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Purification of carp growth hormone and cloning of the complementary DNA

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Key words: Growth hormone; cDNA sequence; Amino acid sequence; Sequence homology; (Carp, *C. carpio*)

The growth hormone (GH) was isolated and purified from common carp (*Cyprinus carpio*) pituitary glands by salt precipitation and HPLC on reverse-phase C18 columns. The carp GH cDNA was synthesized and cloned in *Escherichia coli* using *Eco*RI linkers and pBR322 as vector. The positive clones were selected and sequenced. The full-length carp GH cDNA contains 1187 nucleotide basepairs with an open reading frame coding for the precursor form carp GH of 210 amino-acid residues. The partial amino-acid sequence from the protein completely agrees with that derived from the cDNA, with serine as the first residue in mature carp GH preceded by a 22-residue hydrophobic signal peptide. Comparison of the amino-acid sequence of carp GH with those of various species reveals positional identity at 32.4%, 38.8%, 42.0%, 37.2%, 66%, 55% and 49% with GHs of man, rat, duck, bullfrog, salmon, tuna and yellow tail, respectively.

Growth hormones, which are secreted from pituitary glands, are required for normal growth and development pre-adult. Recently, the primary structure of the GH of the chum salmon has been determined [1,2] and the cDNA for the GHs of chum salmon [3], rainbow trout [4], tuna [5], coho salmon [6,7], red sea bream [8] and yellow tail [9] have been cloned. We have purified carp GH from pituitary glands and have determined the N-terminal amino-acid sequence. The cDNA has been cloned and sequenced. The results are described in this report.

The carp GH has been purified according to previous procedures [10]. It was identified by the precipitin reaction with antiserum against tilapia GH. The amino-acid sequence was determined in a pulse-liquid phase protein sequencer (Applied Biosystems Inc. 477A). The mRNA was prepared by the guanidinium/cesium chloride method [11] followed by two-cycle chromatography on oligo(dT)-cellulose column [12]. Double-stranded cDNA was prepared by the method of Gubler and Hoffman [13], methylated with *Eco*RI methylase, ligated with *Eco*RI linkers, cut with *Eco*RI and then size-fractionated by electrophoresis on 1.0% agarose gel. The cDNA with size of 0.8–1.2 kilobasepairs was recovered from the gel and ligated with *Eco*RI-cleaved pBR322. The resulting plasmids were introduced into *E. coli* HB101 cells.

The cDNA library was screened by Southern blot hybridization with nick-translated salmon GH cDNA. One of the positive clones was sequenced by the method of Maxam and Gilbert [14] and the chain-termination method of Sanger [15] as shown in Fig. 1. The carp GH cDNA contains 1187 nucleotide base pairs with an open reading frame coding for 210 amino-acid residues (Fig. 2). The N-terminal amino-acid sequence was de-

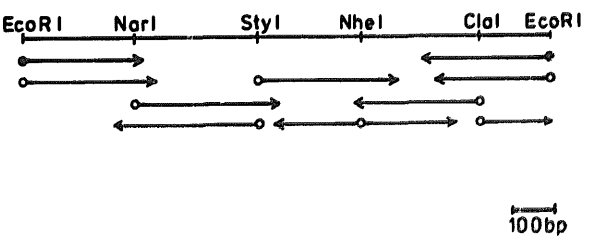

Fig. 1. Restriction map and sequencing strategy of the carp GH cDNA. The solid circles indicate the starting point of dideoxy sequencing, whereas the open ones are the end-labelling sites for chemical sequencing.

The sequence data in this paper have been submitted to the EMBL/Genbank Date Libraries under the accession number x13670.

Abbreviation: GH, growth hormone.

