ADRENOCORTICOTROPIN 45. REVISED AMINO ACID SEQUENCES FOR SHEEP AND BOVINE HORMONES

by

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SUMMARY. The amino acid sequences for the residues 25-32 in the structures of ovine and bovine ACTH molecules have been re-examined. The revised sequences are shown in Figure 2.

It has been known for some time that ovine (1), bovine (2) and human (3) adrenocorticotropins* have identical amino acid compositions but differ in the amino acid sequence (4, 5, 3) between positions 25 and 33. Recently the amino acid sequence of α_h-ACTH in this region has been revised (6, 7). This communication reports our results on the re-investigation of the sequence in this region for α_s-ACTH and α_b-ACTH.

Materials and Methods

ACTH from sheep and bovine pituitary glands were isolated by procedures previously described (8). 50 mg of the hormone and 1.0 mg of crystalline trypsin were dissolved in 5 ml TRIS buffer of pH 8.5; the solution was kept at 37° for 6 hr and evaporated to dryness over HaOH. The digest was then applied in a thin band to the middle of a strip of Whatman 3 MM filter paper and submitted to zone electrophoresis (9) for 4 hr at 400 V with a γ-collidine-

*Abbreviations: α_h-ACTH, human adrenocorticotropin; α_s-ACTH, sheep adrenocorticotropin; α_b-ACTH, bovine adrenocorticotropin.

acetic acid buffer (10) of pH 6.7 at 22° C. After electrophoresis, the paper was dried and development of a guide strip with ninhydrin revealed several bands. The desired band was excised, eluted with H_2O and lyophilized.

Amino acid analyses were performed by the methods of Spackman et al (11) in an automatic amino acid analyzer (Model 120 or 120B, Beckman Instruments). The amino acid sequence of the isolated peptide was determined by the dansyl-Edman method (12, 13) as previously described (14). For the identification of aspartic acid or asparagine and glutamic acid or glutamine, the phenylthiohydration derivatives were determined by thin layer chromatography using the solvent systems of Sjoequist (15).

Results and Discussion

Paper electrophoretic pattern of the tryptic digest of α_s-ACTH may be seen in Figure 1; similar pattern was obtained with the digest of bovine hormone. From previous data (4, 5), the desired peptide was the band (designated as T-1) migrated to the cathode. From 50 mg α_s-ACTH or α_b-ACTH, 8 mg T-1 were obtained. The molar ratios of the amino acids found in T-1 are as follows:

Sheep: $Asp_{1.9}\,Ser_{0.81}\,Glu_{4.2}\,Pro_{2.0}\,Gly_{1.2}\,Ala_{2.8}\,Val_{0.84}\,Leu_{0.91}\,Tyr_{0.87}\,Phe_{2.1}$

Bovine: $Asp_{2.1}\,Ser_{0.9}\,Glu_{4.0}\,Pro_{2.0}\,Gly_{1.1}\,Ala_{3.3}\,Val_{0.9}\,Leu_{1.1}\,Tyr_{0.9}\,Phe_{1.7}$

The NH_2-terminal amino acid sequences of T-1 from sheep and bovine hormone, as revealed by the dansyl-Edman method, are as follows:

Sheep: Val-Tyr-Pro-Asp-Gly-Ala-Glu-Asp-Glu-Ser-Ala-
22 25 30

Bovine: Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-
22 25 30

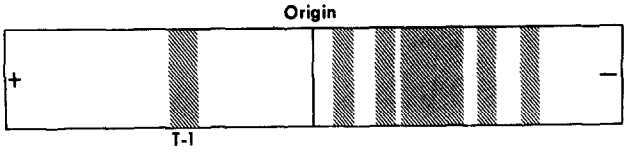
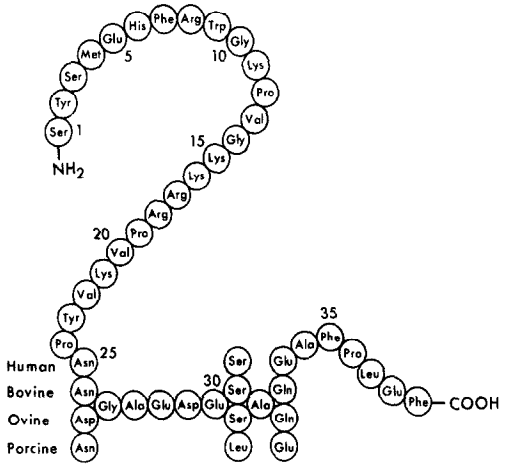


Figure 1. Paper electrophoretic pattern obtained from tryptic digest of α_s-ACTH in collidine acetate (pH 6.7) for 4 hr at 22° C.



AMINO ACID SEQUENCE OF THE ACTH MOLECULE

Figure 2. Structure of adrenocorticotropins.

Thus, these results are somewhat differed from those reported earlier (4,5);

Sheep:	Val-Tyr-Pro-Ala-Gly-Glu-Asp-Asp-Glu-Ala-Ser		
	22	25	30
Bovine:	Val-Tyr-Pro-Asp-Gly-Glu-Ala-Glu-Asp-Ser-Ala-		
	22	25	30

It is of interest to note that the revised sequences (see Figure 2) for α_s-ACTH and α_b-ACTH are almost identical with the revised sequence for the human hormone (6,7). The only differences were in amino acid positions 25 and 33: Aspartic acid instead of asparagine in position 25 for the sheep hormone

H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-
1 5 10

Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-
15 20

Porcine: Asn-Gly-Ala-Glu-Asp-Glu-Leu-Ala-Glu-

Ovine: Asp-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Gln-

Bovine: Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Gln-

Human: Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Glu-
25 26 27 28 29 30 31 32 33

Ala-Phe-Pro-Leu-Glu-Phe-OH
34 39

Structure of Adrenocorticotropins

and glutamic acid instead of glutamine in position 33 for α_h-ACTH. Figure 2 summarizes the revised structure of various adrenocorticotropins; the structure of the pig hormone was taken from references 7 and 16.

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