THE PRIMARY STRUCTURE OF BOVINE PROLACTIN

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1. Introduction

Prolactin is a protein hormone from the anterior pituitary gland. It has a molecular weight of about 22 000. The amino acid sequence of ovine (sheep) prolactin has been determined [1,2] and structural studies on the bovine (ox) [3,4] and human [5,6] hormones have also been reported. Prolactin is structurally homologous with pituitary growth hormone, and comparative studies of amino acid sequences within this protein family provide an interesting example of protein hormone evolution.

In this paper the author summarizes studies on the amino acid sequence of bovine prolactin. The sequence is complete except for assignment of a few amide groups.

2. Experimental

2.1. *Materials*

Bovine prolactin was prepared from pituitary glands by the method of Jiang and Wilhelmi [7] with a modification in the final chromatographic step. Some of the bovine prolactin used was generously provided by the Pituitary Hormone Distribution Program, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Md., USA (batch number NIH-P-B3).

Trypsin (PTCK treated), α-chymotrypsin (3 X crystallized), pepsin and carboxypeptidase A (diisopropylphosphorofluoridate treated) were from Worthington Biochemical Corporation.

[2-^{14}C]Iodoacetic acid was obtained from the Radiochemical Centre (Amersham, Bucks).

2.2. *Preparation and characterization of peptides after cyanogen bromide cleavage*

100 mg of prolactin was dissolved in 10 ml of 70% formic acid, containing 100 mg of cyanogen bromide. The mixture stood at room temperature for 24 hr. The resulting peptides were recovered by concentration on a rotary evaporator, diluting with water, and freeze-drying.

The cyanogen bromide peptides were fractionated by gel filtration on Sephadex G-75, G-50 and G-25 in 50% acetic acid or 0.1 N acetic acid [2]. Some of these peptides were also purified by high voltage electrophoresis. The purified peptides were characterized by amino acid analysis and end group analysis using the 'dansyl' method [8]. Sequences were investigated as described in section 2.4.

2.3. *Preparation and fractionation of tryptic and chymotryptic peptides*

Before enzymic digestion, the disulphide bridges of prolactin were broken by performic acid oxidation or by reduction and aminoethylation. In some cases methionine residues were modified by carboxymethylation with [^{14}C]iodoacetic acid [9,10]. Tryptic and chymotryptic digests of prolactin were prepared using an enzyme: protein ratio of 1:50, in an *N*-ethylmorpholineacetate buffer, pH 8.5 (0.2 M in *N*-ethylmorpholine). Digests were fractionated by high voltage paper electrophoresis and paper chromatography. In some cases a preliminary fractionation using gel filtration on Sephadex G-25 was used. In many cases, peptides were purified directly from peptide maps, as outlined previously [11].

Tryptic and chymotryptic digests of the various

cyanogen bromide peptides of prolactin were also prepared and fractionated by these methods.

2.4. *Characterization of peptides*

Amino acid compositions were determined, after hydrolysis in 6 N HCl (110°C, 24 hr, sealed evacuated tube), on a Locarte amino acid analyser. In many cases, a trace of phenol was added to the hydrolysis mixture, to improve the yield of tyrosine.

N-Terminal amino acid residues were determined by the 'dansyl'-method [8], and N-terminal sequences by the 'dansyl'–Edman technique [12]. C-Terminal residues and sequences were investigated in some cases by digestion with carboxypeptidase A.

For characterization of large peptides (especially those from cyanogen bromide degradation) further enzymic digestion was used in many cases, using trypsin or chymotrypsin. Such secondary digests were fractionated by the methods already outlined.

Location of amide groups was carried out mainly by investigating the mobility of peptides on electrophoresis at pH 6.5.

Identification of methionine-containing peptides was helped in some cases by using prolactin which had been previously labelled with [^{14}C]iodoacetic acid on methionine residues [9,10]. Labelled peptides were detected by autoradiography or scintillation counting [10].

3. Results and discussion

The sequence determined for bovine prolactin is shown in fig. 1. The protein contains a single chain of 199 amino acid residues, with N-terminal threonine and C-terminal '½-cystine'. Some amide assignments have not yet been completed, as indicated in fig. 1.

