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Cloning of the sole (*Solea senegalensis*) growth hormone-encoding cDNA

(Recombinant DNA; fish; evolution; teleost; Northern blot; pituitary gland)

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SUMMARY

We report here the complete nucleotide (nt) sequence of a cDNA clone encoding *Solea senegalensis* growth hormone (sGH) isolated from an expression library prepared from sole pituitary gland poly(A)$^+$ RNA. The library was screened using a flounder *GH* cDNA. The cDNA sequence containing an insert of 769 nt was found to encode a polypeptide of 203 amino acids (aa), including a signal peptide of 17 aa. The 5′- and 3′-untranslated regions of the message are 17 and 119-nt long, respectively. Northern blot hybridization detected a 0.9-kb RNA species. The *sGH* cDNA sequence shows homologies of 80.9, 76.9, 73.8 and 64.2% with the *GH* of tuna, gilthead seabream, flounder and rainbow trout.

INTRODUCTION

Growth hormone (GH), prolactin, placental lactogen and somatolactin are members of a polypeptide hormone family that are structurally and functionally related (Niall et al., 1971; Rand-Weaver et al., 1992). Primary structure analysis of these polypeptides and their cDNAs indicates that they evolved from a common ancestral gene. Thus, they can provide an ideal model system for studying the structure-function relationships, evolution and regulation of gene expression.

In fish, relatively little is known about the structure, regulation and evolution of those genes. Recently, the technology of gene cloning and sequencing has been applied to the *GH* genes of several fish species including those of the salmon (Sekine et al., 1985), rainbow trout (Agellon et al., 1986; 1988), yellow tail (Watahiki et al., 1988), tuna (Sato et al., 1988), gilthead seabream (Funkestein et al., 1991), tilapia (Rentier-Delrue et al., 1989), flounder (Momota et al., 1988), eel (Saito et al., 1988), coho salmon (Nicoll et al., 1987; Gonzalez-Villaseñor et al., 1988), grass carp (Ho et al., 1989), chum salmon (Kawauchi et al., 1986) and common carp (Chao et al., 1989). We are now included in this growing list by clarifying the nt sequence of a cDNA for *Solea senegalensis*.

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Abbreviations: aa, amino acid(s); bp, base pair(s); cDNA, DNA complementary to mRNA; GH, growth hormone(s); *GH*, gene (DNA) encoding GH; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); ORF, open reading frame; pBS, Bluescript plasmid; PCR, polymerase chain reaction; pGH, *Paralichthys olivaceus* GH; PolIk, Klenow fragment of polymerase I; sGH, *Solea senegalensis* GH; saGH, *Salmo salar* GH; spGH, *Sparus aurata* GH; SSC, 0.15 M NaCl/0.015 M Na_3-citrate pH 7.6; *UTR*, untranslated region(s).

EXPERIMENTAL AND DISCUSSION

(a) Isolation and sequencing of a cDNA encoding sGH

Total RNA was isolated from pituitary glands of rapidly growing *Solea* animals. Poly(A)$^+$RNA was prepared by affinity chromatography on oligo(dT)-cellulose

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and used as template for cDNA synthesis. A pituitary cDNA library was constructed in λgt11 vector following standard methods (Sambrook et al., 1989). Briefly, the double-stranded cDNA was inserted at the *Eco*RI site of phage λgt11 DNA and amplified in *E. coli* Y1090 cells. 6×10^4 recombinant phages, were screened for *GH* sequences by hybridization with heterologous flounder *GH* cDNA probe (a gift of Dr. Hideo Ohgai, Otsuka Pharmaceutical, Nishihama Kita-Cho, Japan). It resulted in the isolation of six clones. Analysis of insert size indicated that they were in the range of 0.6 to 0.9 kb.

As digestion with a number of restriction enzymes produced a similar pattern on all of them, their sequences were likely to be identical. In view of this, only one of these clones was subsequently selected for sequencing (Sanger et al., 1977). First, the insert was amplified from the phage by PCR reaction, treated with PolIk, phosphorylated with T4 polynucleotide kinase, and further cloned into plasmid pBS at the *Sma*I site. Several restriction sites indicated in Fig. 1 were used to construct eight overlapping subclones for sequencing. The nt sequence of the *sGH* cDNA, shown in Fig. 1, contains an ORF of 612 nt encoding 203 aa. The *5'-UTR* contains 17 nt and the *3'-UTR* is of 119 nt. The polyadenylation signal AATAAA is 12 nt upstream from the polyadenylation site. It is of particular interest to observe that *sGH* cDNA contains a *Eco*RI site at nt 321. This restriction site was not present in any of the growth hormone cDNAs characterized so far, including fish and mammals.

