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Cloning of a somatolactin-encoding cDNA from sole (*Solea senegalensis*)

(Pituitary; fish; nucleotide sequence homology; amino-acid sequence homology)

Carlos Pendón, Juan Pedro Martínez-Barberá and Manuel M. Valdivia

Department of Biochemistry and Molecular Biology, Facultad de Ciencias, Universidad de Cádiz, 11510 Puerto Real, Cádiz, Spain

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SUMMARY

From a *Solea senegalensis* cDNA expression library, clones encoding somatolactin (SL), a new pituitary hormone belonging to the growth hormone/prolactin family, were isolated and analyzed. Northern blot analysis showed a unique 1.0-kb mRNA species. The sole *SL* 778-bp cDNA encoded full-size *S. senegalensis SL* (ssSL) (230 amino acids), including seven Cys and two potential glycosylation sites. A consensus polyadenylation signal, AATAAA, was found. Protein homology and DNA sequence alignments of *SL* cDNAs from other evolutionarily distant marine fishes suggest that the *SL* sequence is highly conserved.

INTRODUCTION

Somatolactin (SL) is a newly discovered pituitary hormone structurally related to both growth hormone (GH) and prolactin (PRL). Histological studies of the teleost hypophysis have shown that the pars intermedia contains two cell types that can be distinguished by staining with periodic acid/Schiff reagent (PAS) and lead/hematoxylin. PAS-positive cells (PIPAS) can be activated under several environmental conditions, such as black background (Ball and Batten, 1981), acid pH (Wendelaar et al., 1986), low calcium (Olivereau et al., 1981) or low osmolarity (Olivereau et al., 1980) of the ambient water. The response to these changes may include the production of proteins of 25 and 27 kDa and similar ones were indentified in flounder (fl) pituitaries and isolated from the teleost Atlantic cod (*Gadus morhua*). Although in contrast to GH and PRL, the function(s) of SL in teleost are, to date, unknown, this new pituitary hormone was suggested to be involved in those environmental adaptations. Another recent report by Kawauchi (1991) indicates that the SL level correlates well with that of sex steroid hormones, indicating a putative role in reproduction.

SL has been purified from several fishes species (Rand-Weaver et al., 1991b; 1992). Currently, the cDNA clones coding for flounder SL (flSL) (Ono et al., 1990), chum salmon SL (csSL) (Takayama et al., 1991a), Atlantic cod SL (acSL) (Takayama et al., 1991b), lumpfish SL (lfSL) (Iraqi et al., 1993) and halibut SL (htSL) (Iraqi et al., 1993) are known.

We report the cDNA sequence of SL isolated from a *S. senegalensis* pituitary expression library. Screening was a result of searching for *GH* related sequences using a fl *GH* cDNA clone as a probe. The ssSL deduced aa sequence was compared with those other marine fishes SL sequences.

Correspondence to: Dr. M.M. Valdivia, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Cádiz, Apartado 40, 11510 Puerto Real, Cádiz, Spain. Tel. (34-56) 830-363; Fax (34-56) 834-924.

Abbreviations: aa, amino acid(s); acSL, Atlantic cod SL; bp, base pair(s); cDNA, DNA complementary to mRNA; csSL, chum salmon SL; flSL, flounder SL; GH, growth hormone; htSL, halibut SL; kb, kilobase(s) or 1000 bp; lfSL, lumpfish SL; nt, nucleotide(s); ORF, open reading frame; PAS, periodic acid/Schiff reagent; PIPAS, PAS-positive cells; PRL, prolactin; *S., Solea*; SL, somatolactin; *SL*, gene (DNA) encoding SL; SSC, 0.15 M NaCl/0.015 M Na_3-citrate pH 7.6; ssSL, *S. senegalensis* SL; *UTR*, untranslated region(s).

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EXPERIMENTAL AND DISCUSSION

(a) Isolation and sequencing of cDNA clones encoding *ssSL*

A cDNA library was constructed from *S. senegalensis* pituitary poly(A)$^+$RNA and λgt11 as cloning vector (Sambrook et al., 1989). Originally the library was screened for *GH* cDNA clones using a fl *GH* probe. Among several *GH*-positive clones, some were not confirmed by restriction enzyme digestions and Southern-blot hybridization as a *GH* cDNA. However, sequencing experiments indicated high homology of some clones with published *SL* sequences. The inserts were amplify from the λ vector, and the PCR products were subcloned in a pBS SK(-) cloning vector (Stratagene, La Jolla, CA, USA) for subsequent nucleotide (nt) sequence determination. The largest clone was chosen for further characterization.

