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Purification, Characterization, and Molecular Cloning of Gonadotropin Subunits of Silver Carp (*Hypophthalmichthys molitrix*)

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The α and β subunit of silver carp gonadotropin (scGTH-α and scGTH-β) were isolated by high-performance liquid chromatography. Heterogeneity of N-terminal amino acid sequence was observed in scGTH-α but not in scGTH-β. For determining the complete primary structures of scGTH-α and scGTH-β, their cDNAs were cloned. Combining the data of N- and C-terminal sequences determined from proteins and the amino acid sequences deduced from cDNAs, we infer that scGTH-α consists of 95 and/or 93 residues and scGTH-β consists of 115 residues. Both scGTH-α and scGTH-β are glycoprotein. Their carbohydrate content is about 20 g per 100 g protein. The molecular weights of scGTH-α and scGTH-β were calculated to be 12,700 and 15,700 Da, respectively. The amino acid sequences of scGTH-α and scGTH-β are very similar to those of the corresponding subunit of carp GTH, different in only 2 and 4 residues, respectively. In addition, a high extent of homology (70%) was also observed between the α subunits of fish and mammalian GTHs. In the case of β subunit, homology among various species of fish (75 to 98%) is much higher than that between fish and mammal (40%). These data suggest that the α subunit is conserved while the β subunit is diversified during the molecular evolution of vertebrate GTH.

Mammalian anterior pituitary gland synthesizes and secretes three glycoprotein hormones. They are luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH). The former two are collectively designated as gonadotropin (GTH). All of them consist of two nonidentical subunits, the α and β subunit. Upon nonconvalent association of these two subunits, hormonal function is achieved. Within a given species, all of these glycoprotein hormones share the same α subunit while the β subunit is hormone specific (see review of Pierce and Parsons, 1981). Like mammalian GTH, teleostean GTH is also synthesized and secreted from anterior pituitary gland and composed of two nonidentical subunits. The function of teleostean GTH on gametogenesis and steroidogenesis has been well documented (see review of Idler and Ng, 1983).

The primary structures of the α and β subunits of mammalian GTHs from several species had been reported (see review of Pierce and Parsons, 1981). Comparison of known sequences of α subunits indicates that they are highly conserved from species to species. Similarly, homology between β subunit is also apparent. There are regions of striking conservation, not only for the same hormone between species, but also between hormones.

The α and β subunits of GTH from several species of teleost have been purified and characterized (Burzawa-Gerard, 1974; Chang *et al.*, 1988b; Huang *et al.*, 1982; Itoh *et al.*, 1988; Suzuki *et al.*, 1988; Trinh *et al.*, 1986). Compared to those of mammalian GTHs, the primary structures of the α and β subunits of fish GTH are not so extensively studied. Up to now, only the complete sequence of the α subunit of carp GTH (cGTH-α) and the β subunit of cGTH

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(cGTH-β) (Chang *et al.*, 1988b) and salmon GTH (sGTH-β) (Itoh *et al.*, 1988; Trinh *et al.*, 1986) have been determined or deduced from the nucleotide sequence of cDNA. The comparative data show that there is a high extent of homology (70%) in amino acid sequence existing among the α subunit of carp and mammalian GTHs. As for the β subunit, the homology between cGTH and sGTH is high (75%) while that between fish GTH and mammalian GTH is low (40%). Such findings suggest that the α subunit is conserved while the β subunit is diversified during the molecular evolution of vertebrate GTH.

The above findings are only based on the data of two species of fish. In order to provide a wider basis for comparative study, we are attempting to determine the primary structures of the α and β subunit of GTHs from more species of fish. This paper is one of a series of such studies. Here, we report the purification and characterization of the α and β subunit of GTH (scGTH-α and scGTH-β) from silver carp (*Hypophthalmichthys molitrix*) and the determination of their primary structures by molecular cloning.

MATERIALS AND METHODS

Purification of scGTH-α and scGTH-β. The scGTH was purified as previously described (Chang *et al.*, 1988a). Purification of scGTH-α and scGTH-β was performed by high-performance liquid chromatography (HPLC). The scGTH was dissolved in H_2O and chromatographed through a Nucleosil C_{18} column (4.6 × 250 mm, 7 μm) equilibrated with solvent A (22.5% acetonitrile–0.07% trifluoroacetic acid, TFA) and eluted by a linear gradient of solvent B (40% acetonitrile–0.07% TFA) in which 70% of solvent B was achieved at 60 min. The flow rate was 1 ml/min. Operation of HPLC was performed at 40°. Individual peak was collected and recovered by lyophilization.