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                    NcoI
TTGAACCACTACTAGCCATGGATAGAGTTGTCATCGTGCTGTCTGTCCTGTCTGTGGCC  59
                  MetAspArgValValIleValLeuSerValLeuSerValAla  14

GCATCCTCTCAGTCAATCCTAGACCAGCGTCGTTTCTCCATCGCCGTGAGCAGAGTTCAA 119
AlaSerSerGlnSerIleLeuAspGlnArgArgPheSerIleAlaValSerArgValGln  34

CATATTCACCTGCTCGCTCAGAAATACTTCTCAGACTTCGAGAGCTCTCTACAGACTGAG 179
HisIleHisLeuLeuAlaGlnLysTyrPheSerAspPheGluSerSerLeuGlnThrGlu  54

                        PstI
GATCAACGTCAGCTCAACAAAATCTTCCTGCAGGATTTCTGTAACTCTGATGACATCATC 239
AspGlnArgGlnValAsnLysIlePheLeuGlnAspPheCysAsnSerAspAspIleIle  74

AGTCCCATCGATAAACATGAGACTCAACGCAGCTCAGTTCTGAAGCTCTTATCGATCTCT 299
SerProIleAspLysHisGluThrGlnArgSerSerValLeuLysLeuLeuSerIleSer  94

                    EcoRI
GTTCGATTGATTGAATCTTGGGAATTCTCCAGTCGCTTCGTCACATGGAGTACATTTCCC 359
ValArgLeuIleGluSerTrpGluPheSerSerArgPheValThrTrpSerThrPhePro 114

AGGAACCAGATTTCACACAAACTGTCAGAACTAAAAACAGGAATCCGGATGCTGATTGAG 419
ArgAsnGlnIleSerHisLysLeuSerGluLeuLysThrGlyIleArgMetLeuIleGlu 134

GCCAATCAGGATGGAGCAGAAGTGTTCTCTGACAGCTCCACCTTCCAGTTGGCTCCTTAT 479
AlaAsnGlnAspGlyAlaGluValPheSerAspSerSerThrPheGlnLeuAlaProTyr 154

GGAAACTTCTATCAGAGTCTGGGAGGTGATGAATCATTAAGACGCAACTACGAACTCCTC 539
GlyAsnPheTyrGlnSerLeuGlyGlyAspGluSerLeuArgArgAsnTyrGluLeuLeu 174

                                                    HincII
GCCTGCTTCAAGAAGGATATGCACAAGGTGGAAACATACCTGACAGTGGCCAAATGTCGA 599
AlaCysPheLysLysAspMetHisLysValGluThrTyrLeuThrValAlaLysCysArg 194

CTCTCTCCAGAAGCTAATTGTACCCTGTAACCCCACCTCCACACAGTGAGGCCCCTCCCC 659
LeuSerProGluAlaAsnCysThrLeu *                               203

GTTGATGATAGCATTGTGTACATTCTATATCGCTGCCACATGTTTGCTAACCTCACTTGT 719
TCAGCATGTGAAATAAATAGTGTTCATTCAAAAAAAAAAAAAAAAAAAAA            769
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Fig. 1. The nt sequence of *sGH* cDNA. Restriction sites used for subcloning are indicated. Eight overlapping fragments from the *sGH* cDNA were subcloned in the pBS vector and used to sequence overlapping clones in both directions from primers in the vector such as T3 and T7. Sequencing was carried out by the dideoxynucleotide chain-termination method (Sanger et al., 1977). The complete deduced aa sequence is shown. Numbers above the aa relate to the aa sequence (aa 1 to 17, signal peptide; aa 18 to 203, mature GH). The polyadenylation signal AATAAA is 12 nt upstream from the poly(A)$^+$ sequence. TAA (asterisk) is the stop codon. This sequence has been deposited in the EMBL/GenBank data base (accession No. U01143).

Fig. 1 shows a Northern blot hybridization analysis of *So.* pituitary total RNA with the *sGH* cDNA clone isolated. A RNA band of about 0.9 kb was detected with the probe. This mRNA size corresponds well to that described for other fish *GH* (Koren, 1989).

(b) Comparison of *sGH* cDNA sequence to other fish *GH*

The aa sequence predicted from the cDNA of the *sGH* mRNA presently described, bring new information about the GH structure of a teleost.

