THE CARBOXY-TERMINAL PRIMARY STRUCTURE OF THE α SUBUNIT FROM BOVINE AND PORCINE LUTEINIZING HORMONE

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1. Introduction

Luteinizing hormone is composed of two subunits, α and β. Studies of the primary structure of the α subunit have been complicated by the observation that subunit preparations from either bovine or porcine sources are heterogeneous when subjected to starch gel electrophoresis [1–3]. Previous work has demonstrated that the individual electrophoretic components of the porcine subunit preparations have enough similarities in their amino acid composition and peptide maps to warrant primary structure studies without subfractionation [3].

In the present report the amino acid sequence of the carboxy-terminal portion of the porcine α subunit is presented; it is compared to analogous sequence data obtained using an electrophoretically homogeneous subfraction of a bovine α subunit preparation. A good degree of homology is demonstrated in the primary structure of both the porcine and bovine α subunit carboxy-terminal regions; however, a significant sequence variability is demonstrated for the bovine material in spite of its electrophoretic homogeneity. The implications of this heterogeneity are considered and the bovine sequence is compared to that of the α subunit of bovine thyrotropin as recently presented by Pierce et al. [4].

2. Materials and methods

Porcine and bovine LH*, their α subunits and their electrophoretically homogeneous subfractions (A and B) were prepared as described [3, 5]. The isolation of the carboxy-terminal cyanogen bromide fragments as well as the isolation of the tryptic glycopeptides have also been presented elsewhere [3, 5].

Amino acid analyses were performed according to Spackman, Stein and Moore [6] using a Beckman 121 amino acid analyzer. The amino-terminal amino acid sequences were established according to Gray [7]; dansylpeptides were hydrolyzed under the conditions described by Gros and Labouesse [8]. The dansyl-amino acids were identified after thin-layer chromatography on polyamide sheets [9].

Carboxypeptidase A and B were used to analyze carboxy-terminal amino acids and sequences; hydrolyses with carboxypeptidases were conducted as follows. Lyophilized, reduced and alkylated protein or peptide, 0.1 μmole, was dissolved in 100 μl of 0.1 M tris-HCl buffer, pH 8.0. After crystalline suspension (41.5 mg/ml) of carboxypeptidase A (Worthington, DFP treated, 41 U/mg) or solution (6.1 mg/ml) of carboxypeptidase B (Worthington, DFP treated, 105 U/mg) were diluted 10 fold in 10% (w/v) lithium chloride, appropriate additions (10 μl or 50 μl, respectively) were made to the substrate solution. Digestions were conducted at 37° and stopped by freezing at minus 25°. In kinetic experiments, a separate tube, prepared as described above, was allowed for each time point.

* *Abbreviations:*
PLH: porcine luteinizing hormone
BLH: bovine luteinizing hormone

Table 1
Carboxypeptidase digestions of the porcine and bovine luteinizing hormone (α-subunit) and their fragments.

Preparations	Carboxypeptidase used and hydrolysis time			Amino acids (μmole/mg)	
PLH α	A	4 hr	:	Serine	0.032
BLH α A	A	4 hr	:	Serine	0.034
				Tyrosine	0.013
BLH α B	A	4 hr	:	Tyrosine	0.066
				CM-Cysteine	0.066*
				Serine	0.061
				Threonine	0.019
BLH α, CN Br-COOH**	A	2 hr	:	Tyrosine	0.20
				Serine	0.15
				CM-Cysteine	0.07*
BLH α, T-GP IA***	A + B	15 min	:	Tyrosine	0.50
				Lysine	0.34
				Histidine	0.28
	B A	45 min 15 min	:	Tyrosine	0.52
				Lysine	0.27
				Histidine	0.23
BLH α, T-GP IB***	A	15 min	:	Tyrosine	0.35
	A	30 min	:	Tyrosine	0.34
				CM-Cysteine	0.04*
				Threonine	0.04
	A	1½ hr	:	Tyrosine	0.36
				Threonine	0.11
				CM-Cysteine	0.10*
	A	4 hr	:	Tyrosine	0.33
				CM-Cysteine	0.22*
				Threonine	0.18
				Serine	0.10

* Cysteine is identified as its *S*-carboxymethylderivative (CM-Cysteine).
** Cyanogen bromide carboxy-terminal fragment.
*** Glycopeptide resulting from tryptic digestion.

Digests were diluted with 0.2 M sodium citrate buffer, pH 2.2, and analyzed directly using the Beckman 121 instrument. Norleucine was the internal standard in all experiments.

3. Results and discussion

Electrophoretically homogeneous subfractions of BLH α subunit were digested by carboxypeptidase A (table 1). The PLH α subunit has a carboxy-terminal serine residue, a result confirming previous hydrazinolysis data. The bovine A component (BLH α A) has serine, and some tyrosine; the bovine B component (BLH α B) has serine, tyrosine and carboxymethylcysteine in equimolar amounts. Since these preliminary digestions as well as amino acid analyses and peptide maps suggested differences between the bovine A and B components, only the bovine A component (BLH α A) was used for subsequent sequence studies.

