BOVINE AND OVINE PROLACTINS: A DIFFERENCE OF TWO AMINO ACIDS INDICATED BY PEPTIDE MAPS

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SUMMARY. Peptide maps of tryptic digests of ovine and bovine prolactins indicated only two places where the amino acid compositions differed. Bovine prolactin had no histidine at position 164 and contained an alanine instead of valine at position 107. All the residues in the amino acid sequence of ovine prolactin were accounted for on the map.

We have investigated the use of peptide mapping (1) for a comparative study of growth hormone and prolactin of various species. To test the usefulness and reliability of the method we chose first to study hormones whose primary structures were known. Ovine prolactin was one of these (2). In this report we give the amino acid composition of all the peptides found in the map and compare them with those obtained with the bovine hormone.

MATERIALS AND METHODS

Bovine and ovine prolactin obtained from the Endocrinology Study Section, NIH, were purified by preparative gel electrophoresis (3) to get homogeneous material. A 3 to 5 mg. sample of hormone was dissolved in 0.2 ml. of 1% NH_4HCO_3 containing 0.1% sodium dodecyl sulfate, and hydrolyzed with porcine trypsin (Novo Industria A/S, Batch S013) for 2 hours at 37°. Peptide mapping was carried out according to Katz et al (1); detection sprays were those of Easley (4). When the peptides were to be eluted for amino acid analysis, the paper was dipped into a dilute solution of ninhydrin (0.5 g/l of 95% ethanol) and as the spots appeared, they were cut from the paper, washed with acetone and the peptide eluted with 6N HCl. Hydrolysis was done at 110° for 20 hours and the mixture analyzed by the method of Spackman et al (5).

RESULTS

Ovine prolactin. Fig. 1 shows the location of the tryptic peptides of ovine prolactin in a map. The numbering system for the residues is that of Li et al (2). Samuelsson and Li (6) reported a peptide map for ovine prolactin but no amino acid data were given. The porcine trypsin (7) caused three nonspecific cleavages at positions 28 (tyrosine), 168 (tyrosine) and 53 (methionine). This actually was helpful in identification of the spots because when trypsin treated with TPCK (L-1-tosylamide-2-phenylethyl-chloromethyl ketone) was used, a large S-S peptide was produced (residues 49-69 and 164-175) which was difficult to locate and analyze. Analyses of all peptides are given in Table I.

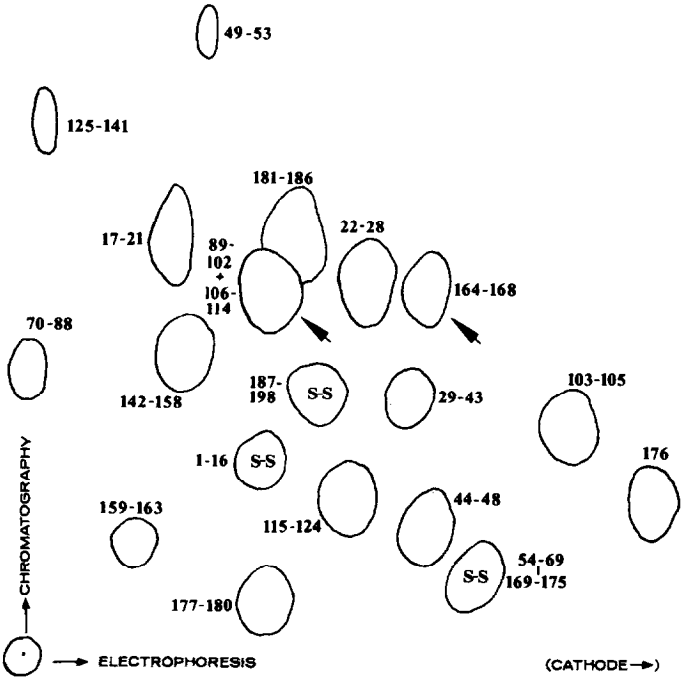

Fig. 1. Peptide map of a tryptic digest of ovine prolactin made by chromatography in butanol-acetic acid-water (4:1:5) and electrophoresis at pH 3.7. The numbers refer to the residues in the amino acid sequence given by Li et al (2). The arrows indicate the two points of difference between the ovine and bovine hormones. The spots with the S-S refer to the cystine peptides.

