THE AMINO ACID SEQUENCE OF BOVINE GROWTH HORMONE

J.A. SANTOMÉ, J.M. DELLACHA, A.C. PALADINI, C.E.M. WOLFENSTEIN
C. PEÑA, E. POSKUS, S.T. DAURAT, M.J. BISCOGLIO
Z.M.M. DE SESÉ and A.V.F. DE SANGÜESA
Facultad de Farmacia y Bioquímica, Departamento de Química Biológica y Centro para el Estudio de las Hormonas Hipofisarias, Junín 956, Buenos Aires, Argentina

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1. Introduction

Bovine growth hormone is one of the most studied growth hormones but its complete primary structure has not been established until now.

For several years it was accepted that the molecular weight of bovine, as well as porcine and ovine growth hormones was approximately 46,000 [1], this value being clearly different from that of the hormones of human and monkey origin that were only half as big [2–4].

Recent measurements have established that the true molecular weights of the hormones obtained from all the abovementioned species are similar and close to 21,000 [5–8]. This value accounts for the number of disulphide bridges and tryptic peptides in bovine growth hormone [8].

This paper reports the amino acid sequence of the bovine growth hormone molecule.

2. Experimental

Bovine growth hormone of high purity was prepared by the method of Dellacha and Sonenberg [9]. Amino acid analyses and oxidation of the protein were done as described previously [10]. The oxidized product was submitted to digestion with trypsin, chymotrypsin or pepsin. The tryptic digestion was carried out as indicated by Santomé. Wolfenstein and Paladini [11]; the chymotryptic digestion was done at pH 8, in a pH-stat during 6 hr at 30°, with a weight ratio of enzyme to hormone of 1:66. The digestion with pepsin was performed in 5% formic acid at 37° during 16 hr, with a weight ratio of enzyme to hormone of 1:10.

The soluble fraction of the various digests was fractionated on ion-exchange resin columns using procedures similar to those described by Margoliash and Smith [12]. Some of these fractions required further purification by high-voltage electrophoresis [13] on paper, using volatile buffers of pH 6.45 and 2.0 and by two-dimensional paper chromatography in the following solvent systems: 1-butanol–formic acid–water (75:15:10, v/v) and 1-butanol–2.8% ammonium hydroxide–ethanol (50:20:15, v/v).

When the size of a polypeptide fragment was considered too big for direct sequencing it was submitted to partial hydrolysis in 12 M hydrochloric acid at 40° during 21 hr. The resulting mixture was resolved by paper chromatography as indicated before.

To avoid the insoluble residue arising in the tryptic digestion of the oxidized hormone this fragmentation was alternatively carried out on the reduced, carbamidomethylated [14] and maleinized protein [15].

The amino acid sequence in the purified peptide fragments was determined from the N-terminal

