

BOVINE GROWTH HORMONE:
EVIDENCE FOR TWO ALLELIC FORMS

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SUMMARY. Peptide maps of tryptic digests of bovine growth hormone isolated from individual pituitary glands were of three types. The hormone contained either an A or a B peptide or a 1:1 mixture of the two. A and B differed by a substitution of valine for leucine. The only difference noted between the ovine and bovine growth hormones was that the ovine hormone had neither A nor B but instead a peptide that differed from A by a replacement of a glycine with valine.

During a study of the peptide map of bovine growth hormone, we noted that the amino acid compositions of two peptides were identical except for one amino acid. Since this suggested there might be two forms of the hormone and that the forms could be characteristic of certain breeds of cattle, we analyzed growth hormone isolated from individual pituitary glands. The results are reported here.

MATERIALS AND METHODS

Growth hormone was isolated from individual glands by homogenization in water to remove blood proteins, extraction at pH 10, chromatography first on Sephadex G-150 and then on DEAE-cellulose (1). In order to separate the two chains the LKB Ampholine column 8100 was used (2). The sample (50 mg) was electrofocused for 72 hours in a pH gradient (pH 7 to 9) containing 4M urea. Separation of the

chains was confirmed by determination of the NH_2 -terminal residue of the two peaks that were separated. Similar results have been reported by Ellis *et al.* (3). A 2-4 mg sample of hormone was dissolved in 0.2 ml of 1% NH_4HCO_3 containing 0.1% sodium dodecyl sulfate, and hydrolyzed for 2 hours at 37° with trypsin treated with L-1-tosylamide-2-phenylethyl-chloromethyl ketone. Peptide mapping was carried out according to Katz *et al.* (4). When the peptides were to be eluted for analysis, the paper was dipped into a dilute solution of ninhydrin (0.05% in ethanol) and as the spots appeared, they were cut from the paper, washed with acetone and the peptide eluted with 6N HCl. After hydrolysis for 20 hours at 110° the mixture was analyzed (5). The ratio of A and B peptides was determined by data from amino acid analysis.

RESULTS

Bovine. By mapping tryptic digests of bovine growth hormone we found that two forms of the hormone are produced by cattle. An individual pituitary gland contained either one of the two modifications by itself or a 1:1 mixture of the two. Evidence for the two forms is shown in Fig. 1. The maps were of three types: they contained peptide A alone, B alone, or both. The amino acid compositions of A and B were identical except that peptide B contained valine instead of leucine (Table I). The spots marked A' and B' had the same amino acid composition, respectively, as A and B and were seen only when the corresponding A or B peptide was present. Because growth hormone has been shown (6) to lose ammonia, and also because A and B were more acidic electrophoretically, we believe that A' and B' are deamidated forms of A and B.

The data on the growth hormone of individual glands were obtained

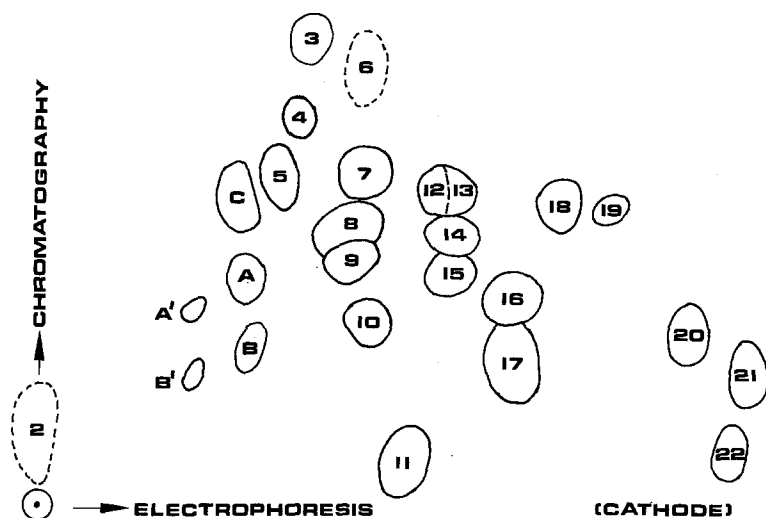


Fig. 1. Peptide map of tryptic digests of bovine and ovine growth hormones made by chromatography in butanol-acetic acid-water (4:1:5) and electrophoresis at pH 3.7. The amino acid compositions of the peptides are given in Table I. The hormones gave identical maps except for peptides A, B, and C. A and B were found in bovine growth hormone; C only in the ovine hormone. Growth hormone from individual bovine pituitaries contained either A or B alone or a 1:1 mixture of the two. A' and B' are thought to be deamidated forms of A and B. The peptides circled with dashes were ninhydrin negative and were located by detection sprays (12).

from two different groups of pituitaries. The first was a group of 12 glands randomly selected from a pool of about 50 pituitaries purchased from a commercial supplier (Ersco, San Mateo, California). No assurance of the breed of the cattle could be given. Eight of the 12 glands had a 1:1 mixture of the A and B forms of growth hormone. The other four had only the A form. The second group of individual pituitaries was made up of 10 glands from three known breeds, Hereford, Jersey, and Holstein. Only females were represented. Peptide maps indicated two Herefords were homozygous for the A form and one the B form; all three Jerseys had the B form; three Holsteins had a mixture of A and B and one the A form.