There was one instance where two peptides were not resolved: peptides

Table I. Amino Acid Composition of Peptides from Map of Ovine Prolactin

Sequence Number[+]	Amino Acid Composition
1- 16	$asp_2\ thr_1\ ser_1\ glu_1\ pro_3\ gly_2\ cys_2\ val_2\ leu_1\ arg_1$
17- 21	$asp_2\ leu_1\ phe_1\ arg_1$
22- 28	$ser_1\ ala_1\ val_2\ met_1\ tyr_1\ his_1$
29- 43	$asp_3\ ser_2\ glu_2\ met_1\ ile_1\ leu_1\ phe_2\ lys_1\ his_1\ arg_1$
44- 48	$glu_1\ gly_1\ ala_1\ tyr_1\ lys_1$
49- 53	$thr_1\ gly_1\ met_1\ ile_1\ phe_1$
54- 69 169-175	$asp_3\ thr_2\ ser_3\ glu_1\ pro_2\ ala_1\ cys_2\ leu_5\ lys_1\ his_2\ arg_1$
70- 88	$thr_1\ ser_1\ glu_5\ gly_1\ ala_1\ val_1\ met_1\ ile_1\ leu_4\ his_2\ arg_1$
89-102*	$asp_2\ thr_1\ ser_1\ glu_1\ pro_1\ val_2\ leu_2\ tyr_1\ trp_1\ his_1\ arg_1$
106-114	$asp_1\ ser_1\ pro_1\ gly_1\ ala_1\ val_1\ ile_1\ leu_1\ arg_1$
103-105	$gly_1\ met_1\ lys_1$
115-124	$asp_1\ glu_4\ ala_1\ ile_2\ lys_1\ arg_1$
125-141	$glu_3\ pro_1\ gly_3\ ala_1\ val_1\ met_2\ ile_2\ leu_2\ phe_1\ lys_1$
142-158	$thr_2\ ser_2\ glu_3\ pro_3\ gly_1\ val_1\ leu_2\ tyr_1\ trp_1\ lys_1$
159-163	$asp_2\ glu_1\ ala_1\ arg$
164-168	$ser_1\ ala_1\ tyr_1\ phe_1\ his_1$
176	arg
177-180	$asp_1\ ser_2\ lys_1$
181-186	$asp_1\ thr_1\ ile_1\ leu_1\ tyr_1\ lys_1$
187-198	$asp_4\ cys_2\ ile_2\ leu_2\ tyr_1\ arg_1$

The tryptophans (90 and 149) were located by detection sprays.
[+] According to Li et al (2).
* Unresolved.

89-102 and 106-114. This was detected when the amino acid composition of the spot was found to correspond to the sum of the two individual peptides. That this spot was a mixture was indicated also by results obtained with the bovine

hormone. It was in one of these peptides (106-114) where there was a difference between the two hormones. Replacement of the valine with alanine in the bovine peptide altered its partition coefficient enough to make it lag behind peptide 89-102. Amino acid analysis of peptide 89-102, free of the coincident peptide, was then made.

There are two lysine-arginine linkages in ovine prolactin, at positions 42-43 and 123-124. In both cases the bond remained intact, that is, the peptide chain of the hormone was cleaved at the carboxyl group of the arginine, not at the lysine. The one arginine-arginine linkage at 175-176 was broken to give a free arginine (residue 176). The other arginine in this linkage remained with the S-S peptide 54-69 and 169-175.

Bovine prolactin. Fig. 2 shows a peptide map of bovine prolactin and the two places where it differed from the ovine map. The first difference was that the bovine map had a spot below the mixed peptide 89-102 and 106-114 found in the ovine map. The new spot had the amino acid composition of 106-114 but was lacking valine and instead had an additional alanine. Therefore, we believe bovine prolactin differs from ovine prolactin at position 107. The replacement of valine with alanine changed the behavior of the peptide during chromatography but not electrophoresis. In the ovine map peptides 106-114 and 89-102 were not resolved but because the bovine peptide 106-114 migrated differently, it was possible to verify the amino composition of 89-102.