Bioassay of scGTH-α and scGTH-β. The GTH activities of scGTH-α and scGTH-β and their recombinant were assayed by the method of androgen production by carp testis *in vitro* as previously described (Huang and Chang, 1980). Reassociation of scGTH-α and scGTH-β was performed by mixing them at a 1:1 molar ratio (final concentration, 1 mg/ml) in 50 m*M* phosphate buffer, pH 7.4, and incubated at 25° for 2 hr.

Chemical analyses. The amino acid composition was determined by the method as described by Chang and Liu (1988). The N-terminal amino acid sequence was determined by a 477A protein sequencer and an on-line 120A phenylthiohydantoin analyzer of Applied Biosystems, Foster City, California (Hewick *et al.*, 1981). For determination of C-terminal amino acid sequence, scGTH-α and scGTH-β were digested with carboxypeptidase Y (Millipore Corp., Bedford, MA) (substrate:enzyme, 30:1, w/w) in 50 m*M* pyridine–acetate, pH 5.4, at 37° for 0, 10, and 20 min by the method of Hayashi (1977). The enzymatic digestion was stopped by the addition of acetic acid. After drying, released amino acids were converted to dimethylaminoazobenzenesulfonyl(DABSYL)amino acid and analyzed (Knecht and Chang, 1986). The carbohydrate content was estimated by using the thiobarbituric acid method for sialic acid (Warren, 1959), the orcinol reaction for neutral sugar (Winzler, 1955), and the *p*-dimethylaminobenzaldehyde reaction for amino sugar (Rondle and Morgan, 1955). The sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) was performed as described by Laemmli (1970). The scGTH and its subunits were quantified by their protein content determined by the method of Lowry *et al.* (1951) with bovine serum albumin as reference.

Radioimmunoassay. The scGTH-α and scGTH-β were used as antigen to induce antisera in rabbit by multiple-site injection (Vaitukaitis *et al.*, 1971). Iodination of protein was performed by the chloramine-T method (Greenwoods *et al.*, 1963). For assay, 10,000 cpm of labeled antigen and a proper dilution of antiserum which bound 30% of labeled antigen in the absence of unlabeled antigen were used. The assay conditions were essentially the same as those described by Roser *et al.* (1984).

Construction of cDNA library. The polyadenylated mRNA of silver carp pituitary glands was prepared from liquid nitrogen frozen tissue by the guanidinium/CsCl method (Ullrich *et al.*, 1977) followed by oligo-dT cellulose column chromatography. Double-stranded cDNA, synthesized by the method of Gubler and Hoffman (1983), was ligated with *Eco*RI linker and subsequently inserted into the *Eco*RI site of pUC 19. The *Escherichia coli* strain JM 101 was used as host.

Screening of cDNA library. The cDNAs encoding cGTH-α and cGTH-β (Chang *et al.*, 1988b) were used as probes to screen the cDNA coding for scGTH-α and scGTH-β, respectively. The cDNAs used for probes were labeled with ^{32}P-dATP by nick translation.

Bacteria containing recombinant plasmids were first grown on nitrocellulose filter, lysed with NaOH, baked, and then hybridized by the method of Grunstein and Hogness (1975). For further confirmation the plasmid DNAs of positive clones were extracted and

digested with *Eco*RI, subsequently subjected to electrophoresis in agarose, and then hybridized *in situ* with labeled probes (Kidd *et al.*, 1983).

DNA sequence analysis. In order to analyze the complete nucleotide sequences of the cloned cDNAs, they were further subcloned. The cDNAs enconding scGTH-α and scGTH-β were cleaved with *Pst*I and *Ava*II, respectively. After separation by electrophoresis in agarose and electroelution, the resulted DNA fragments were subcloned into pUC 19. The nucleotide sequences were determined by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977).

RESULTS

Isolation of scGTH-α and scGTH-β

By the HPLC system used in this study, scGTH could be resolved into four major peaks (Fig. 1). When they were analyzed by SDS–PAGE, peaks 1, 3, and 4 showed one electrophoretic band while peak 2 showed two electrophoretic bands. As described below, the N-terminal amino acid sequence analysis indicated that peak 1 had a similar sequence to that of cGTH-α while peaks 3 and 4 had a similar sequence to that of cGTH-β (Table 1). Consequently, peak 1 was designated as scGTH-α and peaks 3 and 4 were collectively designated as scGTH-β.

