Eur. J. Biochem. *186*, 105–114 (1989)

Pike eel (*Muraenesox cinereus*) gonadotropin

Amino acid sequences of both α and β subunits

Chen-Sheng LIU[1,2], Fore-Lien HUANG[1], Yea-Sha CHANG[1,2] and Tung-Bin LO[1,2]

[1] Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan
[2] Graduate Institute of Biochemical Science, National Taiwan University, Taipei, Taiwan

(Received June 5/August 8, 1989) – EJB 89 0708

The amino acid sequences of pike eel gonadotropin α and β subunits have been determined by standard sequencing analytical methods. The α subunit is composed of 93 amino acid residues while the β subunit comprises 113 amino acid residues. All the invariant half-cystine residues are in the same positions as those found in other gonadotropins.

It is noteworthy that the first, putative glycosylation site (Asn56) found in the α subunit of other gonadotropins was replaced by Asp56 in the α subunit of pike eel gonadotropin. Similarity analyses indicate that both subunits are structurally more similar to other known fish gonadotropin subunits than to those of the mammalian gonadotropins.

Gonadotropin belongs to a family of glycoprotein hormones which are secreted from the anterior pituitary and placenta. It is composed of two subunits, a highly conserved α subunit and an activity-dictating β subunit, while hormonal activity occurs only after noncovalent interaction between an α subunit and a β subunit [1, 2]. From previous cumulative studies on the pituitary gonadotropins of the mammals, birds, reptiles (except snakes) and amphibians [3], it becomes clear that two types of gonadotropins exist, namely follitropin and lutropin. As the names imply, they stimulate testicular and ovarian functions by means of regulation of gametogenesis and steroid hormone synthesis [4]. In contrast, a number of studies on teleost gonadotropins have revealed that it has only a single type of gonadotropin [5–15]. An exceptional case was reported recently which showed two distinct β subunits of chum salmon gonadotropins [16]. Compared to mammalian gonadotropins, there is little information on the primary structures of α and β subunits of fish gonadotropins. So far, structures of cDNA-derived salmon β subunit [14], carp α and β subunits [15], and amino acid sequences of two distinct chum salmon β subunits [16] have been reported.

Huang et al. [12] initiated the isolation and purification of pike eel (*Muraenesox cinereus*) gonadotropin and later on succeeded in obtaining α and β subunits from a phenyl-Sepharose column [17]. We have now completed the amino acid sequencing of both α and β subunits and the results demonstrate that 93 and 113 amino acid residues are present in the α and β subunits, respectively. It is remarkable that only one glycosylation site is found in the α-subunit molecule while other gonadotropin α subunits so far studied have been claimed to have two glycosylation sites [1, 2]. The individual structures are compared to the representative gonadotropin α and β subunits.

Correspondence to C.-S. Liu, Institute of Biological Chemistry, Academia Sinica, P.O. Box 23-106, Taipei, Taiwan 10098, Republic of China

Abbreviations. Dabsyl, dimethylaminoazobenzencsulfonyl; Pth, phenylthiohydantoin; RCM, reduced and carboxamidomethylated.

Enzymes. Carboxypeptidase Y (EC 3.4.16.1); α-chymotrypsin (EC 3.4.21.1); endoproteinase Asp-N (EC 3.4.21.–); endoproteinase Glu-C (EC 3.4.21.19); lysyl endoproteinase (*Achromobacter* protease I) (EC 3.4.14.50); trypsin (EC 3.4.21.4).

Note. The novel amino acid sequence data published here has been deposited with the EMBL sequence data bank.

MATERIALS AND METHODS

Materials

Pike eel gonadotropin α and β subunits were purified as described [17] and also purified by HPLC after acidic dissociation [14]. Homogeneity of the purified subunit was ascertained by N-terminal amino acid sequencing and amino acid analyses. The suppliers of enzymes were as follows: Boehringer, FRG, for endoproteinase Asp-N, endoproteinase Glu-C and trypsin; Wako, Japan, for lysyl endoproteinase; Worthington, USA, for α-chymotrypsin and carboxypeptidase Y. Reagents for alkylation included iodoacetamide (Sigma, USA) and guanidium chloride (Merck, FRG). All other reagents were of the highest grade commercially available.

Enzyme digestion of proteins and purification of peptides

Reduction and alkylation of the purified subunits were carried out essentially by the procedure of Crestfield et al. [18]. Usually, 1.0 mg protein was incubated at 55°C for 3 h in 200 µl 0.4 M Tris/HCl, 0.1% EDTA, 20 mM dithiothreitol, 7 M guanidium chloride solution, pH 8.7, which was purged with argon gas. About 0.8 mg iodoacetamide in 30 µl of the above-mentioned solution was added and incubated for 30 min at 55°C in the dark. Desalting was performed by reversed-phase HPLC using linear gradient elution of 0–60% acetonitrile in 0.07% trifluoroacetic acid employing a Vydac C_4 column. Reduced and carboxamidomethylated protein (RCM protein) was recovered after Speed-Vac concentration. Unless otherwise noted, digestion conditions of RCM proteins using trypsin, lysyl endopeptidase, endoproteinase Glu-

C and endoproteinase Asp-N were the same as described previously [19]. Digestions by α-chymotrypsin and carboxypeptidase Y were carried out as described in the literature [20, 21]. Intact β subunit was used for the C-terminal sequence determination by carboxypeptidase Y and liberated amino acid(s) were converted to dimethylaminoazobenzenesulfonyl (Dabsyl) amino acids [22]. Peptides resulting from enzymatic digestion were purified by reversed-phase HPLC using a C_{18} column with a mobile phase of acetonitrile in 0.07% trifluoroacetic acid.