The second difference between the two maps was the absence of peptide 164-168 from the bovine map. A new spot was seen in the upper part of the map, however. Amino acid analysis of the new spot showed it to be the amino acids of peptide 164-168 but lacking a histidine. Having lost a basic amino acid, the peptide migrated a shorter distance during electrophoresis but farther in the organic phase of the chromatography solvent. Since free

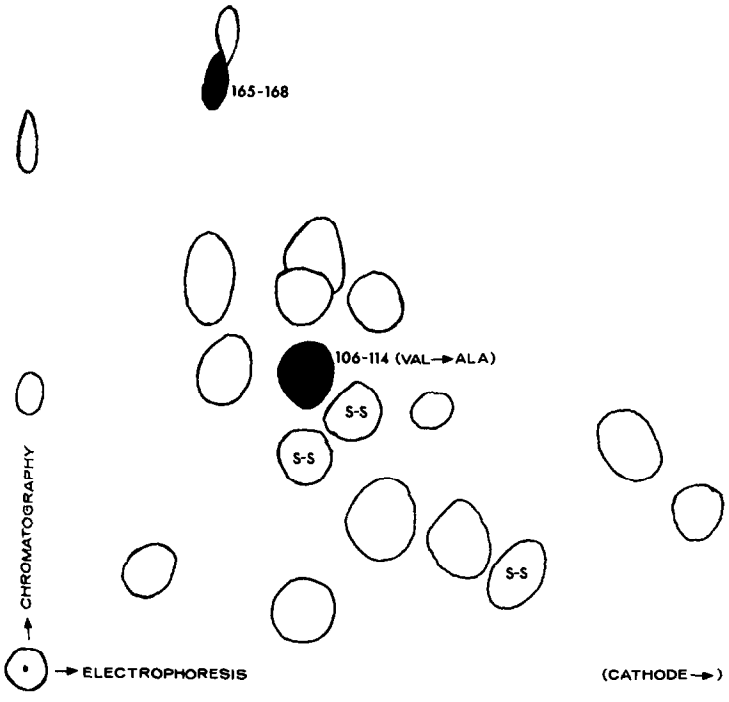

Fig. 2. Peptide map of a tryptic digest of bovine prolactin obtained in the same manner as the map for ovine prolactin. The amino acid compositions of the bovine peptides were identical with those of the ovine hormone except for the two spots that are shaded.

histidine was not detected on the map, we think that histidine has been deleted from the bovine hormone.

Cleavage of one peptide in the bovine hormone by trypsin was at times different from that observed for ovine prolactin. The spot for peptide 29-43 was not always seen in the bovine map and at the same time peptide 187-198 stained more heavily with ninhydrin. Amino acid analysis indicated that the two peptides were unresolved. An explanation for this would be that the arginine at position 43, which is linked to lysine at 42, was sometimes cleaved from the 29-43 peptide. This would make the peptide more acidic and cause it to migrate a shorter distance during electrophoresis. When TPCK-treated trypsin was used, this occurred less frequently.

DISCUSSION

The absence of one histidine from the bovine hormone would be in accord with the disc electrophoretic behavior of the hormones. At pH 9.5 bovine prolactin has a higher mobility than the ovine protein (8). The deletion of a histidine would make bovine prolactin more acidic and increase its rate of electrophoretic migration.

The structural similarity of ovine and bovine prolactin is supported by the immunological studies of Baranyai et al (9). Anti-serum to ovine prolactin produced a precipitin line when tested against an homogenate of bovine pituitaries but no reaction was seen with homogenates of glands from the dog, pig, rabbit, rat, guinea-pig or pigeon. The relatedness of the bovine and ovine prolactins is also true for the growth hormones of these species. Peña et al (10) have shown by amino sequence studies that the growth hormones are very similar, and by peptide mapping (11) we found that the hormones differ with respect to only three peptides.

ACKNOWLEDGEMENTS

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