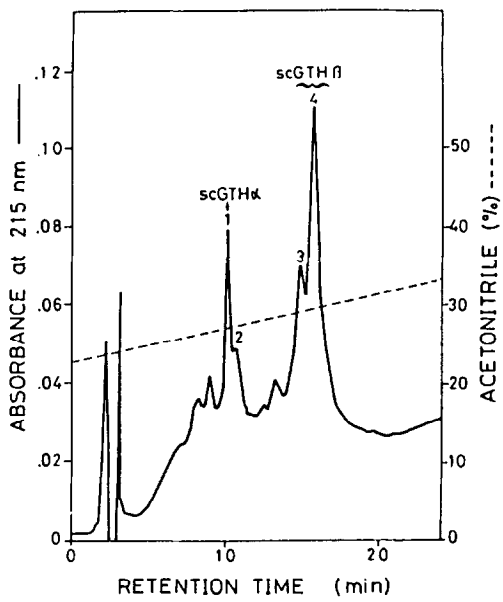

FIG. 1. Reverse-phase HPLC fractionation of the subunits of silver carp GTH. Ten micrograms of scGTH dissolved in H_2O was loaded onto a Nucleosil C_{18} column (4.6 × 250 mm, 7 μm) equilibrated with solvent A (22.5% acetonitrile–0.07% trifluoroacetic acid, TFA). The sample was eluted with a linear gradient in which 70% of solvent B (40% acetonitrile–0.07% TFA) was reached at 60 min at a flow rate of 1 ml/min. Operation of HPLC was performed at 40°.

GTH Acitivity of scGTH-α, scGTH-β, and Their Recombinant

As shown in Fig. 2, either scGTH-α or scGTH-β expressed very low GTH activity while the recombinant of scGTH-α and scGTH-β expressed high GTH activity fully comparable to native scGTH when assayed by androgen production by carp testis *in vitro*. These results further indicate that scGTH-α and scGTH-β isolated by HPLC are the two different subunits of scGTH.

Radioimmunoassay

In order to further characterize scGTH-α and scGTH-β, an immunological approach was also undertaken. Antiserum against scGTH-α (AS-α) and that against scGTH-β (AS-β) were induced in rabbit. Both AS-α and As-β reacted strongly to the homologous antigen but weakly to the heterologous antigen. Calculated at B/B_0 = 50%, AS-α showed 3.7% cross-reactivity toward scGTH-β while As-β showed 5.6% cross-reactivity toward scGTH-α (data not shown).

Chemical Properties of scGTH-α and scGTH-β

The amino acid composition, carbohydrate content, and N- and C-terminal amino acid sequence of scGTH-α and scGTH-β are presented in Table 1. Both scGTH-α and scGTH-β had high content of Asp/Asn, Glu/Gln, Pro, and half Cys. In comparison, the scGTH-α contains more basic but less

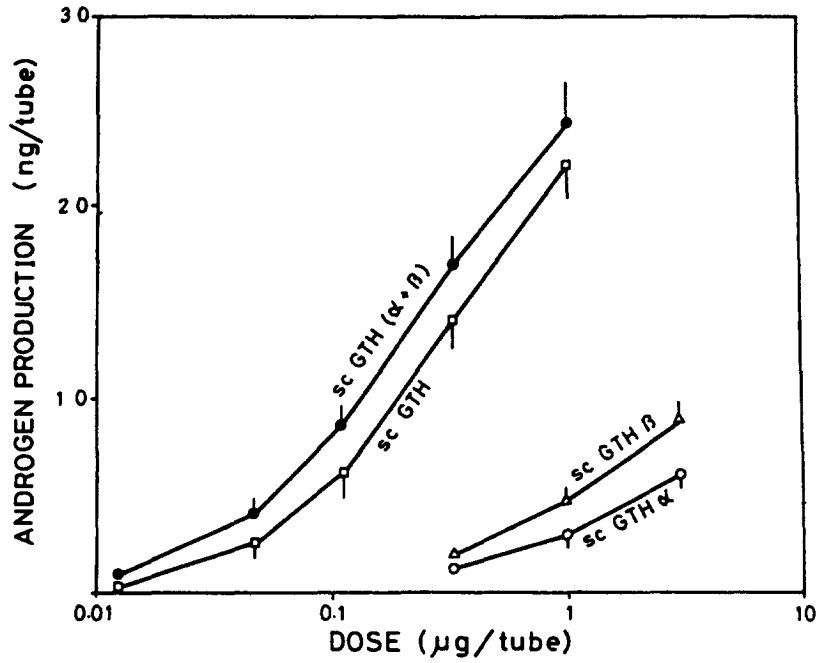

FIG. 2. The GTH activities of native scGTH, its α and β subunits, and the recombinant of α and β subunits. The method of androgen production by carp testis *in vitro* (Huang and Chang, 1980) was used for assay of GTH activity. Each point is the mean of three incubations. Vertical line indicates standard deviation.

acidic amino acid residues than scGTH-β. In addition, scGTH-α had a lower carbohydrate content than scGTH-β, mainly attributable to amino sugar.