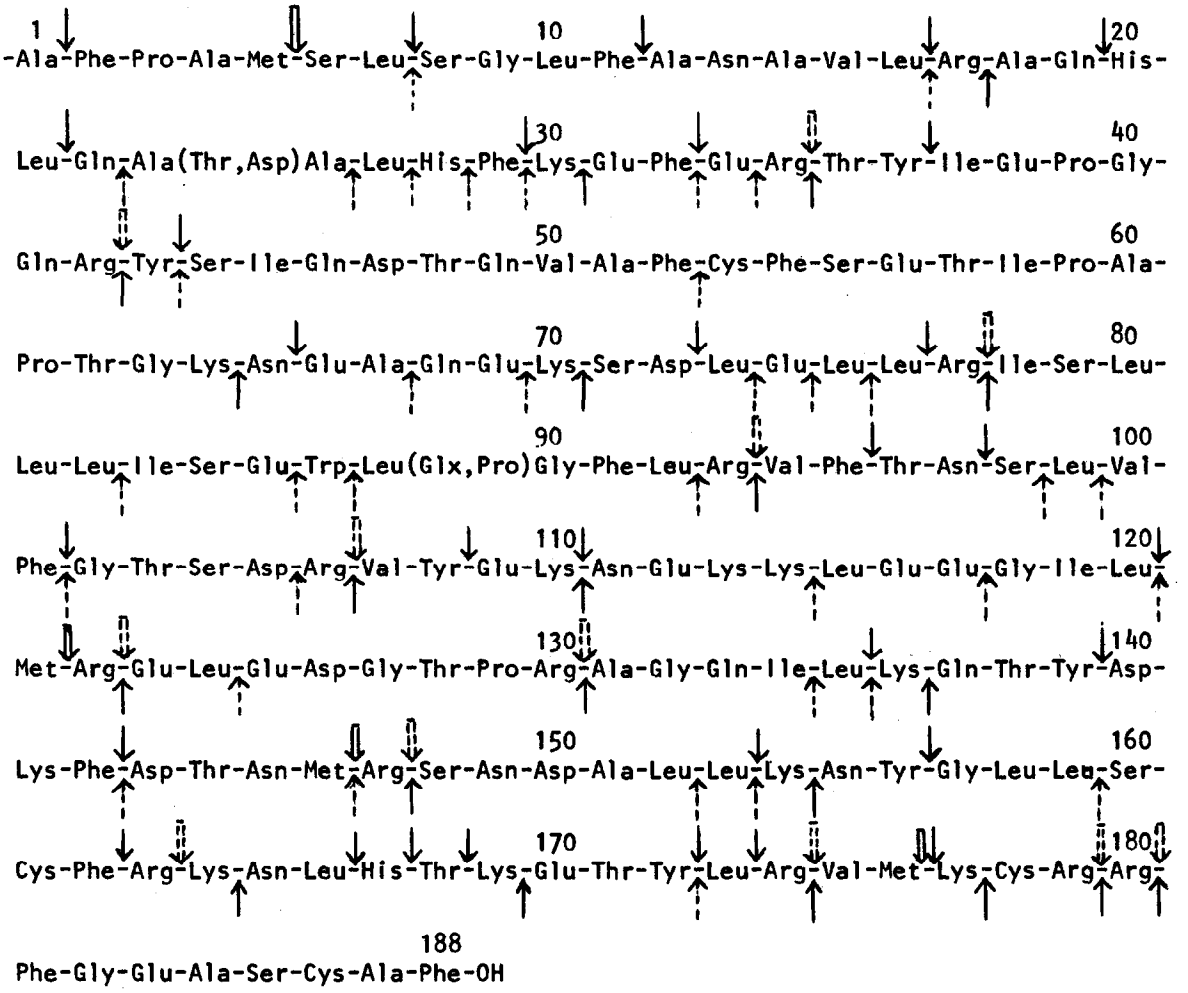

Fig. 1. Amino acid sequence of bovine growth hormone. Numbers indicate the position of the amino acid residues from the amino terminus; ↑ indicates points of tryptic attack; ↓ indicates points of chymotryptic attack; ↑ indicates points of peptic attack; ⇧ indicates points of tryptic attack on the maleinized protein; ⇩ indicates points of attack by cyanogen bromide.

end by the stepwise phenylisothiocyanate method of Edman [16]. The procedures applied were similar to those described by Konigsberg and Hill [17] or by Elzinga [18]. In certain cases the sequence was established by applying the dansyl method of Gray and Hartley [19] to the residual peptide. The C-terminal sequence of the same fragments was studied by digestions with carboxypeptidases A and B [14].

3. Results and discussion

The amino acid sequence indicated in fig. 1 was confirmed by the amino acid composition of the fragments obtained treating the hormone with cyanogen bromide, as previously described [14, 20]. The location of one disulphide bridge was thus established to occur between residues 53 and 161. The disulphide bridge near the C-terminal end of the molecule had already been identified [14]. The single tryptophan residue is in position 86. The three histidine residues are in positions 20, 28 and 167.

Thirty percent of the molecules have valine replacing leucine 124 [21]. This finding substantiates the microheterogeneity suggested by the amino acid composition of a tryptic peptide from bovine growth hormone reported by Fellows and Rogol [22].

The sequence found in the first 21 amino acids from the N-terminal end of the molecule coincides with the results obtained by Fellows, Rogol and Mudge [23].

Wallis [24] has obtained evidence of the existence of two forms of bovine growth hormone, presumably differing only by the presence or absence of the alanine in position 1. We have confirmed these findings in the present investigation.

Bovine growth hormone has about 61% of its amino acid residues identical and in the same sequence as those in human growth hormone, when the comparison is made with the structure reported by Li, Dixon and Liu [25] as revised by Niall [26].

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