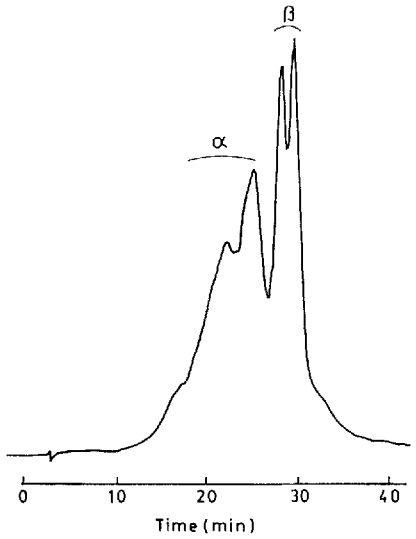

Fig. 1. *HPLC of purified pike eel gonadotropin.* Purified pike eel gonadotropin (DE-80 fraction) was incubated at 37°C for 30 m to 1 h in 0.07% trifluoroacetic acid (solvent A). The aliquot (0.4 mg) was injected on to a Synchropak C_4 column (0.46 cm × 25 cm) with the linear gradient 20–40% solvent B (0.07% trifluoroacetic acid in acetonitrile), run for 80 min and monitored at 220 nm with a flow rate of 1 ml/min. Full absorbance was set at 1.0

Amino acid analysis and sequence determination

Amino acid analyses were accomplished by an accelerated method [23] combining gas-phase hydrolysis at 158°C [24] in a Waters hydrolysis vessel and derivatization with Dabsyl chloride before HPLC analysis of Dabsyl amino acids [22]. Edman degradation was processed in a pulsed-liquid-type sequenator (model 477A, Applied Biosystems) provided with an on-line phenylthiohydantoin (Pth) analyzer. The program 'Normal-I' was used throughout.

Computer analysis of the secondary structure of proteins

Protein sequences were analyzed by using the DNASTAR system provided by American Megatrends Inc. Hydropathy analysis was performed in accordance with Kyte and Doolittle [25] with a window size of 7 amino acids. Another secondary structure expression was provided by the method of Chou and Fasman [26].

RESULTS

Purity of the isolated subunits

Phenyl-Sepharose column chromatography under acidic condition yielded two subunits termed S-I and S-II, the former corresponding to the α subunit and the latter to the β subunit

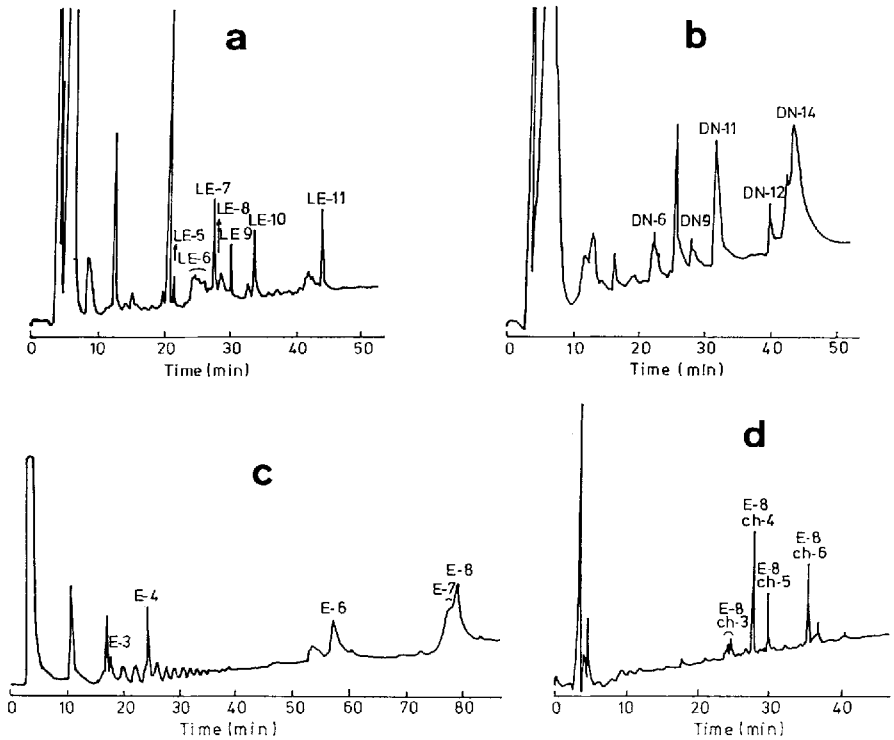

Fig. 2. *Enzymatic digests of RCM pike eel gonadotropin α subunit.* (a) Lysyl endopeptidase digest (0.09 mg) was separated on a Nucleosil C_{18} column with the gradient 0–60% solvent B/60 min. Full absorbance was 0.2 at 220 nm. (b) About 0.2 mg endoproteinase Asp-N digest was separated under the same condition of (a), except full absorbance of 0.1. (c) About 0.1 mg endoproteinase Glu-C digest was separated for 90 min. Other conditions were the same as in (a). (d) α-Chymotrypsin digest of E-8 peptide was separated with 5–60% solvent B for 60 min and full absorbance of 0.1. Other conditions were the same as in (a)

Table 1. *Amino acid composition of RCM pike eel gonadotropin α subunit (RCM PEα), endoproteinase Glu-C digests of RCM pike eel gonadotropin α and chymotryptic digests of the E-8 peptide*
CmCys, carboxymethylcysteine