The amino acid sequence analysis indicated heterogeneity in N-terminal sequence was observed in scGTH-α but not in scGTH-β. Two forms of scGTH-α were found, one with Tyr–Pro–Arg–Asn–Asp– while the other one had Arg–Asn–Asp– as their N-terminal sequence. For scGTH-β, the N-terminal sequence was identified to be Ser–Phe–Leu–Pro–Pro–. The C-terminal sequences of scGTH-α and scGTH-β were determined to be –Lys–Ser and –Phe–Pro, respectively. The molecular weights of scGTH-α and scGTH-β estimated by SDS–PAGE were 17,400 and 22,670 Da, respectively. However, these values are different from those calculated by summing up the molecular weights of constituting amino acid residues and carbohydrate content of the molecule, which yielded 12,700 Da for scGTH-α and 15,700 Da for scGTH-β.

Nucleotide Sequencing of cDNA Encoding scGTH-α

From 548 recombinant cDNA clones, 4 positive clones were obtained when cDNA encoding cGTH-α was used as a probe. The nucleotide sequence and the deduced amino acid sequence of one cDNA encoding scGTH-α are presented in Fig. 3. As shown in Fig. 3, it has 869 base pairs (bp) in length, consisting of 31 bp of the 5′ untranslated region, 354 bp of the open reading frame, and 481 bp of the 3′ untranslated region. The open reading frame encodes a polypeptide of 118 residues which contains a 95-residue protein with the same N- and C-terminal sequences as those determined from scGTH-α. The amino acid composition predicted from cDNA is very close to that determined from scGTH-α (Table 1).

TABLE 1
THE CHEMICAL PROPERTIES OF scGTH-α AND scGTH-β

	α subunit		β subunit	
	Protein[a]	cDNA[b]	Protein[a]	cDNA[b]
Amino acid composition[c]				
Lys	9.2 (9)	10	4.0 (4)	4
His	3.2 (3)	3	3.0 (3)	3
Arg	3.9 (4)	4	4.1 (4)	4
Asx	10.2 (10)	10	9.1 (9)	9
Thr	6.6 (7)	7	10.3 (10)	11
Ser	5.8 (6)	6	7.6 (8)	9
Glx	6.3 (6)	6	11.6 (12)	12
Pro	6.4 (6)	6	13.2 (13)	13
Gly	3.7 (4)	3	4.1 (4)	3
Ala	4.7 (5)	4	2.7 (3)	2
1/2Cys	9.6 (10)	10	12.1 (12)	12
Val	8.4 (8)	8	11.4 (11)	11
Met	1.9 (2)	2	2.0 (2)	2
Ile	2.9 (3)	3	3.2 (3)	3
Leu	5.4 (5)	5	7.1 (7)	7
Tyr	4.2 (4)	5	5.6 (6)	6
Phe	3.0 (3)	3	4.0 (4)	4
Total	(95)	95	(115)	115
Amino acid sequence				
N-terminal	(Tyr–Pro)–Arg–Asn–Asp–		Ser–Phe–Leu–Pro–Pro–	
C-terminal	–Lys–Ser		–Phe–Pro	
Carbohydrate content[d]				
Neutral sugar	10.2		10.6	
Amino sugar	6.6		11.2	
Sialic acid	1.3		2.1	

[a] Determined from protein by amino acid analysis.
[b] Predicted from nucleotide sequence of cDNA.
[c] Number of residues. The integrated number is shown in parentheses.
[d] g per 100 g protein.

Nucleotide Sequencing of cDNA Encoding scGTH-β

There are three positive clones obtained when cDNA encoding cGTH-β was used as a probe for screening of the silver carp pituitary cDNA library. The nucleotide and deduced amino acid sequence of one cDNA encoding scGTH-β are presented in Fig. 4. It has 554 bp in length, consisting of 18 bp of the 5′ untranslated region, 423 bp of the open reading frame, and 110 bp of the 3′ untranslated region. The open reading frame encodes a 115-residue protein with the same N- and C-terminal sequences as those determined from scGTH-β. The data of Table 1 also indicated that the amino acid composition predicted from cDNA is very close to that determined from scGTH-β.

DISCUSSION

The scGTH has been purified and characterized (Chang *et al.*, 1988a). In this paper, we continued our previous work to purify and characterize the subunits of scGTH for the purpose of getting a better understanding of scGTH and of providing a wider basis for comparative study of vertebrate GTHs.

