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ADRENOCORTICOTROPIN 53. THE AMINO ACID SEQUENCE OF THE HORMONE FROM THE OSTRICH PITUITARY GLAND

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SUMMARY: The amino acid sequence of corticotropin from the ostrich pituitary gland has been determined. It consists of 39 amino acids with the following sequence: H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Arg-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-Asn-Gly-Val-Gln-Glu-Glu-Thr-Ser-Glu-Gly-Phe-Pro-Leu-Glu-Phe-OH. This is the first report on the primary structure of corticotropins from avian species.

INTRODUCTION

Naudé and Oelofsen (1) reported recently the isolation and characterization of corticotropin (ACTH) from the pituitary gland of the ostrich Struthio Camelus. This communication describes the complete amino acid sequence of the ostrich ACTH.

MATERIALS AND METHODS

ACTH from ostrich pituitary glands was isolated by the procedure previously described (1). Commercial enzyme preparations of trypsin (Worthington Biochemical Corporation, TRTPCK 36C895) and pepsin (Armour Laboratories, Lot no. 18323) were performed using 2.0 mg ostrich ACTH in 0.3 ml 0.2M ammonium

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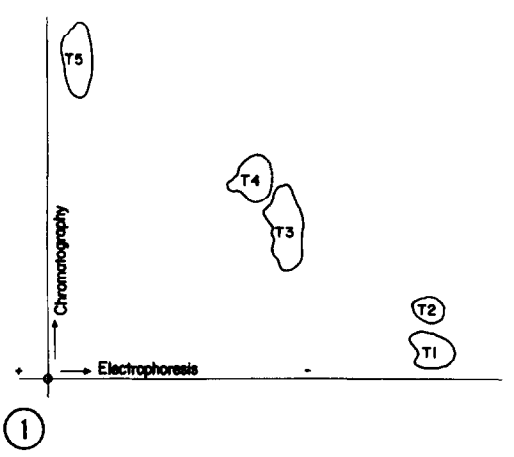
acetate buffer of pH 8.5 at 37° for 8 hr. Digestion using pepsin (E/S=1/100) was performed in 0.2 ml 5% formic acid with 1.0 mg of the hormone at 37° for 2 hr.

The tryptic peptides were isolated from peptide mapping on Whatman 3 mm paper. The first dimension was paper chromatography in the BAW System (upper phase, n-butanol-acetic acid-water, 4:1:5 by volume). The second dimension was high voltage electrophoresis at pH 2.1 (90% formic acid-acetic acid-water, 218:63:719 by volume) for 45 mins. at 2000v. The peptides were detected by spraying with a 0.01% ninhydrin in ethanol solution, and the slightly colored spots were eluted with 0.1N ammonium hydroxide. Appropriate aliquots were submitted to sequence analysis using the dansyl-Edman procedure (2,3) as previously described (4) and amino acid analyses (5). The identification of aspartic acid or asparagine and glutamic acid or glutamine was revealed by paper electrophoresis of the peptides at pH 6.7 as described by Offord (6). Digestions with carboxypeptidase A/B or hydrazinolysis (7) were employed for identification of the COOH-terminal sequence or residue.

For the sequence analysis of the carboxyl portion of ostrich ACTH, 4 mg of the hormone were digested by trypsin as described above. The digest was applied as a band for preparative paper electrophoresis on Whatman 3 mm using pH 6.7 buffer (γ-collidine-acetic acid-water, 8.9:3.1:988 by volume) at 400v for 2 hr. The acidic peptide band (peptide T5, see Figure 1) was detected by dilute ninhydrin on a guide strip, and the peptide was eluted with 0.1N acetic acid.

The peptide T5 (1 mg) was incubated with pepsin and the digest was fractionated by peptide mapping. The first dimension was paper chromatography in the BAW system followed by electrophoresis in pH 6.7 buffer for 2 hr. at 400v. Subsequent



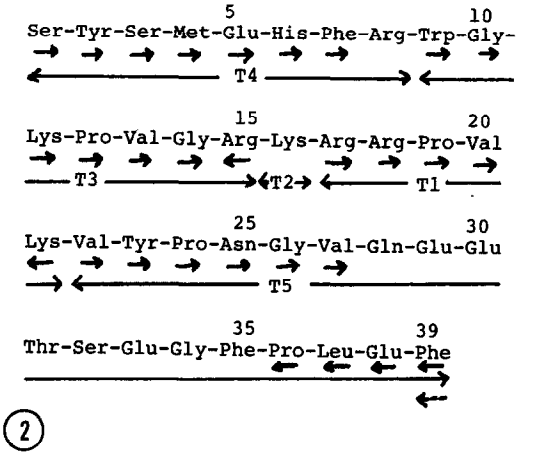


Figure 1. Peptide map of tryptic digest of ostrich ACTH.

Figure 2. Partial sequence of ostrich ACTH. → dansyl Edman; ← carboxypeptidase; ←··· hydrazinolysis.

development for detection, elution, and analyses were performed as described above.

RESULTS

Figure 1 presents the peptide map of the tryptic digest of ostrich ACTH. The amino acid compositions and NH_2-terminal residues of these tryptic peptides are shown in Table 1. From the NH_2-terminal residue of Os ACTH (1) and the sequence analysis of these peptides, the arrangement of these peptides is proposed to be: T4 → T3 → T2 → T1 → T5 as shown in Figure 2.

