[Chem. Pharm. Bull. 23(7)1622—1624(1975)]

UDC 547.466.1.057 : 615.357.011.5

Studies on Peptides. LI.[1,2] Application of the Solid Phase Synthesis for the Preparation of Pro-Analogues of LH and FSH Releasing Hormone

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(Received January 16, 1975)

Solid phase synthesis was applied to prepare a number of LH-FSH-RH analogues. Each constituent amino acid residue of this hypothalamic principle was systematically replaced by Pro. Ten samples thus prepared, after partial purification, were submitted to biological assay. However, none of the inactive peptide, hopefully an inhibitor, was found in these analogues.

Solid phase peptide synthesis introduced by Merrifield[4] has an advantageous feature to prepare various peptide samples for biological screening tests,[5] because of its quick and easy operation.

We applied this technique to prepare a series of peptide samples related to LH–FSH–RH (luteinizing hormone and follicle-stimulating hormone releasing hormone), whose structure (I) was determined by Schally, *et al.*[6] in 1971. Each constituent amino acid residue of LH–FSH–RH was systematically replaced by Pro. When this amino acid is involved in the peptide sequence, the imino hydrogen of peptide bond is vanished. Alternation in biological spectra of this hormone caused by such systematic change of the peptide backbone was examined.

(pyro)Glu–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–Gly–NH_2 (I)

Synthetic outlines we employed are those described previously.[5] Thus ten parallel solid phase syntheses were performed using respective Boc-amino acids. Except for the Pro[1]-analogue, the protected peptides were liberated from the resins by ammonolysis at the nonapeptide stage. Each resulting peptide amide, after treatment with trifluoroacetic acid (TFA), was condensed with Z-(pyro)Glu–OPCP[7] and the product was subsequently hydrogenated to remove all protecting groups. For the preparation of the Pro[1]-analogue, the solid phase synthesis was carried out at the decapeptide stage and the product was liberated in the protected form from the resin by ammonolysis and subsequently hydrogenated. Ten samples were then partially purified by Sephadex G-10 and submitted to biological screening test. The results are listed in Table I.

LH–FSH–RH contains one Pro residue at position 9, therefore the Pro[9]-analogue prepared above corresponds to the sample of LH–FSH–RH itself. As a reference, this partially purified sample of LH–FSH–RH was found to possess the activity of approximately 45% of the sample

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2) Peptides and their derivatives mentioned in this communication are of the L-configuration. Following abbreviations are used: Boc=*tert*-butoxycarbonyl, OPCP=pentachlorophenyl ester, DCC=dicyclohexylcarbodiimide.
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TABLE I. Screening Test of Pro-analogues of LH-FSH-RH

	Activity		Amino acid analysis									
	Dose (ng)	Mean serum LH +S.E. (ng/ml)	Glu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly
Pro^1-LH-RH[a]	200	12.07±1.73		0.66		0.97	0.74	2.20	1.00	0.92	1.60	
Pro^2-LH-RH	200	15.13±4.86	0.55			0.79	1.03	2.30	1.00	0.84	1.39	
Pro^3-LH-RH	200	9.23±2.33	0.40	0.58		0.79	0.69	2.02	1.00	0.65	2.22	
Pro^4-LH-RH	200	9.40±2.46	0.49	0.53			0.75	2.16	1.00	0.74	1.74	
Pro^5-LH-RH	200	24.27±9.11	0.52	0.57		0.65		2.24	1.00	0.78	1.61	
Pro^6-LH-RH[b]	200	5.90±1.42	0.54	0.63		0.79	0.73		1.00	0.61	1.65	1.07
Pro^7-LH-RH	200	10.73±3.64	0.43	0.42		0.64	0.74	2.00		0.70	1.44	
Pro^8-LH-RH	200	16.53±4.97	0.50	0.46		0.67	0.80	2.00	1.00		1.62	
Pro^9-LH-RH	5	19.28±5.37	0.52	0.49		0.70	0.84	2.26	1.00	0.78	0.64	
Pro^{10}-LH-RH[c]	200	18.80±6.78	0.57	0.42		1.00	1.04	1.22	1.00	0.62	1.04	
Synthetic[8]	5	42.30±7.30	(Trp was not determined)									

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synthesized by the conventional method.[8] This figure seems to be the guide of the purity of other partially purified analogues.

Some tendency in biological change caused by such replacement can be seen. As seen in Table 1, the activity dropped in general to approximately 1/40 or less compared to the above Pro^9 reference sample. It seems noteworthy that the Pro^2-analogue, the His-substituted peptide, retains still some biological activity. However none of the inactive peptide was found in these analogues. Thus, effort to search for inhibitory peptides by such approach has to be concluded as unpromising.

Experimental

Solid Phase Synthesis of Pro-Analogues of LH-FSH-RH——The Pro^{10}-analogue was prepared starting with Boc-Pro-resin (2 g, 0.75 mmole). Other nine analogues were prepared starting with Boc-Gly-resin (2 g each, 0.48 mmole). Removal of the Boc group of respective amino acid residues was performed by 50% TFA in methylenechloride (10 ml) in the presence of anisole (1 ml) at room temperature for 40 min. After introduction of the Trp residue, the deblocking reagent containing 0.1% mercaptoethanol[5,9] was employed. For neutralization, 0.5 N Et_3N in methylenechloride (10 ml) was employed. Except for Arg (NO_2) and His (Bzl), respective Boc-amino acids were incorporated to the peptide resin without particular protection for side chain functional groups. In every dicyclohexylcarbodiimide(DCC) coupling step, 5 equivalent of the respective Boc-amino acid and DCC were employed (reaction time for 24 hr). Boc-Ser-OH was introduced by the pentachlorophenyl ester procedure. In order to assure the introduction of the (pyro) Glu residue, each Boc-nonapeptide resin, except the Pro^1-resin, was liberated from the resin by ammonolysis (in 3 ml of MeOH and approximately 4 ml of liquid ammonia for 3 days) and each resulting protected nonapeptide amide, after treatment with TFA (1 ml) in the presence of anisole (0.2 ml) followed by neutralization with the quantitative amount of Et_3N, was allowed to react with Z-(pyro) Glu-OPCP (1.5 equivalent) in dimethylformamide (DMF) (5 ml) for 48 hr. The solvent was evaporated and the product was precipitated with ether and then hydrogenated in MeOH (20 ml) containing 5% AcOH over a Pd catalyst for 8 hr. Each crude product was then applied to a column of Sephadex G-10 (2×40 cm), which was eluted with 10% AcOH. Individual fractions (5 ml each) were examined by UV absorbancy at 275 mμ. Fractions corresponding to the front main peak were collected and the solvent was evaporated. The residue was lyophilized.

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For the preparation of the Pro[1] analogue, the solid phase synthesis was performed at the decapeptide stage and the partially purified as stated above. Amino acid analysis of 10 partially purified samples thus obtained were listed in Table I.

The LH releasing activity of these analogues was measured by the stimulation of release of LH in ovariectomized rats pretreated with estrogen and progesterone.[10]

Acknowledgement Authors express their sincere appreciation to Dr. Osamu Tanizawa, Department of Obstetrics and Gynecology, Osaka University, Medical School, for biological evaluation of these analogues.

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