The two subunits of scGTH prepared in this study seem to be highly purified, as

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CATCTCACTGGAAGTCAAGAACAAAGCCATC ATG TTT TGG ACA AGA TAT GCT GGA  55
                                Met Phe Trp Thr Arg Tyr Ala Gly
                                -23         -20

GCA AGT ATA TTA TTG TTT TTA ATG CTT ATT CAT CTT GGA CAA GTA TAT 103
Ala Ser Ile Leu Leu Phe Leu Met Leu Ile His Leu Gly Gln Val Tyr
                    -10                                  -1  1

CCA AGA AAT GAT ATT ACT AAC TTT GGA TGT GAG GAG TGC AAA CTC AAG 151
Pro Arg Asn Asp Ile Thr Asn Phe Gly Cys Glu Glu Cys Lys Leu Lys
                                 10

GAG AAC AAC ATT TTC TCA AAA CCC GGC GCT CCC GTC TAT CAG TGT ATG 199
Glu Asn Asn Ile Phe Ser Lys Pro Gly Ala Pro Val Tyr Gln Cys Met
         20                                  30

GGA TGC TGC TTT TCC AGG GCT TAC CCC ACA CCC CTG AGG TCC AAG AAA 247
Gly Cys Cys Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys
                        40

ACC ATG CTT GTT CCC AAA AAT ATC ACA TCA GAA GCT ACA TGC TGT GTA 295
Thr Met Leu Val Pro Lys Asn Ile Thr Ser Glu Ala Thr Cys Cys Val
 50                                  60

GCC AAA GAA GTT AAA CGG GTA CTT GTC AAT GAT GTC AAA CTA GTG AAC 343
Ala Lys Glu Val Lys Arg Val Leu Val Asn Asp Val Lys Leu Val Asn
                 70                                      80

CAC ACA GAC TGC CAC TGT AGC ACC TGT TAC TAT CAC AAA TCT TAA AAA 391
His Thr Asp Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser ***
                                90                  95

CACTATGACATTTCAAATTTACTTGTGTTTGCTTACTTACTTATATTCCTGTACCTATTTTTT 454

CTGCAGTGCTTATTTTCTGTTCTTGATTCACAATGACTTACATATTTAAAGTGAAAACATACT 517

GTTAGAAGTTTGCTATATACCCGTACTGTGCAAATTTTCTTCATACTGTCGATTGTTTTAAAC 580

AATTCTTTTTTAAAAGGTTGTCATAATTGTTGTAATTTGTGCCCTACTTCCATAAATAGCTTA 643

AAATGCAATATTTTATCATTTATAAAATGCAAGATAACTTCATTACTATGCTCTGCTTGGTAT 706

TATTTTAAACCCTCTTTTGTTGGTAATTGCTGACTTGTTTTGTCGTCGCTGCTATACGATTGT 769

TCAAATACATTAATTAAAAACAAGATTGCTTATGA(n=66)                        869
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FIG. 3. The nucleotide sequence and the deduced amino acid sequence of cDNA encoding scGTH-α.

evidenced by the following criteria: (1) Both scGTH-α and scGTH-β have only one electrophoretic band in SDS–PAGE; (2) reassociation of scGTH-α and scGTH-β could fully restore the hormonal function (Fig. 2); (3) the antiserum against scGTH-α and that against scGTH-β had very low cross-reactivity toward their counterpart subunits; and (4) both scGTH-α and scGTH-β have their own unique N- and C-terminal sequence (Table 1). However, it should be noted that heterogeneity is found in the N-terminal sequence of scGTH-α. Such phenomenon was also observed in the case of ovine LH-α (Liu *et al.*, 1972).

Molecular cloning has been widely used to determine the primary structure of protein. Combining the data of N- and C-terminal sequences determined from protein and the amino acid sequence deduced from cDNA, we infer that scGTH-α has 95 and/or 93 residues while scGTH-β has 115 residues. The C-terminal residue of scGTH-α is the same one just preceding the stop codon while that of scGTH-β is the one which is two residues ahead of the stop

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 AGAGGGACACCTGTCAAG ATG TTA GCT GTT CGA AAC AAC ATC CTC CTT CTC  51
                    Met Leu Ala Val Arg Asn Asn Ile Leu Leu Leu
                    -24             -20

TTA TTC TGT TTA GTT GTT CTG CTA GTC TTT GCT CAA AGC TCT TTT CTT  99
Leu Phe Cys Leu Val Val Leu Leu Val Phe Ala Gln Ser Ser Phe Leu
           -10                                  -1  1

CCA CCA TGT GAG CCA GTT AAT GAG ACT GTT GCA GTG GAG AAA GAG GGC 147
Pro Pro Cys Glu Pro Val Asn Glu Thr Val Ala Val Glu Lys Glu Gly
                       10