Much of the sequence work was based on the 8 cyanogen bromide peptides derived from the protein. The order of these, and various other aspects of the sequence, were determined from tryptic and chymotryptic peptides. Identification of the methionine-containing peptides, from which the order of the cyanogen bromide peptides was determined, was facilitated by carboxymethylating methionine residues with [^{14}C]iodoacetic acid [9,10].

The assignment of the 3 disulphide bridges (as indicated in fig. 1) was clear from the fact that two, close to the amino- and carboxyltermini respectively, were retained intact in single cyanogen bromide peptides.

The results presented here confirm earlier indications [3,4,13,14] that bovine and ovine prolactions have very similar sequences. Two clearcut differences are apparent when the sequence shown in fig. 1 is compared with that of ovine prolactin [2]. At residue 108 alanine (bovine prolactin) replaces valine (ovine prolactin) and at residue 165 tyrosine (bovine) replaces histidine (ovine). Seavey and Lewis [4], who compared peptide maps from ovine and bovine prolactins, detected differences at each of these positions, but considered that the histidine at position 165 in sheep prolactin is deleted completely in the bovine hormone. The evidence for the presence of a tyrosine at this position in bovine prolactin is good; failure to detect it in the earlier work could be due to preparation of the appropriate tryptic peptides directly from peptide maps – a method which can lead to partial loss of an amino acid in the N-terminal position of a peptide.

An additional difference between the sequence of bovine prolactin (fig. 1) and that of ovine prolactin [2] is an extra residue of leucine at position 88 (so that bovine prolactin possesses 199 residues rather than the 198 reported for the ovine hormone). Whether this represents a true difference between the two species or an error in one of the sequence determinations is not yet clear. The evidence for the presence of this leucine residue in the bovine prolactin sequence is good, being based on: (1) the amino acid composition of the cyanogen bromide peptide (residues 82–105) which includes this region of the sequence, (2) the composition of a tryptic peptide (82–89) derived from this cyanogen bromide peptide, (3) sequential degradation of the cyanogen bromide peptide by the 'dansyl'–Edman method and with the protein sequenator and (4) the amino acid composition and 'dansyl'–Edman degradation of a peptide prepared from a tryptic digest of bovine prolactin, corresponding to residues 86–89 (and presumably resulting from a chymotryptic-like split on the carboxyl side of residue 85).

A further difference between bovine and ovine prolactins probably exists in one of the amide residues which have not yet been assigned for the bovine hormone. This is suggested by the fact that bovine prolactin migrates considerably more rapidly towards the anode in gel-electrophoresis systems than does