Fig. 1 shows the nt sequence of the *ssSL* cDNA determined from a series of overlapping subclones. Several restriction sites shown in Fig. 1 were used for subcloning and sequencing purpose. The *ssSL* sequence contains an ORF of 693 nt (encoding 230 aa), a 5′-*UTR* of 22 nt and a 3′-*UTR* of 63 nt. The polyadenylation signal AATAAA is in 3′-*UTR*, 15 nt upstream from the polyadenylation site (at nt 736). The 5′-*UTR* of *ssSL* cDNA isolated is shorter than that of the *acSL* cDNA by 230 nt and very similar in length to the other *SL* cDNA described previously. The 3′-*UTR* of *SL*s cloned so far, shows in general high variability in sequence.

```
   GAAGACTCCTGACAGAACGCACATGATGACGGCAGTCAAACAGAGTGGTGTATGGGCT  58
                          MetMetThrAlaValLysGlnSerGlyValTrpAla -12

GTCTTGCTATGGCCCTATTTGCTTGCTGTAAGCATCCCACTAGACTGTAGGGATGAGCAG 118
ValLeuLeuTrpProTyrLeuLeuAlaValSerIleProLeuAspCysArgAspGluGln   9

GGCAACATGTCTCGCTGTCCCTTCATCTCCCAAGAAAAACTTCTGGACCGAATCATCCAA 178
GlyAsnMetSerArgCysProPheIleSerGlnGluLysLeuLeuAspArgIleIleGln  29

      SacI
CACGCTGAGCTCATCTCCCGCATCTCAGAAGAATCATGTTCTTTGTTTGAGGAGCTGTTT 238
HisAlaGluLeuIleSerArgIleSerGluGluSerCysSerLeuPheGluGluLeuPhe  49

GTTCCCTTCCCACTGCGGCTTCAGAGAAACACGGTCGGCTACGCATGCATCACCAAGGCC 298
ValProPheProLeuArgLeuGlnArgAsnThrValGlyTyrAlaCysIleThrLysAla  69

TTACCCATCCCTAGCTCCAAGAGTGAAATTCAACAAATATCTGATAAATGGTTGCTGCAA 358
LeuProIleProSerSerLysSerGluIleGlnGlnIleSerAspLysTrpLeuLeuGln  89

                                        PstI
TCTGTGCTGACGCTGGTCCAGTCATGGATCGAGCCTTTGGTCTACCTGCAGACCACACTA 418
SerValLeuThrLeuValGlnSerTrpIleGluProLeuValTyrLeuGlnThrThrLeu 109

GATCGCTACGATAACGCGCCAGACGTGCTGCTCAACAAGACTAAGTGGGTGTCCGAGAAA 478
AspArgTyrAspAsnAlaProAspValLeuLeuAsnLysThrLysTrpValSerGluLys 129

CTGGTCAGTCTGGAGCAAGGCGTGGTCGTCCTTATCAGAAAGATGCTGGATGAAGGAACG 538
LeuValSerLeuGluGlnGlyValValValLeuIleArgLysMetLeuAspGluGlyThr 149

HincII
TTGACTACAACATACAACGAACAAGATCTACTCCAATACGATGTCCTACCAGATATGTTG 598
LeuThrThrThrTyrAsnGluGlnAspLeuLeuGlnTyrAspValLeuProAspMetLeu 169

GAATCTGTTATGAGAGACTATACCCTGCTCAGCTGCTTCAAGAAAGACGCCCATAAGATG 658
GluSerValMetArgAspTyrThrLeuLeuSerCysPheLysLysAspAlaHisLysMet 189

GAGATTTTCCTCAAGCTCCTCAAGTGTCGGCAAACTGACAAATTCAACTGTGCATAAAAC 718
GluIlePheLeuLysLeuLeuLysCysArgGlnThrAspLysPheAsnCysAla *      207

ATAATGTGCAACTTTTAAATAAAACAATGTCTAGCTTTAAAAAAAAAAAAAAAAAAAAAA 778
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Fig. 1. The nt sequence of *ssSL* cDNA. Sequence was obtained from overlapping clones covering both strands of the cDNA by the dideoxynucleotide chain-termination method (Sanger et al., 1977) using T3 and T7 universal primers. Restriction sites used for subcloning are indicated. The nt are arranged with the Met codon (ATG) at nt 23 and the stop codon (TAA) (asterisk) at nt 715. The polyadenylation signal AATAAA (nt 736–741) is underlined. The aa sequence is numbered beginning with the putative signal peptide of 23 aa, and the coding sequence for mature ssSL starts with Ile 1. This sequence has been submitted to the EMBL/GenBank under the accession No. U06753.

(b) Northern blot analysis of the *ssSL*