Amino acid	RCM PEα	Amino acid composition of peptide								
		E-3	E-7	E-8	E-4	E-6	E-8			
							Ch-5	Ch-6	Ch-3	Ch-4
	mol/mol									
Asx	9.9 (10)	2.9	3.3	2.0		3.8 (4)	1.0			1.2 (1)
Glx	6.7 (6)	2.0	2.3	2.0	1.5	1.4 (1)		1.0		1.2 (1)
CmCys	9.4 (10)	0.9	3.6	2.7	1.7	2.9 (3)		2.2		
Ser	8.3 (8)	1.3	5.3	5.0		2.3 (2)		2.0	1.0 (1)	2.0 (2)
Thr	8.0 (7)		2.8	2.6	0.8	3.0 (3)			0.9 (1)	1.6 (2)
Gly	3.9 (3)	2.5	1.6	1.5				1.0		
Ala	4.5 (4)		2.3	2.2	2.0			0.8	1.0 (1)	
Arg	5.2 (5)	1.3	2.7	2.4	1.2				1.1 (1)	1.2 (1)
Pro	6.0 (6)	1.2	5.1	5.0				2.0	2.2 (2)	1.3 (1)
Val	4.5 (4)		2.2	2.0	0.8	1.2 (1)		0.8		0.7 (1)
Met	2.6 (2)		1.1	1.1		1.3 (1)				0.5 (1)
Ile	3.0 (3)	0.9	2.0	1.8				0.8		0.9 (1)
Leu	5.6 (5)		3.3	1.9		2.0 (2)			1.0 (1)	1.3 (1)
Phe	4.6 (4)		4.3	3.9			2.0	2.1		
Lys	8.3 (9)		5.7	5.0		3.0 (3)	1.0	1.2		2.2 (3)
His	3.1 (3)					3.2 (3)				
Tyr	3.0 (4)	0.6	1.0	0.8		1.3 (2)			0.8 (1)	
Sequence location		1–3	14–60	19–60	61–68	69–93	19–22	23–37	38–45	46–60

Table 2. *Amino acid composition of lysyl endopeptidase digest of RCM pike eel gonadotropin α*
CmCys, carboxymethylcysteine; ND, not determined

Amino acid	Amino acid composition of peptide						
	LE-8	LE-7	LE-11	LE-9	LE-10	LE-5	LE-6
	mol/mol						
Asx	3.3 (3)				1.0 (1)	1.9	1.8 (2)
Glx	2.0 (2)		1.6 (1)		2.3 (2)		1.4 (1)
CmCys	1.8 (2)		2.1 (3)		1.9 (2)		1.8 (3)
Ser	1.2 (1)	1.2	2.8 (3)		1.0 (1)		2.2 (2)
Thr			1.6 (1)	1.3 (1)	3.1 (3)		1.8 (2)
Gly	2.3 (2)		1.8 (1)				
Ala			2.5 (2)		2.3 (2)		
Arg	2.3 (2)		1.4 (2)		1.2 (1)		
Pro	1.0 (1)		4.0 (4)	1.3 (1)			
Val			1.0 (1)	0.9 (1)	2.0 (2)		
Met				0.9 (1)		0.9	
Ile	0.7 (1)		0.9 (1)		1.0 (1)		
Leu	1.0 (1)		1.2 (1)	1.0 (1)		1.1	1.2 (1)
Phe		1.9	1.4 (2)				
Lys	1.0 (1)	1.0	0.6 (1)	1.0 (1)	1.0 (1)	1.0	1.0 (1)
His							2.4 (3)
Tyr	0.8 (1)		ND (1)				1.7 (2)
Sequence location	1–17	21–24	25–48	50–55	56–71	72–76	77–93

[17]. By means of SDS/PAGE, S-II was always found to be essentially homogeneous, while S-I was sometimes contaminated by S-II. Hence, the major supply of α subunit was provided by reversed-phase HPLC of the DE-80 fraction [12] after acidic dissociation (Fig. 1).

Identical amino acid compositions of the first two peaks indicated the presence of two α peaks which were closely followed by two β peaks for the same reason. Rechromatography of the α peak, which emerged first under the same conditions, disclosed only one symmetrical peak at the same retention time (not shown).

Sequencing of α subunit

RCM α subunit was fragmented separately by endoproteinase Glu-C, endoproteinase Asp-N and lysyl endopeptidase. In all cases, digestion was conducted in 1 M guanidium chloride, 0.06 M Tris/HCl, pH 8.7, immediately after re-

Table 3. *Amino acid composition of endoproteinase Asp-N digest of RCM pike eel gonadotropin α*
CmCys, carboxymethylcysteine

Amino acid	Amino acid composition of peptide				
	DN-9	DN-14	DN-12	DN-6	EN-11
	mol/mol				
Asx	2.6	2.3	1.0 (1)	3.5	1.4
Glx	2.1	1.4	1.8 (2)	1.4	
CmCys	1.4	2.9	1.5 (2)		3.3
Ser	1.4	4.2	1.1 (1)		1.6
Thr		1.8	2.4 (3)	1.1	1.3
Gly	2.2	1.5			
Ala		2.4	1.9 (2)		
Arg	1.2	2.3	1.1 (1)		
Pro	1.2	5.0			
Val		1.6	1.6 (2)		
Met		0.8		1.3	
Ile	0.9	1.0	0.8 (1)		
Leu	1.0	1.5	1.0 (1)	1.0	
Phe		3.8			
Lys	1.2	3.6	1.1 (1)	0.9	1.0
His				0.9	1.9
Tyr	1.1	1.1			1.7
Sequence location	1–17	18–55	56–72	73–81	82–93