TGT CCA AAA TGT CTG GTG TTT CAG ACC ACC ATC TGC AGT GGC CAC TGC 195
Cys Pro Lys Cys Leu Val Phe Gln Thr Thr Ile Cys Ser Gly His Cys
20                                  30

CTA ACA AAG GAG CCT GTA TAC AAG AGC CCA TTT TCC ACT GTC TAC CAA 243
Leu Thr Lys Glu Pro Val Tyr Lys Ser Pro Phe Ser Thr Val Tyr Gln
                 40                                  50

CAC GTG TGC ACT TAC CGG GAC GTC CGC TAT GAG ACA GTC CGC TTG CCA 291
His Val Cys Thr Tyr Arg Asp Val Arg Tyr Glu Thr Val Arg Leu Pro
                               60

GAC TGT CCT CCC GGG GTG GAC CCC CAT ATC ACT TAC CCG GTG GCT CTC 339
Asp Cys Pro Pro Gly Val Asp Pro His Ile Thr Tyr Pro Val Ala Leu
         70                                  80

AGC TGC GAC TGC AGC CTC TGC ACC ATG GAC ACG TCC GAC TGT ACC ATC 387
Ser Cys Asp Cys Ser Leu Cys Thr Met Asp Thr Ser Asp Cys Thr Ile
                       90

GAA AGC CTG CAG CCT GAT TAC TGC ATG TCT CAG AGG GAG GAT TTC CCT 435
Glu Ser Leu Gln Pro Asp Tyr Cys Met Ser Gln Arg Glu Asp Phe Pro
100                                 110

GTG TAT TAG CCTACAGGAGTACTGTCCTGTCATCAAACCACAAAGCCCACTCTAAATCAG 494
Val Tyr ***
    117

ATAAATGTCACATAGATGTATATCAATAAAAAACTACATACTTCATA(n=13)           554
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FIG. 4. The nucleotide sequence and the deduced amino acid sequence of cDNA encoding scGTH-β.

codon. Consequently, there must be a post-translational modification of scGTH-β by proteolytic cleavage of two residues (–Val–Tyr) from the precursor. Post-translational modification at the C-terminal part is also observed in cGTH-β (Chang *et al.*, 1988b) and mammalian LH-β (Maurer, 1985) and TSH-β (Maurer *et al.*, 1984), but not in sGTH-β (Itoh *et al.*, 1988; Trinh *et al.*, 1986) and mammalian FSH-β (Esch *et al.*, 1986).

Recently, two distinct GTHs, designated as GTH I and II, from chum salmon pituitary glands were isolated by Suzuki *et al.* (1988). They share a common α subunit but have a different β subunit. The homology of the amino acid sequence between these two subunits is very low, only about 30% (Itoh *et al.*, 1988). Therefore, duality of GTH was hypothesized. However, only one type of GTH was isolated and characterized from other species of fish, including silver carp (Burzawa-Gerard, 1974; Chang *et al.*, 1988a,b; Donaldson *et al.*, 1972; Farmer and Papkoff, 1977; Huang *et al.*, 1981; Pierce *et al.*, 1976). Such discrepancy may be due to either species differences or different methods used for GTH extraction and purification. If two types of β subunits of GTH are present in silver carp, then two types of corresponding cDNAs should be expected. Because the probe we used for

screening scGTH-β was the cDNA encoding cGTH-β, which is highly homologous to the β subunit of salmon GTH II, therefore only one type of cDNA encoding scGTH-β was obtained. In order to investigate whether duality of GTH is also present in silver carp or not, we are currently rescreening the cDNA library of silver carp with other probes.

The amino acid sequence of scGTH-α is very similar to that of cGTH-α, with a difference of 2 residues. In addition, scGTH-α bears a high extent of homology (72%) to that of the α subunit of mammalian glycoprotein hormones (Tables 2 and 4). The extent of homology between the α subunit of fish and mammalian GTH is close to that between the α subunit of pituitary glycoprotein hormones from different species of mammal. Like the α subunit, homology of amino acid sequences between scGTH-β and cGTH-β is extremely high, only different in 4 residues. When scGTH-β is compared with sGTH-β, the homology in the amino acid sequence decreased to 75% (Table 4). The β subunit of Cyprinidae GTH (scGTH and cGTH) and Salmonidae GTH (sGTH) share a long homologous region in the middle part of the molecule (Table 3). However, their N- and C-terminal sequences are different from each other. These results reveal the phylogenetic rela-

