chinook salmon (5)	I--S--M-----R-----K-----M-----				
Rainbow trout (6)	I--S--M-----R-----K-----M-----				
<u>Q. mossambicus</u> I (7)	-PI-E-----H-----T-QE-----I---I---				
<u>Q. niloticus</u> I (8)	-PI-E-F----H-----T-QE-----I---I---				
<u>Q. mossambicus</u> II (9)	-PI-E-F----H-----T-QE-----I---I---				
<u>Q. niloticus</u> II (10)	-PI---IY---Q----A--M--QE-G-EAF-ID--LA				
Chicken (11)	LPICPIGSVNCQ-S-GE-FD--VK--HYI-Y--SEIF-EF-ERYAQGRGFIT				
Sheep (12)	TPVCNPNGPGDCQ-S-R--FD--VMV-HYI-N--SEMF-EF-KRYAQGKGFIT				
Human (13)	LPICPGAARQC-T-R--FD--VV--HYI-N--SEMFSEF-KRYTHGRGFIT				

vertebrate PRLs are located in the segment of the first 12 N-terminal residues which are lacking in fish PRLs. All the four cysteine residues of fish PRLs can be aligned at the same positions of PRLs of higher vertebrates. In salmon PRL, two disulfide linkages are also formed between the corresponding cysteine residues of mammalian PRLs, i.e., Cys₄₆₋₁₆₀ and Cys₁₇₇₋₁₈₇ (Yasuda *et al.*, 1986). Whether such disulfide linkages are present in cyprinid PRLs remains to be studied.

ACKNOWLEDGMENT

This work was in part supported by the National Science Council, Republic of China.

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