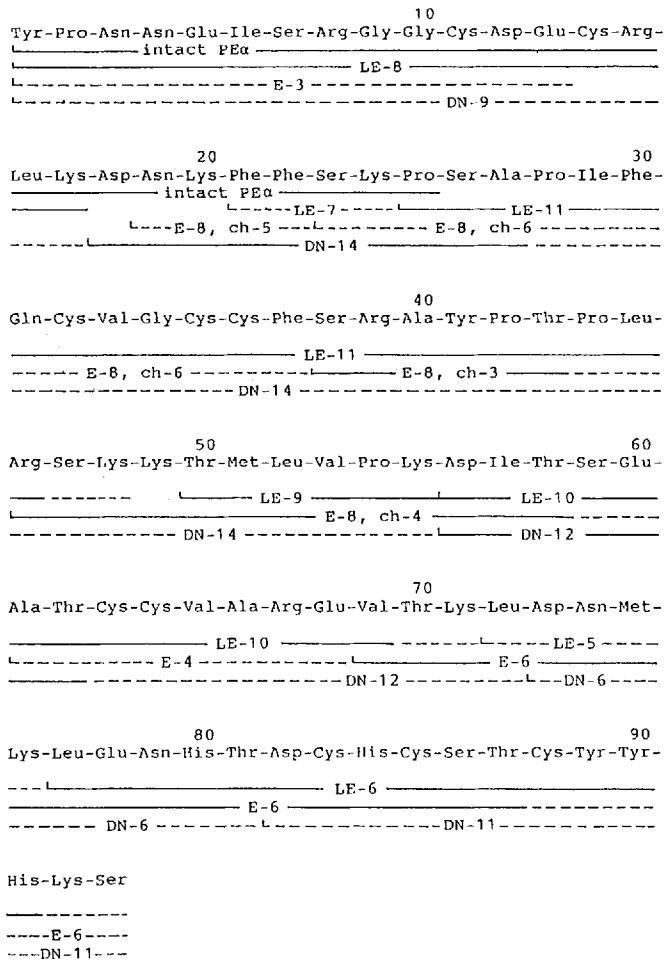

Fig. 3. *Amino acid sequence of pike eel gonadotropin α subunit.* Results of sequencing are shown in the solid-line sections while the dotted-line sections show the sequence inferred from the amino acid analyses

duction and carboxamidomethylation, by adding 6 vol. H_2O. The large peptide, E-8, obtained after HPLC separation of endoproteinase Glu-C was subfragmented by α-chymotrypsin. The four kinds of peptide maps obtained by reversed-phase HPLC were displayed collectively in Fig. 2a–d.

The amino acid composition data for the above-mentioned enzymatic digests as well as that of the RCM α subunit are compiled in Tables 1–3. These data attest to the certainty of the results obtained by the automatic sequenator work on the individual peptides. N-Terminal sequencing of the intact, native pike eel gonadotropin α subunit established the arrangement of the initial 25 amino acid residues. Sequencing of the peptides from different sources complemented each other to complete the residual alignment of the molecule which had 93 amino acid residues (Fig. 3). The two C-terminal amino acids, Lys-Ser, were assigned in accordance with the amino acid compositions (such as DN-11 peptide) as well as other fish gonadotropin α subunits (see Fig. 8).

The two presumed N-linked glycosylation sites in gonadotropin α subunit [1, 2] happened to be Asp-Ile-Thr (56–58) and Asn-His-Thr (79–81) in the present molecule. Asn 79 could not be detected in the sequencing process while Asp56 appeared as Pth-Asp in three cases; LE-10 peptide, E-8, ch-4 peptide and DN-12 peptide. The seven successive degradation patterns of DN-12 peptide were shown in Fig. 4. The amounts of Pth-modified amino acid at each step were calculated: Asp (456 pmol)-Ile(63 pmol)-Thr(160 pmol)-Ser(92 pmol)-Glu(50 pmol)-Ala(63 pmol)-Thr(79 pmol).

Sequencing of the β subunit

The three HPLC chromatograms generated by endoproteinase Asp-N, trypsin and α-chymotrypsin digestions of RCM β subunit, are shown in Fig. 5a–c. The resulting amino acid composition data as well as that of RCM β subunit were placed in Tables 4 and 5. For the chymotryptic peptides, only those needed for overlapping purpose were documented (see Table 5). Determination of the 39 N-terminal amino acid residues was accomplished by the sequencing of RCM pike eel gonadotropin β subunit. The successive linkings of the individual peptides derived from the same source were performed with the aid of other peptides, ending up in 113 amino acid residues (Fig. 6). The missing structural gap of 58–60 between T3 and T2 peptides was filled by the ch-2 peptide (57–61) and the DN-7 peptide (58–67) successively. Since the C-terminal proline failed to appear in the sequencing of DN-10 peptide, its presence was ascertained by carboxypeptidase Y action on the intact molecule as indicated in Fig. 6. The one potential glycosylation site was assumed to be in Asn-Glu-Thr(10–12).

DISCUSSION

Fish gonadotropins are unique in two respects: (a) each contains one species-specific gonadotropin compared to the coexistence of lutropin and follitropin in mammals; by the rather high gonadotropin content of the pituitary, e.g. 4.2% in carp [27], 7.6% in sturgeon [28] and 8% in pike eel [12] with reference to 0.6% of ovine lutropin [29] and 0.003% of ovine follitropin [30].

In the pike eel, gonadotropin activity was expressed in several electrophoretically different forms on SDS/PAGE system having the potency ratio of 3:1 between the highest and the lowest potencies [12]. The starting material used in this study was therefore the DE-80 fraction with the highest po-