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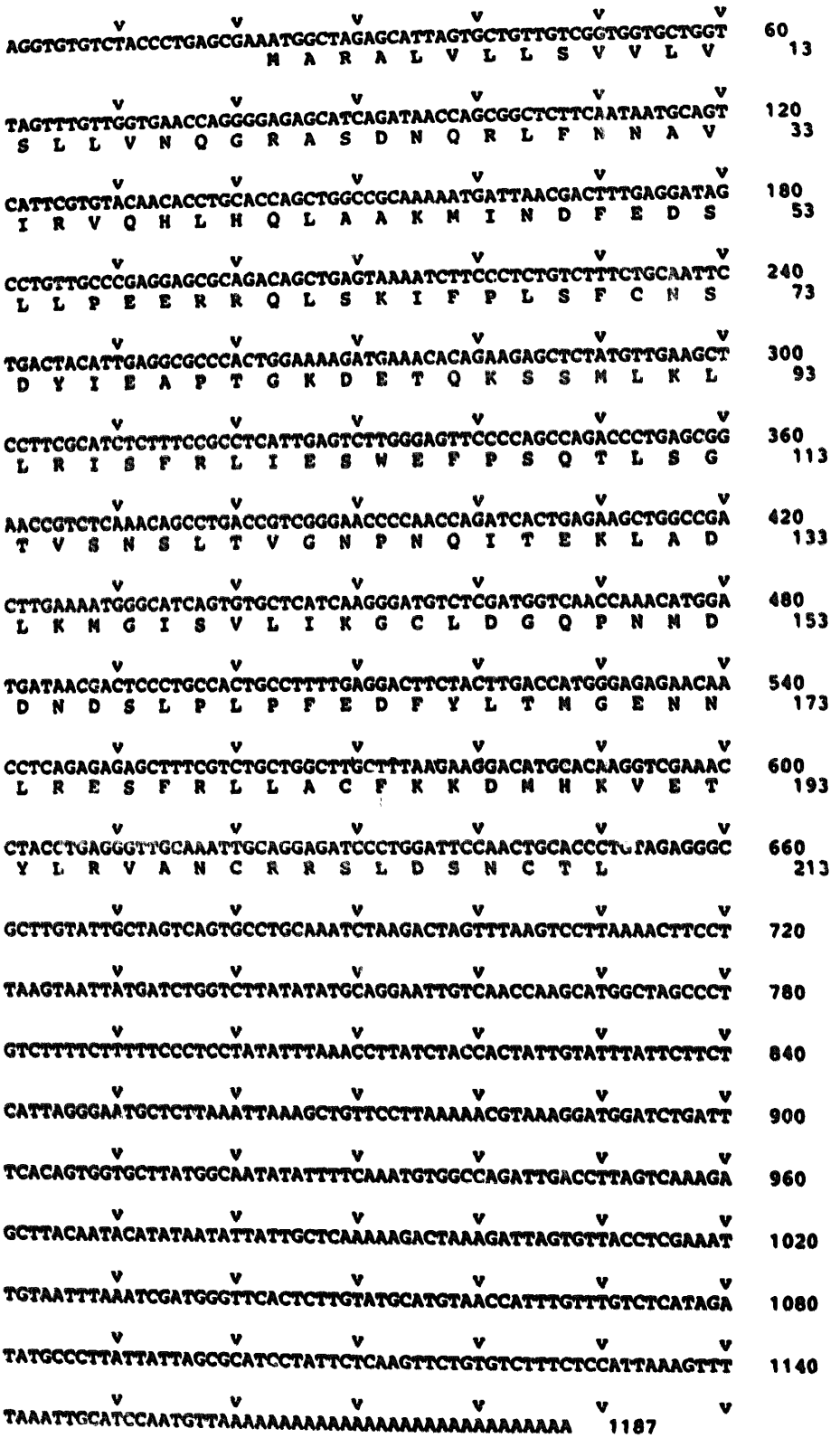

Fig. 2. The nucleotide sequence of the carp GH cDNA with the derived amino-acid sequence.

termined with an automatic protein sequencer as shown below:

$$H_2N\text{-}SDNQRLFNNAVIRVQHLHQLAAKMINDFEDSLLP\text{-}\cdots\cdots\cdots$$

The sequence completely agrees with the amino-acid sequence derived from the nucleotide sequence, with serine as the first residue in mature carp GH.

Comparison of amino-acid sequence between carp GH and other GHs (Fig. 3) reveals that carp GH shares

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                      **   *  *   **
Carp                    SDNQRLF NNAVIRVQHLHQLAAKMINDFEDSLLPEER
Human                 FPTIPLSRLFDN AMLRAHRLHQLAFDTYQEFEEAYIPKEQ
Rat                   FPAMPLSSLFAN ALLRAQHLHQLAADTYKEFERAYIPEGQ
Duck                TFPAMPLSNLFAN AVLRAQHLHLLAAETYKEFERSYIPEDQ
Bullfrog            FPQMSLSNLFTN AVIRAQHLHQMVADTYRDYERTYIPEDQ
Salmon                  IENQRLF NIAVSRVQHLHLLAQKMFNDFDGTLLPDER
Tuna                ITDSQ  RLF SIAVSRVQHLHLLAQRLFSDFESSLQTEEQ
Yellow tail         ITDSQ  HLF SIAVSRIQNLHLLAQRLFSQFESTLQTEDQ