Tryptic peptide T5 was further digested with pepsin and the peptic peptides were separated by two-dimensional chromatography electrophoresis as shown in Figure 3. The peptic peptides were submitted to amino acid and NH_2-terminal residue (Table 2) and sequence analyses. Figure 4 presents the proposed primary structure of T5 from these analytical data. Thus, the complete amino acid sequence of ostrich ACTH is shown in Figure 5.

Table 1: Amino acid composition and NH_2-terminal residues of tryptic peptides

Amino Acid	T1	T2	T3	T4	T5	Sum	Os-ACTH[a]
Lys	1.1	1.0	0.9			3.0	3
His				1.0		1.0	1
Arg	1.7		1.1	1.0		3.8	4
Asp					1.0	1.0	1
Thr					1.0	1.0	1
Ser				1.7	0.9	2.6	3
Glu				1.2	4.8	6.0	6
Pro	0.9		1.0		1.7	3.6	4
Gly			1.9		2.0	3.9	4
Val	0.9		1.0		2.2	4.1	4
Met				0.9		0.9	1
Leu					0.9	0.9	1
Tyr				1.0	0.8	1.8	2
Phe				1.0	1.8	2.8	3
Trp			1.0			1.0	1
NH_2-terminal residue	Arg	Lys	Trp	Ser	Val		

[a] Taken from (1)

DISCUSSION

The data herein described represents the first report on the primary structure of ACTH from avian species. When compared with known structures of ACTH from other species (8,9) including human (10,11,12) and dogfish Squalus acanthias (13), the sequence

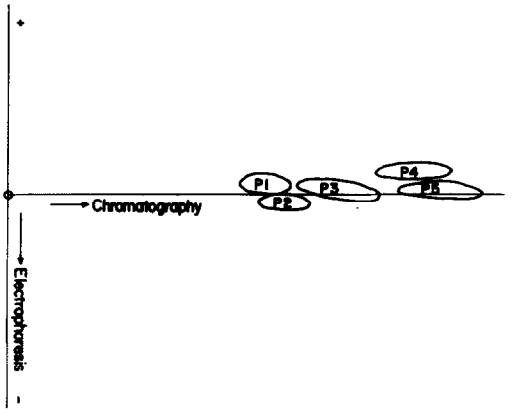


Figure 3. Peptide map of peptic digest of T5.

Table 2: Amino acid composition and NH_2-terminal residue of peptic peptides of T5

Amino Acid	P1	P2	P4	P5
Asp	1.0	1.0		
Thr	0.5		0.6	
Ser	0.6		1.2	
Glu	3.2	2.2	2.2	1.2
Pro	0.9	1.3	0.7	
Gly	1.2	1.0	1.0	0.8
Val	2.0	2.5		
Leu			0.9	
Tyr	0.6	0.8		
Phe			1.0	
NH_2-terminal residue	Val	Val	Glu	Glu

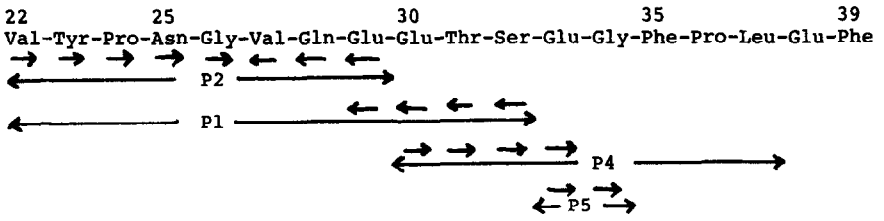


Figure 4. Amino acid sequence of T5. → dansyl Edman; ← carboxypeptidase.

5 10 15 20

Ostrich: H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Arg-Lys-Arg-Arg-Pro-Val-

25 30 35

Lys-Val-Tyr-Pro-Asn-Gly-Val-Gln-Glu-Glu-Thr-Ser-Glu-Gly-Phe-Pro-Leu-Glu-Phe-OH

Dogfish: H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met-Gly-Arg-Lys-Arg-Arg-Pro-Ile-

Lys-Val-Tyr-Pro-Asn-Ser-Phe-Glu-Asp-Glu-Ser-Val-Glu-Asn-Met-Gly-Pro-Glu-Leu-OH

Porcine: H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-

Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Leu-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe-OH

Ovine: H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-

Lys-Val-Tyr-Pro-Asp-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Gln-Ala-Phe-Pro-Leu-Glu-Phe-OH

Bovine: H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-

Lys-Val-Tyr-Pro-Asn-Gly-Ala-Gln-Asp-Glu-Ser-Ala-Gln-Ala-Phe-Pro-Leu-Glu-Phe-OH

Human: H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-

Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe-OH

Figure 5. Primary structures of ACTH from ostrich, dogfish, porcine, ovine, bovine and human pituitary glands. Residues different from that of the human molecule are underlined.

of the first twelve amino acids for all species is identical. Considerable variations occur at the COOH-terminal portion of

the molecule (see Figure 5). Indeed, corticotropic activity of ACTH resides in the NH_2-terminal region of the hormone (9). It is apparent that the conservation of the NH_2-terminal sequence during the evolution from dogfish to ostrich to man is to preserve the biological function of the ACTH molecule.

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