The expression of the pituitary *ssSL* gene was examined by Northern hybridization. The probe used was a 0.4-kb *ssSL* cDNA subclone. It was found to hybridize with a single 1.0-kb mRNA at very high-stringency wash conditions, as shown in Fig. 2. This mRNA size correlates well with that described for cs and fl SLs. However, in contrast with fl where several mRNAs *SL* emerging from a putative alternative splicing, a single one was found in *S. senegalensis*, even with very long exposure time of the autoradiography film. The existence of multiple mRNA for the same gene has also been observed in other hormones such as human folicle-stimulating hormone (FSH), human thyroid-stimulating hormone (TSH) (Jameson et al., 1988; Wood et al., 1987), and potential functions could involve RNA stability, intracellular transport of RNA, or translational efficiency. It is however thus evident that *ssSL* expression differs from that of fl in some aspect to be determined by further characterization of the function of this hormone in *S. senegalensis*.

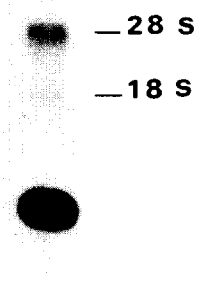

Fig. 2. Northern blot analysis of *SL* mRNA from *S. senegalensis* pituitary. Total RNA (30 μg) from mature fish was denatured and separated on a 1.2% agarose-2.2 M formaldehyde gel and transferred to a nylon membrane. The blot was hybridized at 55 C with a 0.4-kb [^{32}P]*SL* cDNA probe. Filters were washed at 55 C for 30 min and for high-stringency conditions at 75 C for 60 min. Exposure time was 18 h.

It appears that the *SL* gene is highly expressed in adult teleost pituitary as it was found by Rand-Weaver and colleages (1991a) that SL is a major protein in the cod pituitary. The fact that the *ssSL* gene is highly expressed in *Solea* pituitary (suggested by the signal on the Northern analysis) would explain the isolation by a *GH* screening routine analysis. On the other hand sequence comparison of *ssGH* and *ssSL* showed a 41% nt sequence homology.

(c) Comparison of ssSL to other fish SLs

The aa sequence of *S. senegalensis* SL deduced from the cDNA coding region is shown in Fig. 3. This figure also compares the SL polypeptide sequences of flSL, csSL, lfSL, htSL and acSL. Alignment of aa sequences of SL from these six fish species shows that seven Cys residues were conserved in all the species. One aditional Cys residue was found in csSL. A potential *N*-glycosylation site (Asn-Lys-Thr, aa 189–191) was found in all species except cs. Other additional potential *N*-glycosylation site (Asn-Ser-Ser) changes from fish to fish species. These putative *N*-glycosylation sites remain to be confirmed by direct isolation of the ssSL native hormone or the expression of the *ssSL* cDNA in a suitable eukaryotic expression system, where proper and accurate glycosylation can occur (Rand-Weaver et al., 1992b). The role of glycosylation could be assessed better by site-directed mutagenesis once the function of SL is better understood.

The function of SL is currently largely unknown.

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                                                                  60
ssSL  ...MMTAVKQSGVWAVLLWPYLLAVSIPLDCRDEQGNMSRCPFISQEKLLDRIIQHAELI
htSL  .MNMMT.VKQ.GVWAALLWPYLLAASIPLDCKDEQGSFSACPSISQEKLLDRVIQHAELI
flSL  .MNMMT.VKQQGVWAALLWPYLLTASIPLDCKEEQGSLSRCPSISQEKLLDRVIQHAELI
lfSL  ..MHLVSVIQRGVWAVLLWPNLLASSVPLDCREEQGILSRCPSISQEKLLDRVIKHAELI
