LOCALIZATION OF A MICROHETEROGENEITY IN THE AMINO ACID SEQUENCE OF BOVINE GROWTH HORMONE

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Thirty per cent of the molecules in bovine growth hormone (BGH) preparations obtained from pooled glands have valine replacing leucine 124 [1]. This heterogeneity was first detected by Fellows and Rogol [2] by amino acid analysis of a tryptic peptide obtained from a cyanogen bromide cleaved fragment. Subsequently, Seavey et al. [3] obtained positive evidence for its allelic origin in the bovine population. The announcement by these authors [3] of a more extensive study in several breeds of cattle, prompted us to report the experimental data supporting the exact localization of this heterogeneity within the polypeptide chain of BGH, whose amino acid sequence has been recently communicated [1].

The cyanogen bromide fragment of BGH containing the microheterogeneity was obtained and purified as indicated recently for the homologous fragment from ovine growth hormone [4]. Its tryptic hydrolysis gave a mixture of peptides that could be resolved by column chromatography on a sulphonic resin [5]. One peptide peak, T_3, contained the microheterogeneity as shown by the individual amino acid analysis of its component fractions (table 1). The values obtained suggest that the peak is formed by a mixture, in varying proportions, of two identical peptides, except for the replacement of leucine for valine in 30% of the molecules. Fraction 4 (table 1), contains in pure form the leucyl variant.

Partial acid hydrolysis [5] of T_3 (all fractions pooled) gave free aspartic acid and two peptides, H_1 and H_2, well resolved by column chromatography on a sulphonic resin [5]. The amino acid composition and Edman degradation of T_3H_1 is shown in table 2.

The simultaneous disappearance of the leucyl and valyl residues in the third step of the degradation confirms that both residues are present in the same position in the otherwise identical tetrapeptides composing the mixture T_3H_1. The sequence of this peptide identifies unequivocally its location within the cyanogen bromide fragment since the sequence of this pentacosapeptide has been established in the study of the primary structure of BGH already published [1]. The microheterogeneity occurs then, in position 124. No evidence for this type of heterogeneity was detected in ovine growth hormone [3] where the homologous position of its chain is occupied only by leucine [5].

A replacement of leucyl residues for valyl residues, due to allelomorphism, has also been described in bovine carboxypeptidase A [6].

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Table 1
Amino acid composition of the chromatographic peak corresponding to peptide T_3.
See text for details of the fractionation.

Fraction no.	Amino acids (mole/mole peptide)							
	Leu	Val	Asx	Thr	Glx	Pro	Gly	Arg
1	0.74	0.37	1.13	1.02	1.55	0.82	1.39	n.d.
2	0.43	0.57	1.01	0.98	1.96	n.d.	1.01	n.d.
3	0.75	0.22	1.01	1.21	1.90	1.04	1.20	n.d.
4	1.02	0	1.07	1.37	2.00	0.91	1.25	n.d.
Pool	0.70	0.29	1.02	1.16	1.90	0.99	1.18	n.d.

n.d.: present but not determined.

Table 2
Edman degradation of peptide T_3H_1.

Step	Amino acids (subtractive method)				
	Arg	Glx	Leu	Val	Glx
0	1.07	0.96	0.73	0.28	0.96
1	0	0.97	0.74	0.32	0.97
2	0	0	0.72	0.25	1.00
3	0	0	0	0	1.00

Values are expressed in moles per mole of peptide. The amino acids are represented in their correct sequence; half of the total value found in the analyses was assigned to each glutamic acid residue.

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