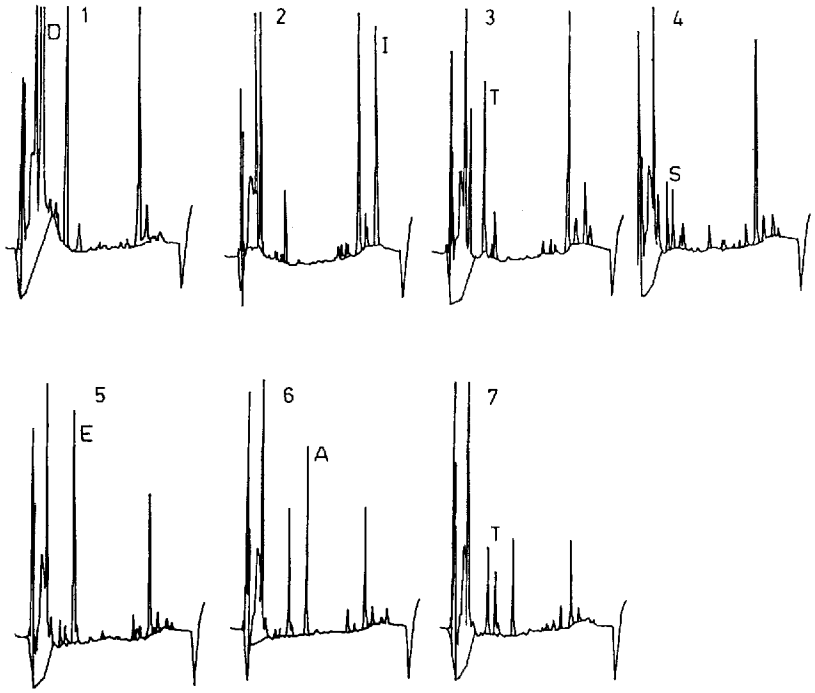

Fig. 4. *Direct sequence analysis of DN-12 peptide from Fig. 2b.* One-letter amino acid notation was used to indicate Pth-modified amino acids

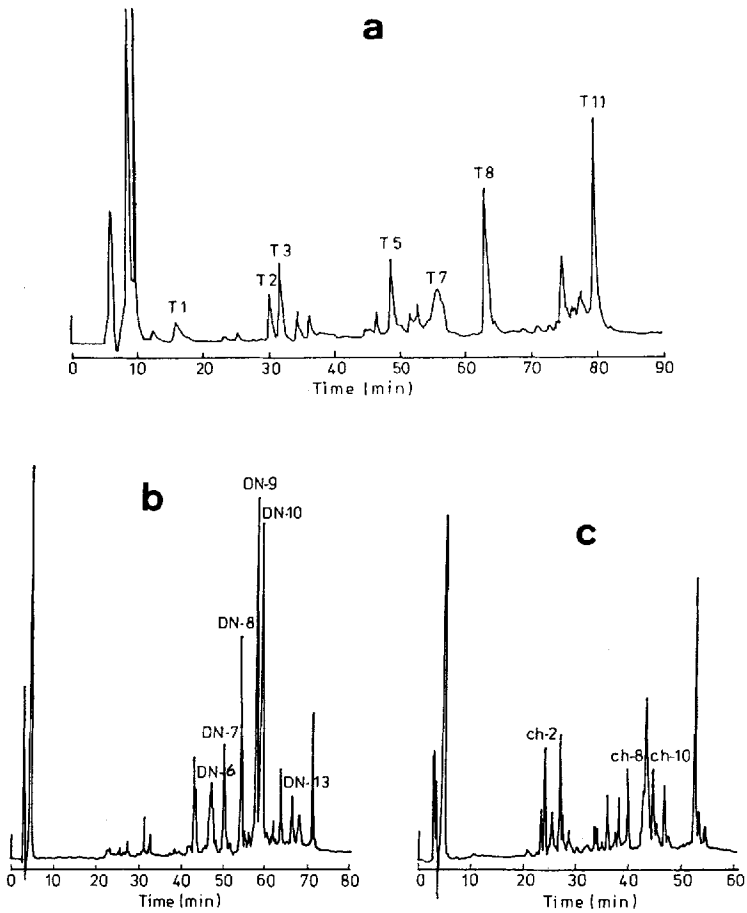

Fig. 5. *Enzymatic digests of RCM pike eel gonadotropin β subunit.* (a) Tryptic digest (0.25 mg) was separated on a Nucleosil C_{18} column eluted by linear gradient of 5–65% solvent B within 120 min. Full absorbance was 1.0 at 220 nm. (b) Endoproteinase Asp-N digest (0.7 mg) was eluted by 5–50% solvent B within 90 min. Other conditions were the same as described in (a). (c) Chymotryptic digest (0.25 mg) was eluted by 5–65% solvent B within 90 min. Other conditions were the same as described in (a)

Table 4. *Amino acid composition of RCM pike eel gonadotropin β subunit (RCM PEβ) and tryptic peptides of RCM pike eel gonadotropin β*
CmCys, carboxymethylcysteine

Amino acid	Amino acid composition of							
	RCM PE β	T7	T1	T8	T5	T3	T2	T11
	mol/mol							
Asx	11.5 (11)	1.3 (1)	1.3 (1)	1.1 (1)				6.6 (7)
Glx	8.6 (9)	3.7 (4)		1.2 (1)	1.2 (1)		1.3 (1)	2.0 (2)
CmCys	8.7 (12)	1.1 (1)	1.3 (1)	3.0 (3)		1.0 (1)		6.0 (6)
Ser	8.9 (11)	2.0 (2)		2.6 (3)	1.9 (2)			3.3 (4)
Thr	6.7 (9)	1.0 (1)		1.8 (2)	0.9 (1)	1.2 (1)	1.0 (1)	2.8 (3)
Gly	2.9 (3)		1.1 (1)	1.2 (1)				1.2 (1)
Ala	3.7 (3)							2.4 (3)
Pro	6.2 (7)				1.0 (1)	1.2 (1)	1.1 (1)	2.7 (3)
Val	11.2 (11)	3.2 (2)	1.0 (1)	0.9 (1)	1.0 (1)			5.3 (6)
Met	10.0 (10)	1.9 (2)		0.9 (1)	0.9 (1)	1.0 (1)	1.0 (1)	2.7 (3)
Ile	2.0 (2)							2.0 (2)
Leu	5.6 (5)	1.8 (2)		1.8 (2)				0.9 (1)
Phe	6.3 (7)	1.0 (1)		1.0 (1)	1.1 (1)			3.6 (4)
Lys	2.7 (3)			0.9 (1)				1.8 (2)
His	4.1 (4)	1.1 (1)	1.2 (1)	1.7 (2)				
Tyr	1.8 (2)			0.9 (1)				0.8 (1)
	3.3 (4)			0.8 (1)	1.0 (1)	1.2 (1)	1.1 (1)	
Sequence position		1–17	18–22	23–43	44–52	53–57	61–65	66–113