csSL  .MNMMQ.VMQSVVWAVLLWPCLVSLGVPLECKDEQGSIILCASISKEKLLDRVIQHAELI
acSL  MHTLAAVVVLQVCWAAVLWPCPPTHSSPVDCREEQAGSSQCPTISQEKLLDRVIQHTELI
Con          V     WA  LWP       P  C  EQ     C  IS EKLLDR I H ELI

                                                                 120
ssSL  SRISEESCSLFEELFVPFPLRLQRNTVGYACITKALPIPSSKSEIQQISDKWLLQSVLTL
htSL  YRVSEESCSMFEEMFVPFPLRLQRNQAGYACITKALPIPSSKSEIQQISDTWLLHSVLLL
flSL  YRVSEESCSMFEEMFVPFPLRLQRNQAGYACITKALPIPSSKSEIQQISDTWLLHSVLML
lfSL  YRVSEESCSLYEDMFI..PLQFQRNQVGYACITKTLPVPSSKNEIQQISDKWLLHSVLML
csSL  YRVSEESCTLFEEMFVPFPMRSQRNQAGYTCATKAFPIPGSKSEIQQISDKWLLHSVLIL
acSL  YRVSEESCSMFEDMFVPFPVRLQRNQAGNTCITKDFPIPTSKNELQQISDTWLLHSVLML
Con    R SEESC   E  F   P   QRN  G  C TK  P P SK E QQISD WLL SVL L

                                                                 180
ssSL  VQSWIEPLVYLQTTLDRYDNAPDVLLNKTKWVSEKLVSLEQGVVVLIRKMLDEGTLTTTY
htSL  VQSWIDPLVYLQTTLDRYDNASEMLLNKTKWVSDKLISLEQGVVVLIRKMLDEGMLTATY
flSL  VQSWIEPLVYLQTTLDRYDNAPDMLLNKTKWVSDKLISLEQGVVVLIRKMLDEGMLTATY
lfSL  VQSWIEPLVYLQTSLDRYNAAPEMLLNKTKWVSEKLISLEQGVVVLIKKMLDEGMLTINH
csSL  VQSWIEPLVYLQTTLDRYDDAPDTLLKKTKWVSEKLLSLEQGVVVLIRKMLDDDMLTTSY
acSL  VQSWIEPLVYLQTTLDRYDDVPDVLLNKTKWMSEKLISLEQGVVVLIRKMLDGAILNSSY
Con   VQSWI PLVYLQT LDRY      LL KTKW S KL SLEQGVVVLI KMLD   L

                                                                 235
ssSL  NEQDLLQYDVLPDMLESVMRDYTLLSCFKKDAHKMEIFLKLLKCRQTDKFNCA..
htSL  NEQGLFQYDVLPDMLESVMRDYTLLSCFKKDAHKMEIFLKLLKCRQTDKYNCP..
flSL  NEQGLFQYDAQPDMLESVMRDYTLLSCFKKDAHKMEIFLKLLKCRQTDKYNCA..
lfSL  SEQGLLQNGVQPQMLESVMRDYTLLSCFKKDAHKMEAFLKLLKCRQTDRYNCS..
csSL  YEQGVAPYALQPEVLESVLRDYTLLSCFKKDAHKMETFLKLLKCRQTDKYSCFLH
acSL  NEYSAVQLDVQPEVLESILRDYNVLCCFKKDAHKIETILKLLKCRQIDKYNCALY
Con    E         P  LES  RDY  L CFKKDAHK E  LKLLKCRQ D   C
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Fig. 3. The aa alignment of SL from six fish species. Residues are numbered as a reference beginning with the putative start Met for acSL. Seven Cys residues are conserved in all the sequences as it is shown in concensus (con). ssSL shows a 86, 84, 77, 74 and 68% aa sequence identity to flSL, htSL, lfSL, csSL and acSL, respectively.

Several physiological activities in fish, such as background adaptation (Ball et al., 1981) and ion regulation (Olivereau et al., 1980; 1981) have been proposed for a presumed polypeptide from the PIPAS cells such as SL. In contrast, in higher vertebrates the presence of SL-producing cells is still uncertain. For clarification of SL function, availability of the *SL* cDNA clones should enable us to carry on many interesting questions on induction and regulation of the *SL* gene at the molecular level. Further, use of transgenic fish might prove quite useful in elucidating the action of this new hormone.

(d) Conclusions

(*1*) The *ssSL* nt sequence, including 22 bp upstream from the start codon, has been determined.

(*2*) The results showed that the ssSL contains seven Cys residues as in other fish SLs, probably essential for the biological activity of the hormone.

(*3*) A unique 1.0-kb mRNA species was observed by Northern blot analysis, in contrast to several *SL* mRNAs species found in other fishes species.

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