A comparison of sGH to other fish species GH aa sequences as derived from the cDNA is presented in Fig. 3. The first 17 aa at the N terminus of sGH, like those of gilthead seabream, red seabream and tuna GH

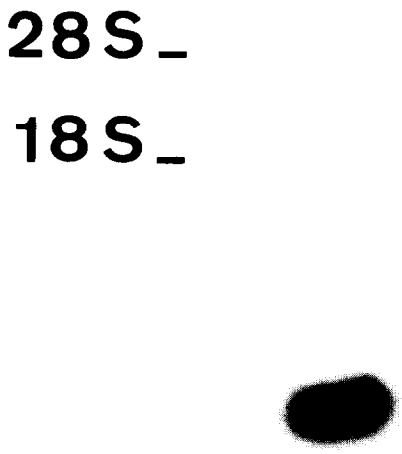

Fig. 2. Northern blot hybridization of *So.* pituitary total RNA to ^{32}P-labelled *GH* cDNA probe. 30 µg of *Solea* pituitary total RNA were electrophoresed on a 1.25% agarose-2.2 M formaldehyde gel, transferred onto nylon filter (Sambrook et al., 1989), prehybridized for 3 h at 60°C in a solution containing 0.9 M NaCl/0.09 M Na_3-citrate/5 × Denhardt's mixture (0.1% Ficoll/0.1% polyvinyl pyrrolidone/0.1% bovine serum albumin)/0.1% SDS/100 µg per ml yeast tRNA. The ^{32}P-labelled probe was then added and hybridization was carried out in the above solution for 16 h at 60°C. The filter was washed twice for 30 min with 1 × SSC/0.1% SDS at 70°C. A RNA band of 0.9 kb was detected with a *Solea* cDNA probe.

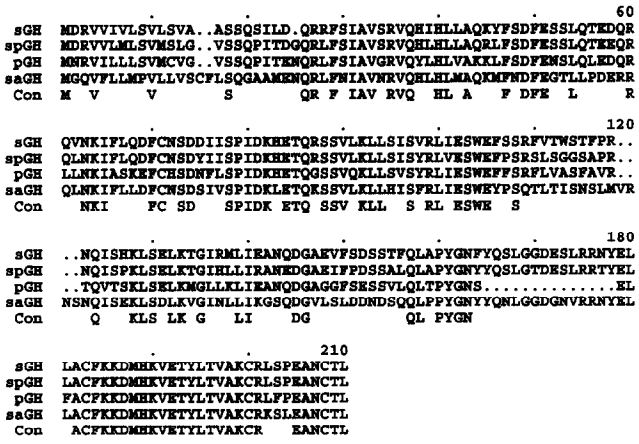

Fig. 3. Comparison of deduced aa sequences between sGH, spGH, pGH and saGH. Dots indicate gaps which were introduced into the sequences to maximize homologies. Consensus aa are shown. The signal peptide is from aa 1 to 17 and the mature hormone from aa 18 to 203 for sGH.

sequence, probably represent the signal peptide of the pre-GH, which is cleaved upon hormone secretion. This fish signal peptide, showed however to be shorter to that of the mammalian GH (Koren, 1989). As it was described by others, the sequence of the signal peptide, is more divergent among species than that of the mature GH polypeptide (Koren, 1989). For sole and gilthead seabream, the similarity of aa and nt sequence in the signal peptide is 64% and 74.5% respectively, as compared to 83.3% and 79.3% in the mature GH polypeptide. Fig. 3 also shows that sGH shares structural features which have been observed not only in other fish GH but also in mammalian GH. Four Cys residues (Cys^{68}, Cys^{176}, Cys^{193} and Cys^{201}) in sGH occur at nearly identical positions as those in mammalian GH (Dayhoff et al., 1978). The resulting disulfide linkages may also play the same essential role for the maintenance of the biological activity of the hormone (Lewis et al., 1980; Paladini et al., 1981). These specific disulfide bonds are crucial for biological activity as it has been shown by in vitro binding assay (data not shown) and by in vivo studies (Lewis et al., 1980). Comparison of aa sequence of sGH to those of mammalian GH shows in average, a homology of 35% (Miller et al., 1980; Seeburg, 1982; Miller and Eberhardt, 1983). However, this is increased when functional aa distribution was compared (data not shown). Further, sGH shares with other GH, domains at the N- and C-terminal regions, which may be important for the hormone function. So, there is one Asn-Cys-Thr motif in sGH aa sequence which is a potential site for *N*-linked glycosylation as have also been observed in the salmon GH (Sekine et al., 1985).

(c) Conclusions

(*1*) The *sGH* nt sequence, including 17 bp upstream from the start codon, has been determined.

(*2*) The results showed that the *sGH* cDNA, similary to other fish *GH*, contains four Cys residues probably essential for the biological activity of the hormone.

(*3*) A unique 0.9-kb mRNA species was observed by Northern blot analysis.

(*4*) Surprisingly, *sGH* contains an internal *Eco*RI site not shown in any other *GH* cDNAs described so far, including mammalian and fish species.

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