C-TERMINAL SEQUENCE OF AMINO ACIDS[+] IN BOVINE GROWTH HORMONE

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The sequence of amino acids in the C-terminal end of bovine growth hormone has been established using the preparation of Dellacha and Sonenberg (1964) which has a greater purity than previous ones.

Recent measurements by Andrews and Folley (1963) using a gel-filtration method, indicate a molecular weight of 20,000 ± 2,000 for the hormone. This finding has been confirmed in our laboratory by Dellacha, Enero and Faiferman (pers.comm.) who found the value 20,800 ± 400 by sedimentation velocity and diffusion measurements. Accordingly, the results obtained in this work are expressed as residues per 20,800 g of protein.

MATERIAL AND METHODS: Bovine growth hormone prepared in this laboratory by the method of Dellacha and Sonenberg (1964) was found to meet the physical, chemical and biological criteria of purity indicated

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The oxidation of the carboxypeptidase A treated-hormone was done by the following procedure: to 100 mg of protein dissolved in 30 ml of cold performic acid (Schram et al. 1954) was added 0.5 ml of methanol; the mixture was kept 4 hours at -10° (Hirs,1956) and then diluted with 250 ml of water at 0° and immediately freeze-dried. The powder was suspended in 30 ml of water and freeze-dried a second time.

The carboxypeptidases were obtained from Worthington Biochemical Corp.: the A type enzyme was COA-DFP, lot No.6129 and the B type was COB, lot No.6069. Prior to its use the B type enzyme was treated with diisopropylfluorophosphate. The incubations were done at pH 8.6, according to Fraenkel-Conrat et al.(1958), using 10 per cent trichloroacetic acid to stop the reaction.

The method of Bradbury (1956) was used to perform hydrazinolysis.

In all cases free amino acids were determined in the Technicon AutoAnalyzer by the procedure of González Cadavid and Paladini (1964).

RESULTS AND DISCUSSION: The incubation for 20 hours of the native hormone with carboxypeptidase A (ratio enzyme/hormone: 1/50, by weight), liberated 0.8 mole of phenylalanine and 0.09 mole of alanine per 20,800 g of protein. The identical result was obtained by incubation of the hormone with a mixture of carboxypeptidase A and B, each enzyme being present in the proportion indicated before.

By hydrazinolysis 0.6 mole of phenylalanine were detected. Since this method is only approximate, the amount of C-terminal amino acid obtained is considered a good confirmation of the value reached by digestion with carboxypeptidase.

Carboxypeptidase A rapidly liberates phenylalanine and alanine from the native hormone in the presence of 0.1 per cent of sodium dodecyl sulphate: in 15 minutes 0.8 mole of each amino acid were released per 20,800 g of hormone with a ratio enzyme/hormone of 1/100. This shows the effectiveness of the detergent in exposing the C-terminus of the molecule to the action of the enzyme. No other amino acids were detected in significant amount.

As a further check of the presence of alanine as the second amino acid in the chain an experiment was done in which the terminal phenylalanine was eliminated by incubation of the hormone with carboxypeptidase A in the absence of sodium dodecyl sulphate. The modified protein was dialyzed, freeze-dried and submitted to hydrazinolysis. This treatment released 0.6 mole of alanine per 20,800 g of hormone.

The third amino acid in the C-terminal sequence was detected by the following procedure: phenylalanine and alanine were removed from the hormone by incubation with carboxypeptidase A in the presence of sodium dodecyl sulphate. The modified protein was dialyzed, freeze-dried and oxidized with performic acid as described. The hydrazinolysis of this product revealed 0.65 mole of cysteic acid per 20,800 g of hormone.

All the results obtained in this work indicate the presence of approximately 1 residue of the corresponding amino acid per mole of hormone. This would indicate that there is only one polypeptide chain in the C-end of the molecule and that its sequence is:

R-Cys.Ala.Phe.OH

This sequence is quite different from the one suggested by Harris et al.(1954) and Li (1956): R-(Val,Thr,Ser)Leu.Ala.Phe.Phe.OH on the basis of 1.8 mole of phenylalanine and

amounts lower than 0.5 mole, per 45,000 g of hormone, for the other amino acids liberated by carboxypeptidase A. Although both works agree on the nature of the terminal amino acid, they differ on the others; when the values found by Harris et al.(1954) for the subterminal amino acids are recalculated for a molecular weight of 20,800 they decrease to very low levels. This fact suggests that they may have arisen from contaminating proteins.

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