The partial amino acid sequence of the carboxy-terminal, cyanogen bromide fragment of the porcine

Table 2
Amino- and carboxy-terminal sequences of the porcine and bovine luteinizing hormone peptides.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Porcine, LHα COOH-terminal CN Br fragment.	Gly	Asn[a]	*Ala*	Arg	Val	Glu	Asx	*Ser*	Thr	Glu[a]	Cys (Tyr 1.0; Cys 1.9; His 2.9; Tyr 2.0; Lys 1.1)									Ser	COOH
Bovine, LHα COOH-terminal CN Br fragment	Gly	Asx	*Val*	Arg	Val[b] Glx	Glx	Asx	SER[c]	Thr	Glx	Cys (Tyr 0.7; Cys 1.3; His 1.4; Tyr 1.5; Lys 0.7; SER[c])										
BLHα, T-GP IA					Val (Glx)	Glx	Asx	Ser[c]	Thr	Glx	Cys (His 2; Cys 2; Thr 2)						Tyr	Tyr	His	Lys	COOH
BLHα, T-GP IB					Val (Glx)	Glx	Asx	His	Thr	Glx	Cys	His	Ser	Cys	Thr	Cys	(Tyr	COOH 1.3[d])			
BLHα, most elongated COOH-terminal sequence	Gly	Asx	Val	Arg	Val Glx	Glx	Asx	Ser His	Thr	Glx	Cys	His	His Ser	Cys	Thr	Cys	Tyr	Tyr	His	Lys	Ser	COOH

[a] Asparagine and glutamic acid were identified previously [2] as their phenylthiohydantoin derivatives.
[b] In BLH α, T-GP IA, amino-terminal Glx is in trace amounts while in BLH α, T-GP IB, amino-terminal Glx is present in sufficient amounts in addition to valine as to create non-integrality for those residues (Val 1.5; Glu 2.4) in the composition of the cyanogen bromide fragment.
[c] After correction for losses during hydrolysis, serine amounts to 2.6 residues in the amino acid composition of bovine LH α, cyanogen bromide, carboxy-terminal fragment (see text), while an integral residue is determined in the composition of BLH α, T-GP IA.
[d] Tyrosine amounts, determined after acid hydrolyses in the presence of phenol. This non-integrality, for tyrosine, together with the data of carboxypeptidase digestions, demonstrate that not all the peptides referred to as BLH α, T-GP IB, have a Tyr–Tyr carboxy-terminal sequence.

LH α subunit is given in table 2. All amino acids are present as integral residues and its amino-terminal sequence is without ambiguity up to step 11. Serine is identified as the carboxy-terminal residue of the peptide. This fragment contains one of the polysaccharide units of LH α subunit.

In contrast, the composition (table 2) of the bovine cyanogen bromide, carboxy-terminal fragment exhibits non integral numbers for several amino acids. An ambiguity is revealed in its amino-terminal sequence at position 5. Its carboxypeptidase digestion (table 1) reveals both serine and tyrosine in amounts which do not permit a clear identification of the carboxy-terminal residue. This fragment also contains one of the polysaccharide units of the subunit. When compared to that of the porcine peptide (table 2), its sequence reveals substitution at position 3 (alanine) by valine.

Digestion of BLH α A by trypsin yielded several glycopeptides, the amino acid sequences of the carboxy-terminal end of the bovine LH α subunit being elucidated by the isolation and characterization of two of them. One of the tryptic glycopeptides (BLH α, T-GP IB) does not contain lysine, a claer indication of its carboxy-terminal location in the subunit molecule. The second peptide (BLH α, T-GP IA) contains lysine but its amino terminal sequence is homologous to that of BLH α, T-GP IB. Both amino-terminal sequences start at the variable position observed in the bovine carboxy-terminal, cyanogen bromide fragment. The carboxy-terminal deletion observed for the peptide population referred to as BLH α, T-GP IB extends for either 3 or 4 amino acids when compared to the most elongated carboxy-terminal sequence of the subunit. Location of His in position on the carboxy side of Asx (position 7) was established taking into account the data from its amino acid analysis and carboxy-terminal sequence determination. The non integrality of the numbers of serine and histidine residues observed in the amino acid composition of the bovine cyanogen bromide carboxy-terminal fragment was thus in agreement with two different carboxy-terminal sequences for the bovine LH α subunit. In table 2, the most elongated sequence of the carboxy-terminal region of bovine LH α subunit is proposed and the observed points of variability are described.

Conclusions

1) The primary structure of the carboxy-terminal portion of both porcine and bovine α subunits have been compared and a good degree of homology is demonstrated.

2) The bovine material, though selected for its electrophoretical homogeneity, is considerably more heterogeneous in its primary structure than the porcine LH α subunit. An ambiguous position is observed at position 5 of the carboxy-terminal, cyanogen bromide fragment. In addition a deletion of either 3 or 4 amino acids is demonstrated at the carboxy-terminal end of a significant part of the peptide population. Whether those observed phenomena are due to the genetic heterogeneity of the animal population or to an intrinsic heterogeneity of the protein itself must be determined by additional investigations.

3) At the former Laurentian Hormone Conference (1970), Pierce et al. [4] presented the structure of the bovine TSH α subunit. Because of similarities in amino acid compositions, peptide maps and cyanogen bromide fragments, TSH α and LH α subunits were presumed to share a high degree of homology. In agreement with their data, the amino acid sequences presented here confirm the similarity of the carboxy-terminal sequence of TSH α and LH α subunits.

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