A STUDY OF THE PEPTIDES OF A TRYPTIC HYDROLYZATE OF LACTOSOMATOTROPIN

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TABLE 1. Amino Acid Compositions and N-Terminal Amino Acids of Some Peptides of STH and LSTH

	1	Peptide No.							
Amino acid	1		2		3		4		
	STH	LSTH	STH	LSTH	STH	LSTH	STH	LSTH	
Lysine Histidine	+	+		+	+			+	
Argenine Aspartic acid Threonine Serine Glutamic acid	++++++	+++	+++++++++++++++++++++++++++++++++++++++	++	++	+++	++++++++++++	+ + +	
Proline Glycine Alanine Valine Methionine	+++	+	++++	++	+ + +	+	+++++++++++++++++++++++++++++++++++++++	+	
Isoleucine Leucine Tvrosine	+	+		+	+++		+	+	
Phenylalanine N-Terminal	+ Ser	+ Leu	Asp	Ile	Glu	Gly	+ Phe	Ile	

Making use of the quantitative method of obtaining hypophyseal hormones that we have developed [1], we have isolated a preparation of lactosomatotropin (LSTH) possessing, in addition to growth activity (1 unit/mg), lactogenic activity (8 units/mg by the pigeon test), in which the N-terminal amino acid is threonine and the C-terminal acid glycine. A comparison of the LSTH preparation with the preparation of somatotropic hormone (STH) obtained previously [2] was carried out by studying the amino acid composition and the N-terminal residues of the peptides isolated from tryptic hydrolyzates of STH and LSTH and having similar chromatographic and electrophoretic mobilities.

The STH and LSTH samples were hydrolyzed at a ratio of enzyme to substrate of 1:33 (the trypsin was treated with Carpenter's inhibitor) at 37°C in 0.05 M triethylamine carbonate buffer (pH 8) for 5 h, and 5 mg of each freeze-dried hydrolyzate was deposited on Whatman 3 mm paper (54 × 57 cm). After chromatography in the amyl alcohol-isobutanol-propanol-pyridine-water (1:1:1:3:3) system and electrophoresis in a pyridine-acetate buffer with pH 6.5, the chromatograms were sprayed with a 0.1% solution of fluorodinitrobenzene (FDNB) in a mixture of butanol and acetone. The excess of FDNB was removed with benzene, and the yellow spots were cut out and eluted with 1% acetic acid solution, the eluates being evaporated to dryness. The DNP-peptides were hydrolyzed and the ether-soluble DNP-amino acids were identified by thin-layer chromatography on silica gel.

The aqueous solutions of the hydrolyzates after treatment with ether and butanol were deposited on a paper chromatogram for the qualitative determination of the amino acid compositions of the peptides. All the results obtained, which are given in Table 1, permit the assumption that the STH and LSTH preparations contain fragments differing in their amino acid composition.

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