TABLE 2
THE AMINO ACID SEQUENCES OF THE α SUBUNIT OF SILVER CARP AND CARP GTH AND HUMAN AND BOVINE LH

scGTH	Tyr	Pro	Arg	Asn	Asp	Ile	Thr	Asn	Phe	Gly	Cys	Glu	Glu	Cys	
cGTH	—	—	—	—	—	Met	Asn	—	—	—	—	—	—	—	
hLH								Val	Gln	Asp	—	Pro	—	—	
bLH	Phe	—	Asp	Gly	Glx	Phe	—	Met	Glu	—	—	Pro	Gln	—	
scGTH	Lys	Leu	Lys	Glu	Asn	Asn	Ile	Phe	Ser	Lys	Pro	Gly	Ala	Pro	
cGTH	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
hLH	Thr	—	Gln	—	—	Pro	Phe	—	—	Gln	—	—	—	—	
bLH	—	—	—	—	—	Lys	Tyr	—	—	—	—	Asp	—	—	
scGTH	Val	Tyr	Gln	Cys	Met	Gly	Cys	Cys	Phe	Ser	Arg	Ala	Tyr	Pro	
cGTH	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
hLH	Ile	Leu	—	—	—	—	—	—	—	—	—	—	—	—	
bLH	Ile	—	—	—	—	—	—	—	—	—	—	—	—	—	
scGTH	Thr	Pro	Leu	Arg	Ser	Lys	Lys	Thr	Met	Leu	Val	Pro	Lys	Asn	
cGTH	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
hLH	—	—	—	—	—	—	—	—	—	—	—	Gln	—	—	
bLH	—	—	Ala	—	—	—	—	—	—	—	—	—	—	—	
scGTH	Ile	Thr	Ser	Glu	Ala	Thr	Cys	Cys	Val	Ala	Lys	Glu	Val	Lys	
cGTH	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
hLH	Val	—	—	—	Ser	—	—	—	—	—	—	Ser	Tyr	Asn	
bLH	—	—	—	Glx	—	—	—	—	—	—	—	Ala	Phe	Thr	
scGTH	Arg	Val	Leu	Val	Asn	Asp	Val	Lys	Leu	Val	X	Asn	His	Thr	
cGTH	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
hLH	—	—	Thr	—	Met	Gly	Gly	Phe	Lys	—	Glu	—	—	—	
bLH	Lys	Ala	Thr	—	Met	Gly	Asn	Val	Arg	—	Glx	—	—	—	
scGTH	Asp	Cys	His	Cys	Ser	Thr	Cys	Tyr	Tyr	His	Lys	Ser			
cGTH	—	—	—	—	—	—	—	—	—	—	—	—			
hLH	Ala	—	—	—	—	—	—	—	—	—	—	—			
bLH	Glx	—	—	—	—	—	—	—	—	—	—	—			

Note. sc, silver carp; c, carp; h, human; b, bovine; —, residue identical to that of scGTH; X, gap insertion for half cystine alignment. References: cGTH from Chang *et al.* (1988b); hLH from Fiddes and Talmadge (1984); bLH from Cornell and Pierce (1974).