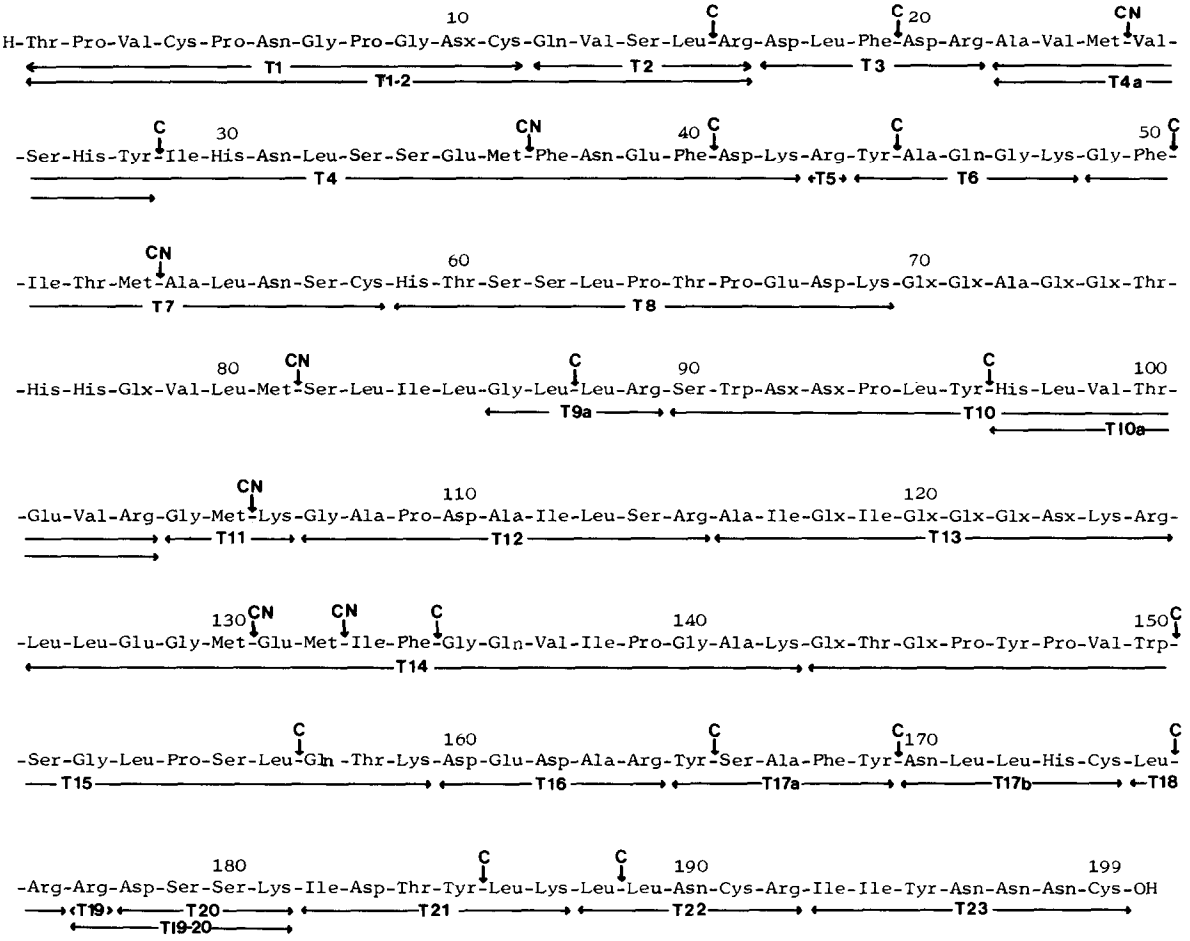

Fig. 1. The primary structure of bovine prolactin. T1, T2 etc. indicate peptides derived from tryptic digests of the aminoethylated protein. A few of the 'tryptic' peptides have clearly resulted from chymotryptic-like splits occurring during digestion with trypsin. C ↓, CN ↓ indicate points of cleavage by chymotrypsin and cyanogen bromide respectively. Disulphide bridges connect residues Cys 4 to Cys 11, Cys 58 to Cys 174 and Cys 191 to Cys 199.

the ovine hormone [15]. This might be due to the histidine/tyrosine substitution at residue 165, but this seems unlikely because the electrophoretic difference is clearly seen at pH 8 and above (when neither histidine nor tyrosine side chains would normally carry a charge). It seems more probable that a glutamine/glutamic acid or asparagine/aspartic acid substitution remains to be discovered. The remaining amide assignments in bovine prolactin are currently being investigated.

Gráf et al. [16] reported an N-terminal sequence for bovine prolactin, which agrees well with the present sequence. They found asparagine at residue 10. From the present work, the evidence about the amide assignment at this position is ambiguous, possibly because of ready deamidation of an asparagine residue. Li et al. [2] reported aspartic acid at residue 10 in ovine prolactin, but Niall [6] found asparagine.

The primary structure of human prolactin has also been investigated [5,6,17,18]. From the sequences available it is clear that there are a considerable number of differences between the bovine (or ovine) and human hormones. Human and bovine prolactins appear to differ at about 20% of all residues. The difference

is considerably less, however, than that seen between human and bovine growth hormones [11,13] though ovine and bovine prolactins are slightly more different than the corresponding growth hormones. Studies on the sequence of pig prolactin have also been reported [18].

The sequences of growth hormones and prolactins show some homology [13,17,19,20] and indicate that the two hormones can be considered members of a single family of protein hormones. This is clear when the sequences of bovine growth hormone [11] and bovine prolactin (fig. 1) are compared. About 25% of all residues are identical when the two hormones are aligned (allowing for a few gaps corrsponding to short deletions in one hormone or the other). Growth hormone is several residues shorter than prolactin at the N-terminus (and the N-terminal disulphide loop of prolactin has no counterpart in growth hormone), while prolactin is two residues shorter than growth hormone at the C-terminus.

Prolactin is also homologous with human placental lactogen [13,17,19,20] but the degree of homology between these two lactogenic hormones is no greater than that between prolactin and growth hormone, while human placental lactogen and human growth hormone show a high degree of homology.

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