Bovine growth hormone isolated from pooled rather than individual

Table I. Peptides from Maps of Bovine and Ovine

Growth Hormones		
Peptide Number ⁺	Amino Acid Composition	
A	asp thr glu ₂ pro gly leu arg	Bovine
B	asp thr glu ₂ pro gly val arg	
C	asp thr glu ₂ pro val leu arg	Ovine
1*	See footnote	
2	asp ₂ thr ₃ ser ₃ glu ₃ pro gly ₂ ala ₂ cys ₂ val ile ₂ leu ₃ tyr(2?) phe ₂ lys arg	
3	asp ser glu leu ₃ arg	
4	asp ₂ thr ₂ ser ₂ gly val ₂ leu phe arg	
5	asp ₂ ser ala leu ₂ lys	
6	asp glu ₂ gly met ile leu ₄ lys arg	
7	thr ₂ glu leu tyr arg	
8#	glu ₂ phe arg + peptide No. 15 lacking 1 arginine	
9	thr glu ₃ pro gly ile tyr arg	
10	asp ₃ thr ₂ glu met tyr phe lys arg	
11	asp glu ₄ ala lys	
12	asp ala ₂ val leu arg	Poorly resolved**
13	glu gly ala ile leu lys	
14	glu val tyr lys	
15	ser glu gly ala ₂ cys ₂ phe arg ₂	
16	asp thr glu ₂ ala ₂ leu ₂ phe lys his	
17	asp leu lys his	
18	val met lys	
19	leu lys	
20	arg	
21	lys	
22	asp leu lys ₂ his	
<hr/>		
+ Corresponds to number given in Fig. 1.		
* Peptide 1 gave inconclusive analyses; this is being studied further.		
# After reduction and carboxymethylation of the hormone, glu ₂ phe arg remained but the cystine-peptide was found elsewhere.		
** At times peptide 13 is seen without arginine-positive peptide 12.		

glands always had the two peptides A and B in a ratio of about 2:1 (A:B).

Two such pools of 24 glands each were analyzed. Likewise, growth

hormone obtained from the Endocrinology Study Section, representing a

large pool of pituitaries, contained the two peptides in a 2:1 ratio. The

relative amounts of the peptides found in pooled material would explain

the 2:1 ratio for leucine and valine found by Fellows and Rogol (7) in their

peptide Tp_{1,2} from fragment C. As an explanation of this observation, they suggested that the tryptic peptide could be a mixture of two peptides which were identical except for a replacement of valine for leucine. Our data are in complete agreement with this explanation.

It was possible that the A and B peptides indicated a difference in the two chains (8) of the hormone but this was not found to be the case. After separation of the two chains by isoelectric focusing both were found to contain peptides A and B in about a 2:1 ratio, the same as noted in the hormone before electrofocusing. That the two chains of growth hormone did not account for the leucine-valine allelism was indicated also by our finding that growth hormone having only peptide A still had alanine and phenylalanine as NH₂-terminal residues. The work of Peña et al. (8), where no evidence for an allelic origin of the two chains was found, also supports this view.

Ovine. The peptide maps of bovine and ovine growth hormones differed only with respect to peptides A and B. Instead of either of these two peptides, the ovine hormone had a peptide (C in Fig. 1) whose composition was the same as peptide A of the bovine protein except that glycine was replaced with valine (Table I). Curiously, no deamidated form of peptide C was seen. Dellacha et al. (9) reported that maps of bovine growth hormone had five spots, the composition of which were not determined, that were not in the ovine hormone. Also, the ovine hormone contained one spot that was absent from bovine growth hormone. Our data are in agreement with these results except for the one very alkaline peptide noted by Dellacha and co-workers which we did not observe in our bovine map.

DISCUSSION

Besides the bovine and ovine growth hormones, we have determined

the amino acid composition of peptides seen in maps of the prolactins of these species, human growth hormone and rat growth hormone. No evidence for the type of allelism seen with bovine growth hormone was noted. Such an allelism has been reported, however, by Terry *et al.* (10) for human immunoglobulin kappa chains. Seven individuals had valine at position 191 whereas three had both leucine and valine. Recently Mross *et al.* (11) found a glycine-serine allelism in a fibrinopeptide from individual gibbons.

The number of individual glands and breeds that we examined was too small to be able to make definite statements concerning the distribution of the two forms of growth hormone throughout the bovine population. If the two forms were expressed in simple Mendelian fashion, we should find equal quantities of the A and B peptides in growth hormone from pooled glands, instead of the 2:1 ratio that was noted. As more individuals become available to us, we shall be able to tell if some breeds have exclusively one form.

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