                             * *  *  *       *      **  *
Carp                RQ LSKIFPLSFCNSDYIEAPTGKDET QKSSMLKLLRISF
Human               KYSFLQNPQTSLCFSESIPTPSNREETQQKSNL ELLRISL
Rat                 RYS IQNAQAAFCFSETIPAPTGKEEAQQRTDM ELLRFSL
Duck                RHT NKNSQA FCYSETIPAPTGKDDAQQKSDM ELLRFSL
Bullfrog            REK QTLLISVYCYSETIPAPTDKDNTHQKSDI DLLRFSL
Salmon              RQ LNKIFLLDFCNSDSIVSPVDKHET QKSSVLKLLHISF
Tuna                RQ LNKIFLQDFCNSDYIISPIDKHET QRSSVLKLLRISY
Yellow tail         RQ LNKIFLQDFCNSDYIISPIDKHET QRSSVLKLLSISY

                     *  **              *                * **  *
Carp                RLIESWEFPSQTLSGTVSNSLTVGNPNQ ITEKLADLKMGI
Human               LLIQSWLEPVQFLRSVFANSLVYGASDSNVYDLLKDLEEGI
Rat                 LLIQSWLGPVQFLSRIFTNSLMFGTSDR VYEKLKDLEEGI
Duck                VLIQSWLTPVQYLSKVFTNNLVFGTSDR VFEKLKDLEEGI
Bullfrog            TLLQSWMTPIQIVNRVFGNNQVFGNIDR VYDRLRDLDEGL
Salmon              RLIESWEYPSQTL  IISNSLMVRNANQ ISEKLSDLKVGI
Tuna                RLVQSWEFPSRSL       SGGS APRN QSPKLSDLKTGI
Yellow tail         RLVQSWEFSSRFL       SGGS ALRN ISPKLSDLKTGI

                      *     *
Carp                SVLIKGCLDG  QPNMDDNDSLP LPFEDFY  LTMGENNLRES
Human               QTLMGRLEDG  SPRTGQIFKQT YSKFDTN   SHNDDALLKN
Rat                 QALMQELEDG  SPRIGQILKQT YDKFDAN   MRSDDALLKN
Duck                QALMRELEDR  SPRGPQLLKPT YDKFDIH   LRNEDALLKN
Bullfrog            HILIRELDDG  NVRNYGVLTFT YDKFDVN   LRSEEGRAKN
Salmon              NLLITGSQDG  VLSLDDNDSQQ LPPYGNYYQNLGGDGNVRRN
Tuna                HLLIRANQDGAEMFADSSALQLAPYGQYYQS   LGADESLRRS
Yellow tail         NLLITGSQDGAEMFSDVSALQLAPYGQFYQS   LGGDDLLRRN

                      ** ** **  * ** *    **      *
Carp                FRLLACFKKDMHKVETYLRVANCRRSLDSNCTL
Human               YGLLYCFRKDMDKVETFLRIVQCR SVEGSCGF
Rat                 YGLLSCFKKDLHKAETYLRVMKCRRFAESSCAF
Duck                YGLLSCFKKDLHKVETYLKVMKCRRFGESNCTI
Bullfrog            YGLLSCFKKDMHKVETYLKVMKCRRFVESNCTF
Salmon              YELLACFKKDMHKVETYLTVAKCRKSLEANCTL
Tuna                YELLACFKKDMHKVETYLTVAKCRLSPEANCTL
Yellow tail         YELLACFKKDMHKVETYLTVAKCRLSPEANCTL
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Fig. 3. Comparison of amino-acid sequences of GHs of carp (the present study), human [16], rat [17], duck [10], bullfrog [18], salmon [3], tuna [5] and yellow tail [9]. The salmon sequence is identical to that of GH of rainbow trout [4]. Gaps are introduced to maximize similarity. Asterisks indicate the invariant residues for all sequences.

32.4%, 38.8%, 42.0%, 37.2%, 66.0%, 55% and 49% sequence similarity with the GHs of human [16], rat [17], duck [10], bullfrog [18], salmon [3], tuna [5] and yellow tail [9], respectively. The invariant residues are predominantly located within the α-helices which are necessary to maintain the structural integrity of these proteins and are not required for species specificity [19]. It is interesting that the 3'-untranslated region of carp GH mRNA as well as salmon GH mRNA is longer than those of mammalian GHs. In another respect, G and C are highly favored in the third position of the codons for rat [17] and human [16] growth hormones, but are less favored in carp GH.

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