Molecular Cloning of Silver Carp and Bighead Carp Prolactin

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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), 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 λgt10. 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 AvaII for subcloning. The nucleotide sequences were determined by the dieoxy chain termination method (Sanger et al., 1977) using supercoiled plasmid DNA as template (Chen and Seeburg, 1985).

![Image of nucleotide sequence and deduced amino acid sequence of bcPRL cDNA]

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).
Silver carp (1)  VGLNDLLERASQLSDKLHSLSTSLTNDLDHFPPVGRVM
Bighead carp (2)  -----------------------------------------
Carp (3)  -----------------------------------------
Chum salmon (4)  I--S--M----R---------N----M---------R---
Chinook salmon (5)  I--S--M----R---------N----M---------R---
Rainbow trout (6)  I--S--M----R---------N----M---------R---
O. mossambicus I (7)  -P1-E--------H-------T--QK------N1--
O. niloticus I (8)  -P1-E--------H-------T--QK------N1--
O. mossambicus II (9)  -P1-E--------H-------T--QK------N1--
O. niloticus II (10)  -P1-E--------H-------T--QK------N1--
Chicken (11)  LPICPGSVNCQ-S-QE-FD---VK--HYI-Y--SEIF-EF--EYRAGKBGFIT
Sheep (12)  TPVCPNGPGDCQ-S-R--FD--VMV--HYI--N--SEMF--EF--KRAQKGIFIC
Human (13)  LPICPGGAARCQ-T-R--FD---VV--HYI--N--SEMFSEF--KRYTHGRGFIT

1. PRPSMCHTSSLQIPNKDQALVKPEDELLSLARSLLLLAWSDPLALLASSEASLHXPERN
2.  -----------------------------------------
3.  -----------------------------------------
4.  -----------------------------------------
5.  -----------------------------------------
6.  -----------------------------------------
7.  -----------------------------------------
8.  -----------------------------------------
9.  -----------------------------------------
10. -----------------------------------------
11. KAVNG--------TT-E--K--QQIIHED--N-VGVR-RS-N--N-A--VQRIKE-DTIL
12. MALNS--------PT-E--E--QQTHHEV-M--ILG--RS-N--N-VTG-MKVQ-DAIL
13. KAINS--------AT-E--E--QQMNQKDF----IV-I-RR--N-VTG-MVQEA-AIL

1. INSDKTEGDNLSLGAGIELVHVMKSSSDNLSSLLPSFISNLXGQDKTXRLVNFHFLISC
2.  -----------------------------------------
3.  -----------------------------------------
4.  -----------------------------------------
5.  -----------------------------------------
6.  -----------------------------------------
7.  -----------------------------------------
8.  -----------------------------------------
9.  -----------------------------------------
10. -----------------------------------------
11. WKAIEE--NEKLIME--MEKIVGRVHS---DAGNEY--HDWGLP--QIA--FD---PA--YN--N-
12. SPRAI----EENKH--MEMVEFQVIP--ARETEFYFVWSGLP--QTK--EDAX--HSA--YN--N-
13. SKEVIE--ETKRIE-ME-IYSQVHVPSTRENEIYFVWSGLP--QMA--EE--X--SAYNN--N-

170 180 187
1. FRRDSHKIDFLKVLRCRAAKKRPEMC
2.  -----------------------------------------
3.  -----------------------------------------
4.  -----------------------------------------
5.  -----------------------------------------
6.  -----------------------------------------
7.  -----------------------------------------
8.  -----------------------------------------
9.  -----------------------------------------
10. -----------------------------------------
11. N---NY---K----LIXHDIN
12. L---S---TY--L-N---LIXYNNN
13. L---L-N--L-K---IXXHNN

263
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., Cy546-150 and Cy5177-187 (Yasuda et al., 1986). Whether such disulfide linkages are present in cyprinid PRLs remains to be studied.

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REFERENCES

Molecular cloning of two cDNAs and expression in *Escherichia coli*. DNA 8, 261–270.


