

Identification of a novel Sex Hormone Binding Globulin (SHBG) in the rainbow trout (*Oncorhynchus mykiss*) ovary

by

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ABSTRACT. - In the present study, we report and characterize the sequence of a novel gene putatively encoding for a previously uncharacterized sex hormone binding globulin that we called SHBGb. Unlike the classical SHBG protein (SHBGa), this gene is expressed in the ovary. In addition, microarray-based expression profiling studies have demonstrated that SHBGb mRNA is temporally co-expressed with ovarian aromatase mRNA during sex differentiation, previtellogenesis, late-vitellogenesis and post-vitellogenesis.

Key words. - Salmonidae - Ovary - SHBG - Aromatase.

Introduction

In the course of two recent genomic studies carried out using 9-K rainbow trout cDNA microarrays, a previously uncharacterized cDNA was identified. The corresponding transcripts exhibited an expression profile extremely similar to ovarian aromatase expression profile. This correlation was observed during sex differentiation (Baron *et al.*, 2007) and during late- and post-vitellogenesis (Bobe *et al.*, 2006). The protein encoded by this cDNA had sequence similarities with vitamin k-dependent protein S, growth arrest 6 and sex hormone binding globulin (SHBG). The present study aimed at sequencing this novel cDNA in order to allow the identification of the corresponding protein.

Methods

The cDNA putatively encoding for rainbow trout SHBGb was obtained from CRB GADIE resource center (Jouy-en-Josas, France, <http://www-crb.jouy.inra.fr/>). The cDNA was fully sequenced on both strands and the corresponding sequence deposited in GenBank (Acc # EF577269).

Results and discussion

The shbgc cDNA was 1796 bp in length and contained one open reading frame (Fig. 1). The ATG codon existed at position 103 of the cDNA, and a stop codon was present at position 1341 (Fig. 1). The open reading frame presumably encodes a protein of 412 amino acids with a calculated

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1   ATC GGA CCT TAC AAG TAT AGA ACA TAA ACG CCA TAA GGA ACG TCT GGT TAA GAG AAG CAA GTG CCC CAA AGG ACC GTG AAA AGA AGT AGA
 91   CCT TAG TAG ATC ATG TTT GCA TTG AAG AGG TTT GTG GTG GCT CTG CTG ACC CTA GGG ATT TGG GTT CAT CCA ACG CTT GGA CGG ACA CTG
    M F A L K R F V V A L L T L G I W V H P T L G R T L
181 GAG CCA TCA CCA GAA TGC TAC TAT TTT GTT GAG TCC AGG TCC TCA CAT CTC CTG TAC ACT GGG AAC AGC TCT GTG GGG AAT GTC CCC ATT
 27   E P S P E C Y Y F V E S R S S H L L Y T G N S S V G N V P I
271 CTG GAG TAC AAG GTC ACA GAG TTG ACC AGC TTT GAC TCT GAG TTT GAG CTG CGT ACT CTG GAC CCT GAG GGG GTG ATA TTC TTT GGG GAC
 57   L E Y K V T E L T S F D S E F E L R T L D P E G V I F F G D
361 ATC GGC GGG CAG CAG AAC TAC TTC CTG CTT GCT GTG ATC CAA GGG AAC CTG AGC GTC CAG ACG AGC TGC GGG GAT GGA CAG GTC CTC GTC
 87   I G G G Q N Y F L L A V I Q G N L S V Q T S C G D G Q V L V
451 ACC TCC GGG CCC AAG ATC AGT GAC GGG GAG TGG AAG AAG ATT GCA GTG ATG AAA CAT GAG GGA GCT GTG GCT GTA CGC TGT GGC TCG GAG
117   T S G P K I S D G E W K K I A V M K H E G A V A V R V G S E
541 ACA GCC TGC ACA GTG CAG CAG TCT GCT GAG AGC CAG AGG GCC GAG ATA GGC AAC GGC ATG CTG AGA ATC TCT ATC GGA GGC CTG CTC CCA
147   T A V T V Q Q S A E S Q R A E I G N G M L R I S I G G L L P
631 GAT AGC GGA GTT ACC TTG GGG CTG AAC CCT CCT CTG GAT GGC TGC ATG CGG AGC TGG GAC TGG GTC AGA CAG GAC TCC AGC ATC CTA GAG
177   D S G V T L G L N P P L D G C M R S W D W V R Q D S S I L E
721 CGG ACC CTG CAG GAT TCC AAG GTG CAG AGG TGC TGG GAA CAT ATT GCC CCC GGG TCC TAC TTT ACT GGA GTC GGC TCT GTT GGT TTC AGC
207   R T L Q D S K V Q R C W E H I A P G S Y F T G V G S V G F S
811 TCT CTA GCC TTG CTG GGA AAC TCT TCA GCT GAG CTA GAT GGT GCA GAC TGG ACA CTC TCT GTG GAG CTA GCT CTC AGA ACG GTT TCA GCC
237   S L A L L G N S S A E L D G A D W T L S V E L A L R T V S A
901 AGA GGC TTT CTC TTT ATT CTG CTA GAC ACT CAG AAC GAC TAC ATC CTC TCC TTG AAA CTC AAC CAC CCT TCA CAG GAA CTG ATG CTA CGT
267   R G F L F I L L D T Q N D Y I L S L K L N H P S Q E L M L R
991 TTG AGG GGG ACT CTA TTC TGG TCG CGC AGC TAC CCC CAG ACC CTC TGC TCA GGT GAG AGT CAG TTC CTG CAG CTG GAG GTG AGG CCA GGC
297   L R G T L F W S R S Y P Q T L C S G E S Q F L Q L Q V R P G
1081 CAG CTG GTG ATT GGC ATG GGC ATA ACC AAG GCA ACC ATG AGG TTG ACG GAT GGG GAC TAC GAG CTC CTG AAG AGG GTG TTG AGC CAA CCA
327   Q L V I G M G I T K A T M R L T D G D Y E L L K R V L S Q P
1171 GGG AGC AGG GTG TAC CTG GGG GGC GGG CCA GCG GGT CTG TCC AGC TTC CAG GGC TGT CTC CAG GCC AAG ATC CAG GGT GTC AAC GTT GAC
357   G S R V Y L G G G P A G L S S F H G C L Q A K I Q G V N V D
1261 CTT GAC CTG GCA GAG GTC AAA CAC GGG GAT GTC CGC TCT CAC AGC TGC CCT GCC GCC CTC GAC ATC AGA GAT GGG AAG TAA AGG AGC CGC
387   L D L A E V K H G D V R S H S C P A A L D I R D G K *
1351 AGT TAC ACC CGA CAC TGC TTA TAA GAC TAC ACA AGG CTA CAC TGT TTT CTG TTT GGC TAT TGT TGT TCT CTC AAC ATT TTA CTG TCT CAT
1441 GGC TAT CTA TGT AAC AAG ATG TGA CAC AGT ACT GCA GAA TGC AGT AAC ATT GTA TTT GTA TTG AAG CAT CTC TGA CAT GTG GGG GAT GTT
1531 CAT TTG AAG AAC AAG AGA AGG ATT GCG TTT TCT TTG GGT AGA TTG TTT TGC ATA CAT TTT TGG CAA ACT TGG TAG TTA CTG CAA AAC CGT
1621 AAC ATG GCC TCT TTT TTT GCT GGA TAT TGT TTT TGT AAT TTC TAG CAG AAT TCC CCT ATG AGG TAA AGC TAA ACA ACA GTA CAG CTG
1711 AAT TAC CAT GTC CAC TCT TGT TAA GTC TGT GTG AAT GGC AAT AAA GCA TTT GCT GCT TTT TCT AAA AAA AAA AAA AAA AAA AAA AAA