Table 5. *Amino acid analyses of endoproteinase Asp-N and α-chymotryptic digest of RCM pike eel gonadotropin β*
CmCys, carboxymethylcysteine

Amino acid	Amino acid composition of peptide								
	DN-6	DN-8	DN-9	DN-7	DN-13	DN-10	CH-2	CH-8	CH-10
	mol/mol								
Asx	1.2 (1)	0.9 (1)	1.0 (1)	1.0 (1)	3.3 (4)	2.5 (2)	1.3 (1)	4.7 (4)	1.5 (1)
Glx	5.0 (4)	1.0 (1)	1.0 (1)	0.9 (1)		2.5 (2)		1.4 (1)	0.7 (1)
CmCys	0.7 (1)	3.3 (4)	0.9 (1)		3.8 (4)	1.6 (2)		3.3 (4)	1.0 (1)
Ser	1.9 (2)	1.9 (2)	3.0 (3)		1.3 (1)	2.0 (2)		2.0 (2)	1.5 (2)
Thr	0.8 (1)	2.0 (2)	2.1 (2)	1.0 (1)	2.0 (2)			1.9 (2)	
Gly		2.5 (2)			1.4 (1)				
Ala					1.3 (1)	2.3 (2)		1.0 (1)	1.1 (1)
Arg			2.4 (2)	2.0 (2)	1.2 (1)	1.5 (2)	2.0 (2)		1.4 (2)
Pro	2.3 (2)	1.3 (1)	2.5 (2)	1.4 (1)	3.4 (3)	2.2 (2)			1.5 (2)
Val	1.6 (2)	0.8 (1)	1.7 (2)	1.7 (2)	3.0 (3)		1.0 (1)		
Met					1.2 (1)	1.0 (1)		1.0 (1)	1.0 (1)
Ile	1.7 (2)	1.5 (2)				0.8 (1)		0.9 (1)	
Leu	0.9 (1)	0.9 (1)	1.1 (1)	1.0 (1)	2.3 (2)	0.9 (1)		0.9 (1)	1.1 (1)
Phe		1.0 (1)			1.4 (1)	1.0 (1)			1.0 (1)
Lys	1.0 (1)	1.7 (2)	1.1 (1)						
His		1.0 (1)			1.0 (1)				
Tyr			2.4 (3)	0.6 (1)			1.0 (1)		
Sequence position	1–17	18–38	39–57	58–67	68–92	96–113	57–61	84–100	101–113

tency. Another fraction, DE-50, was sometimes used for confirmatory purpose. All the evidences verify that there is only one α-subunit form and one β-subunit form in the gonadotropin of the pike eel.

Initial subunit separation using a C_{18} HPLC column failed to achieve a satisfactory recovery of α subunit and thereafter a C_4 HPLC column was used instead.

In Fig. 1, the pike eel gonadotropin α subunit appeared just before the β subunit; this order was reversed in the case of salmon gonadotropin [14]. By separate HPLC separation of the human chorionic gonadotropin α and β subunits, Grego and Hearn [31] showed the position of two α peaks just ahead of two β peaks. The cause of double peaks was attributed to either partial deamidation of the subunit during the isolation procedure or to differences in the glycosylation state [31].

Since the α subunit seemed to be absorbed considerably by a reversed-phase silica gel column in the course of chromatography, its enzymatic digestion was carried out im-

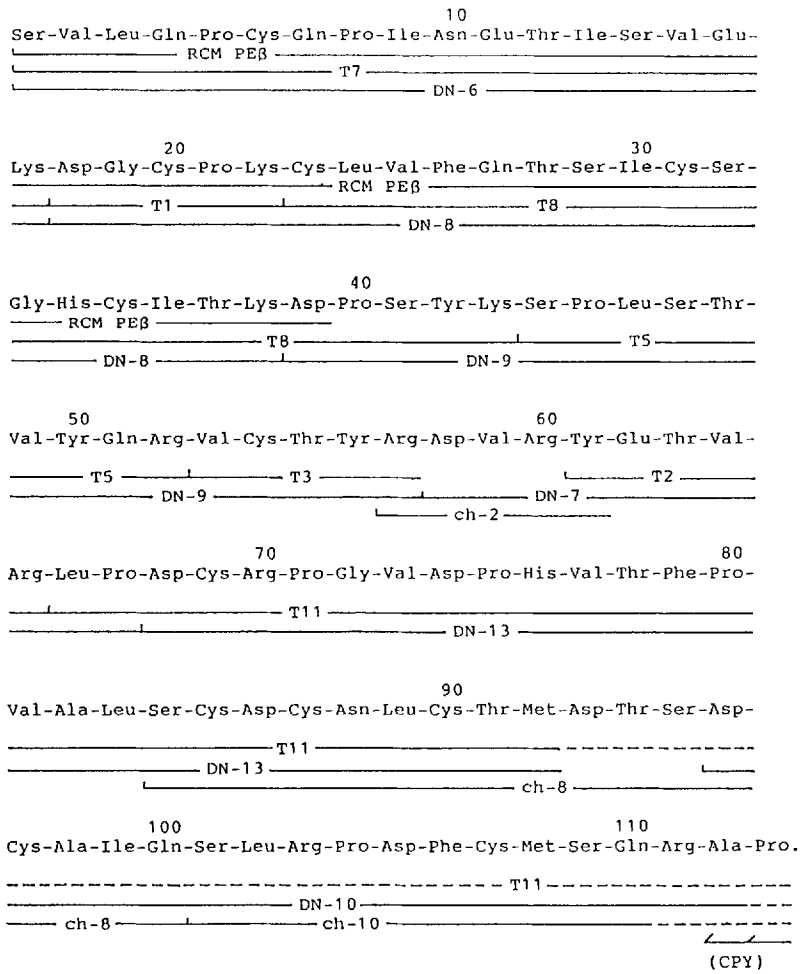

Fig. 6. *Amino acid sequence of pike eel gonadotropin β subunit.* Results of sequencing were expressed in solid-line sections while dotted-line sections show the sequence inferred from the amino acid analyses