TABLE 3

THE AMINO ACID SEQUENCES OF THE β SUBUNIT OF SILVER CARP, CARP, AND SALMON GTH AND BOVINE LH AND FSH

scGTH				Ser	Phe	Leu	Pro	Pro	Cys	Glu	Pro	Val	Asn	Glu	
cGTH				—	Tyr	—	—	—	—	—	—	—	—	—	
sGTH				—	Leu	Met	Gln	—	—	Gln	—	Ile	—	Gln	
bLH	Ser	Arg	Gly	Pro	Leu	Arg	—	Leu	—	Gln	—	Ile	—	Ala	
bFSH									—	—	Leu	Thr	—	Ile	
scGTH	Thr	Val	Ala	Val	Glu	Lys	Glu	Gly	Cys	Pro	Lys	Cys	Leu	Val	
cGTH	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
sGTH	—	—	Ser	Leu	—	—	—	—	—	—	Thr	—	—	—	
bLH	—	Leu	—	Ala	—	—	—	Ala	—	—	Val	—	Ile	Thr	
bFSH	—	Ile	Thr	—	—	—	—	Glu	—	Gly	Phe	—	Ile	Ser	
scGTH	Phe	Gln	Thr	Thr	Ile	Cys	Ser	Gly	His	Cys	Leu	Thr	Lys	Glu	
cGTH	Leu	—	—	—	—	—	—	—	—	—	—	—	—	—	
sGTH	Ile	Arg	Ala	Pro	—	—	—	—	—	—	Val	—	—	—	
bLH	—	Thr	—	Ser	—	—	Ala	—	Tyr	—	Pro	Ser	Met	Lys	
bFSH	Ile	Asn	—	—	Trp	—	Ala	—	Tyr	—	Tyr	—	Arg	Asp	
scGTH	Pro	Val	Tyr	Lys	Ser	Pro	Phe	Ser	Thr	Val	Tyr	Gln	His	Val	
cGTH	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
sGTH	—	—	Phe	—	—	—	—	—	—	—	—	—	—	—	
bLH	Arg	—	Leu	Pro	Val	Ile	Leu	Pro	Pro	Met	Pro	—	Arg	—	
bFSH	Leu	—	—	Arg	Asp	—	Ala	Arg	Pro	Asn	Ile	—	Lys	Thr	
scGTH	Cys	Thr	Tyr	Arg	Asp	Val	Arg	Tyr	Glu	Thr	Val	Arg	Leu	Pro	
cGTH	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
sGTH	—	—	—	—	—	—	—	—	—	Met	Ile	—	—	—	
bLH	—	—	—	His	Glu	Leu	—	Phe	Ala	Ser	—	—	—	—	
bFSH	—	—	Phe	Lys	Glu	Leu	Val	—	—	—	—	Lys	Val	—	
scGTH	Asp	Cys	Pro	Pro	Gly	Val	Asp	Pro	His	Ile	Thr	Tyr	Pro	Val	
cGTH	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
sGTH	—	—	—	—	Trp	Ser	—	—	—	Val	—	—	—	—	
bLH	Gly	—	—	—	—	—	—	—	Met	Val	Ser	Phe	—	—	
bFSH	Gly	—	Ala	His	His	Ala	—	Ser	Leu	Tyr	—	—	—	—	
scGTH	Ala	Leu	Ser	Cys	Asp	Cys	Ser	Leu	Cys	Thr	Met	Asp	Thr	Ser	
cGTH	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
sGTH	—	—	—	—	—	—	—	—	—	Asn	—	—	—	—	
bLH	—	—	—	—	His	—	Gly	Pro	—	Arg	Leu	Ser	Ser	Thr	
bFSH	—	Thr	Glu	—	His	—	—	Lys	—	Asp	Ser	Asp	Ser	Thr	
scGTH	Asp	Cys	Thr	Ile	Glu	Ser	Leu	Gln	Pro	Asp	Tyr	Cys	Met	Ser	
cGTH	—	—	—	—	—	—	—	—	—	—	Phe	—	—	—	
sGTH	—	—	—	—	—	—	—	—	—	—	Phe	—	Ile	Thr	
bLH	—	—	Gly	Gly	Pro	Arg	Thr	—	—	Leu	Ala	—	Asp	His	
bFSH	—	—	—	Val	Arg	Gly	—	Gly	—	Ser	Tyr	—	Ser	Phe	
scGTH	Gln	Arg	Glu	Asp	Phe	Pro									
cGTH	—	—	—	—	—	Leu									
sGTH	—	—	Val	Leu	Thr	Asp	Gly	Asp	Met	Trp					
bLH	Pro	Pro	Leu	Pro	Asp	Ile	Leu								
bFSH	Arg	Glu	Ile	Lys	Glu										

Note. sc, silver carp; c, carp; s, salmon; b, bovine; —, residue identical to that of scGTH. References: cGTH from Chang *et al.* (1988b); sGTH from Trinh *et al.* (1986); bLH from Maurer (1985); bFSH from Esch *et al.* (1986).

TABLE 4
HOMOLOGY ANALYSIS OF VERTEBRATE GTHs

Subunit	Type of GTH	Homology (%)
α	scGTH vs cGTH	98
	scGTH vs bLH	72
	bLH vs hLH	74
β	scGTH vs cGTH	97
	scGTH vs sGTH	75
	scGTH vs bLH	42
	scGTH vs bFSH	40
	sGTH vs bLH	41
	sGTH vs bFSH	35
	bLH vs bFSH	38

Note. References: cGTH-α and cGTH-β from Chang *et al.* (1988b); bLH-α from Cornell and Pierce (1974); bLH-β from Maurer (1985); hLH-α from Fiddes and Talmadge (1984); sGTH-β from Trinh *et al.* (1986); bFSH-β from Esch *et al.* (1986).

tionship of the β subunit of fish GTH. The data of Tables 3 and 4 indicate that homology between the β subunit of fish and mammalian GTH is around 40%, which is close to that between the β subunit of LH and FSH of a given species of mammal. Although the amino acid sequences of the β subunit of fish and mammalian GTHs are diversified, all of them contain 12 half-cystines and these residues can be aligned at the same positions.

The homology analyses of the α and β subunit between fish and mammalian GTH are summarized in Table 4. These comparative data gave further indication that the α subunit is conserved while the β subunit is diversified during the molecular evolution of vertebrate GTH.

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