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Figure 1. - Nucleotide and amino acid sequences of rainbow trout SHBGb. Nucleotide bases and amino acids residues are numbered. ATG start codon underlined; * indicates stop codon; Genbank Accession # EF577269.

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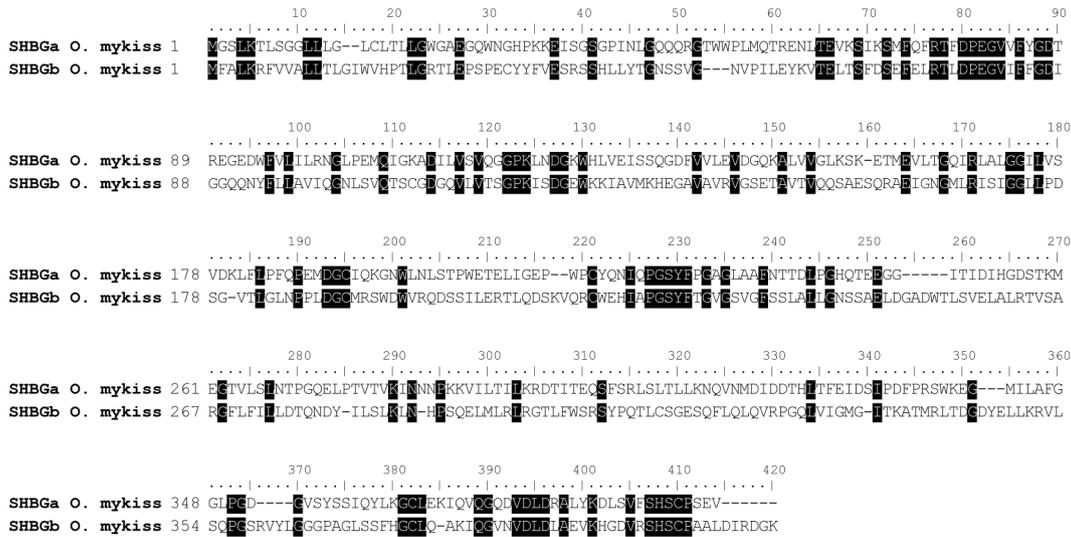


Figure 2. - Alignment of the amino acid sequence of rainbow trout SHBGb with the previously characterized SHBG protein (SHBGa). Conserved residues are shaded.

molecular mass of 45 kDa. The deduced SHBGb amino acid sequence displayed 26% and 29% identity with rainbow trout and zebrafish SHBG respectively (Fig. 2).

Conclusions

In the present study, we report and characterize the sequence of a novel gene putatively encoding for a previously uncharacterized sex hormone binding globulin that we named SHBGb. Unlike the classical SHBG protein (SHBGa), this gene is expressed in the ovary. In addition, expression profiling studies have demonstrated that SHBGb mRNA expression is extremely well correlated with ovarian aromatase mRNA expression during sex differentiation and

late-vitellogenesis and post-vitellogenesis. Further studies are needed to investigate the expression and steroid binding capacities of this novel sex hormone binding globulin.

References

BARON D., MONTFORT J., HOULGATTE R., FOSTIER A. & Y. GUIGUEN, 2007. - Androgen-induced masculinization in rainbow trout results in marked dysregulation of early gonadal gene expression profiles. *BMC Genomics*, Submitted.
 BOBE J., MONTFORT J., NGUYEN T., & A. FOSTIER, 2006. - Identification of new participants in the rainbow trout (*Oncorhynchus mykiss*) oocyte maturation and ovulation processes using cDNA microarrays. *Reprod. Biol. Endocrinol.*, 4: 39.