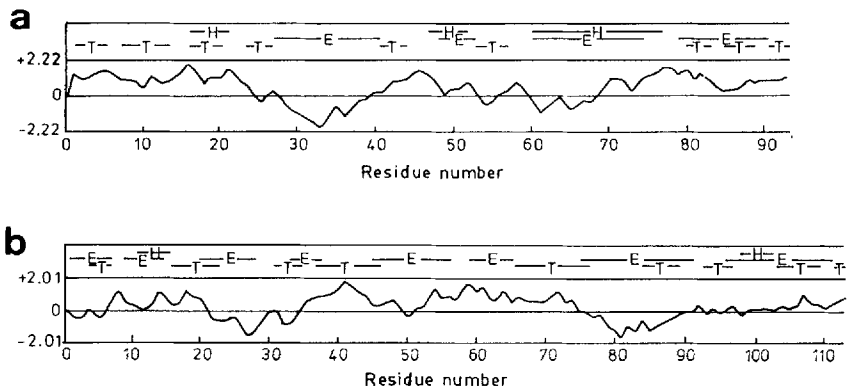

Fig. 7. *Secondary structure of pike eel gonadotropin α subunit (a) and pike eel gonadotropin β subunit (b).* (H) α-Helix; (E) extended structure or β-sheet; (T) β-turn according to Chou and Fasman [26]. Hydropathicity index was calculated according to Kyte and Doolittle [25] with the window size of 7 amino acid residues

mediately after reduction and alkylation in the presence of 1 M guanidium chloride to obviate the loss caused in the process of desalting through HPLC. Nevertheless, the recovery of α-subunit peptides generated after enzyme digestion was in general much lower than that of the β subunit peptides when they are compared in correlated terms of peak height, amount and absorbance unit (for example, compare Fig. 3b and Fig. 5b).

Maghuin-Rogister et al. (1975) reported the presence of two tryptic glycopeptides corresponding to sequence positions 56–67 and 80–95 in all α subunits of porcine lutropin, follitropin and thyrotropin [32]. Prior to this, they already showed that one glycopeptide, respectively, for β subunits of bovine and porcine lutropins were present in the sequence positions 8–15 and 9–16 [33]. Structural data so far documented [2] show that there are two potential N-linked

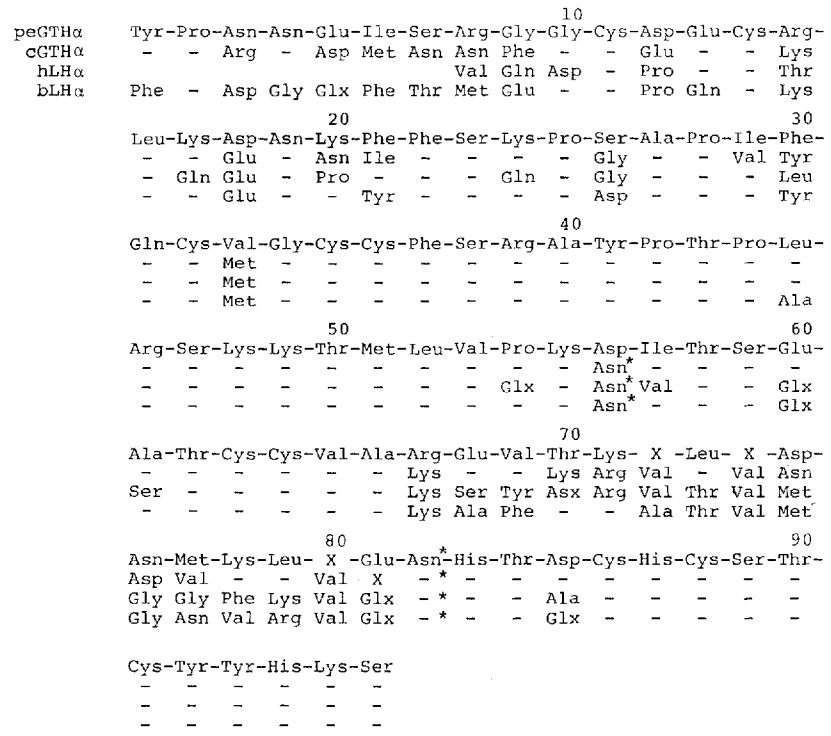

Fig. 8. *Amino acid sequence comparison of pike eel gonadotropin α (peGTHα) with carp gonadotropin α (cGTHα), human lutropin α (hLHα) and bovine lutropin α (bLHα): (*) presumed glycosylation site. (×) Deletion of amino acid residue*

glycosylation sites in the gonadotropin α subunits and one or two similar sites in the gonadotropin β subunits. Hence were surprised to know that one of the two potential glycosylation sites (Asn56 in the present sequence) in the α subunit was found to be an Asp residue in place of an Asn residue. The results of sequencing work on LE-10, E-8, ch-4 and DN-12 peptides involving this residue showed unanimously evidence of Asp residues (see also Fig. 4). It is worth noting that the DN-12 peptide was generated by employing endoproteinase Asp-N which specifically cleaves the peptide bond N-terminally at aspartic acid. Throughout the entire study of the α subunit, no single peptide containing Asn56 residue, in an amount detectable by amino acid analysis, was found. We were also puzzled by the low bioassay potency of the present α and β subunits in terms of steroidogenic activity (data not shown) as compared to the original bioassay data. Since there is still no evidence showing the indispensability of the first site glycosylation in the gonadotropin α subunit to the gonadotropin bioactivity, it is hard to assess the possible cause of its present low activity.

The pike eel gonadotropin α subunit is composed of 93 amino acid residues and the length of the β subunit, with 113 amino acid residues, is the shortest among known fish gonadotropin β subunits. The secondary structure elucidated by computer analysis shown in Fig. 7 indicates 29% and 8% α-helical content, 28% and 62% β-sheet content, 25% and 22% β-turn content, and 18% and 8% random coil content, respectively, for α and β subunits according to Chou and Fasman regulations [26]. When there were redundant expressions, the priority order for calculation was (a) α-helix (b) β-sheet and (c) β-turn. As with other known gonadotropins β [1], the remarkable feature of the β subunit resides in its low content of α-helix, implying the structural flexibility of the β subunit to meet the hormonal specificity.

The pike eel gonadotropin α is compared with carp gonadotropin α [15], human lutropin α [34] and bovine lutropin α [35] (see Fig. 8) while pike eel gonadotropin β is compared to carp gonadotropin β [15], salmon gonadotropin β [14], human lutropin β [36] and human follitropin β [37] (see Fig. 9). Similarity indices were calculated: 74% for carp gonadotropin α/pike eel gonadotropin α, 71% for human lutropin α/pike eel gonadotropin α, 67% for bovine lutropin α/pike eel gonadotropin α, 80% for carp gonadotropin β/pike eel gonadotropin β, 71% for salmon gonadotropin β/pike eel gonadotropin β, 45% for human lutropin β/pike eel gonadotropin β and lastly 35% for human follitropin β/pike eel gonadotropin β. It is worth mentioning that pike eel gonadotropin β is much more similar to that of carp than that of salmon. When compared to the mammalian gonadotropin α subunits, pike eel gonadotropin α had deletions at residues 72, 74 and 80, whereas carp gonadotropin α had only one deletion at residue 81.

By chemical modification studies, the C-terminal pentapeptide of the α subunit (Tyr-Tyr-His-Lys-Ser-COOH) apparently is required for receptor-hormone interaction [1]. From the competitive studies using synthetic peptides, residues 34–49 of the α subunit, a highly conserved section, possess a receptor-binding domain. Likewise the sequences between cysteines, 35–54 and 90–95 of the β subunit, are involved in the receptor binding [2]. So far, scarce information is available as to the regions of both α and β subunits involved in the dimer formation. In this regard, the homologous sequences of β 31–37 and β 66–69 are assumed to participate in the association process [2].

We are grateful for the typing work of Ms Mei-Chin Chen and also to the technical assistance of Ms Jin-Min Chen, Mr Chen-Hsien Chang and Mr Shun-Wen Chen. The financial support of National

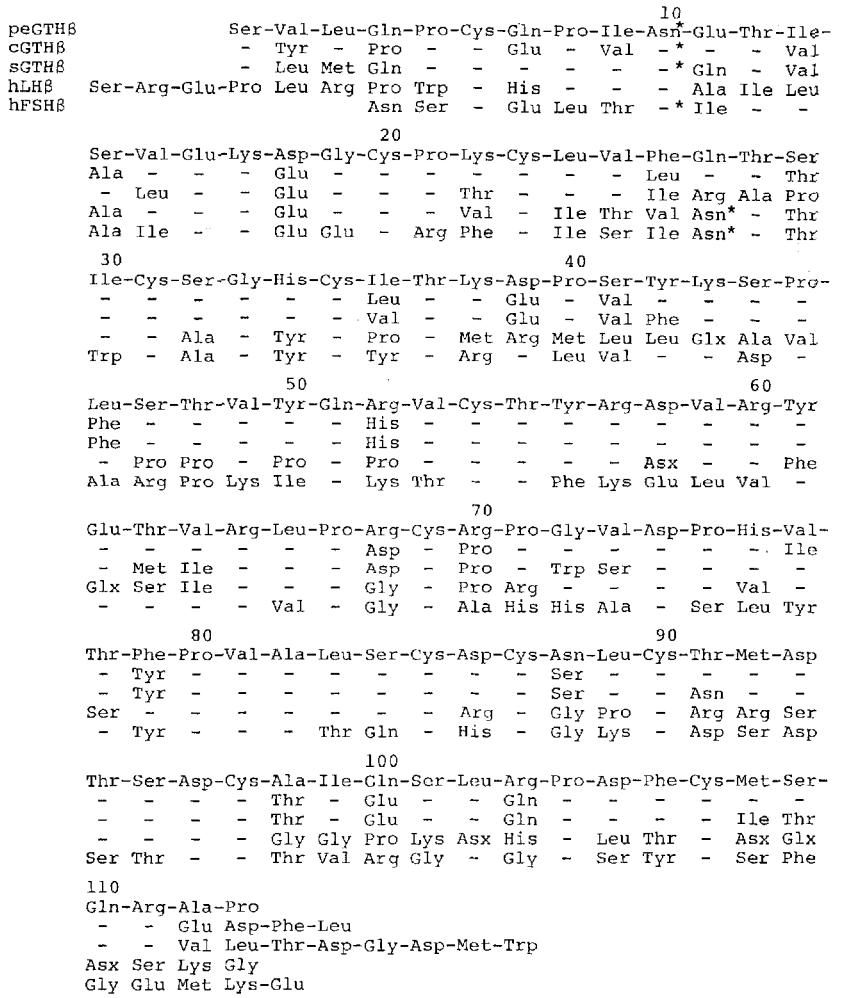

Fig. 9. *Amino acid sequence comparison of pike eel gonadotropin β subunit (peGTHβ) with carp gonadotropin β subunit (cGTHβ), salmon gonadotropin β (sGTHβ), human lutropin β subunit (hLHβ) and human follitropin β subunit (hFSHβ): (×) Deletion of amino acid residue; (*) presumed glycosylation site*

Science Council, Taipei, Taiwan, Republic of China is much appreciated.

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