THE AMINO ACID SEQUENCE OF THE BOVINE LUTEINIZING HORMONE β SUBUNIT

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1. Introduction

Luteinizing hormone (LH) consists of 2 chemically dissimilar subunits, α and β (LHα and LHβ). While our work on the structure of bovine luteinizing hormone subunit (B-LHβ) was in progress, complete amino acid sequences for ovine LHβ (O-LHβ) were independently presented by Liu et al. [1] and by Papkoff et al. [2]. Our work being now complete, it is now possible to determine the extent of similarity between ovine and bovine sequences. This task is complicated by discrepancies appearing in the ovine sequences presented by the other two groups. Our previous data concerning the structure of bovine LHα chain (B-LHα) have focused attention on sequence variability, particularly obvious around the carbohydrate attachment sites [3]. We have looked for such a phenomenon in B-LHβ and compared the composition of its polysaccharide unit to that of the ovine molecule.

2. Experimental

2.1. *Methods*

B-LHβ was prepared as described [3]. The methods used for amino acid and sugar analyses, reduction and alkylation with iodoacetic acid of the protein, cyanogen bromide cleavage, tryptic hydrolysis, end group analyses and sequence determinations were also described [3]. The chymotryptic digestions were carried out at pH 8.5 in 0.2 M sodium phosphate buffer for 2 hr at 37°, with a weight ratio of enzyme to peptide of 1:100. Maleylation of lysine residues was performed at pH 9.4 in a borate buffer [4]. The various digests were fractionated by chromatographic procedures, i.e. gel-filtration on Biogel P6 (Calbiochem) and on Sephadex G-25, G-15 (fine, Pharmacia) and ion exchange chromatography on SE- or QAE-Sephadex (C25 and A25, Pharmacia) [3]. Further purification of peptides were done by electrophoresis at 75 V/cm on Whatman 3 MM paper using pyridine–acetate buffer at pH 3.7 (pyridine – 99% acetic acid – water, 3:20:77).

The cyanogen bromide fragments were separated on a 2 m column of Sephadex G-50 (fine, Pharmacia). The largest tryptic peptide from the maleylated protein (MT_2) was obtained by the same gel-filtration procedure.

2.2. *Nomenclature*

T_i indicates tryptic peptides; MT_i, tryptic peptides resulting from trypsin digestion of the maleylated protein; T_iC_i, peptides resulting from chymotrypsin digestion of tryptic (T_i) peptides; CNBr, the cyanogen bromide fragments individualized by roman numerals.

3. Results and discussion

The amino acid sequence, as indicated in fig. 1, was established by sequential degradation of the various tryptic peptides, whose compositions are given in table 1. Ordering the sequence resulted from the determination of the composition (table 2) and

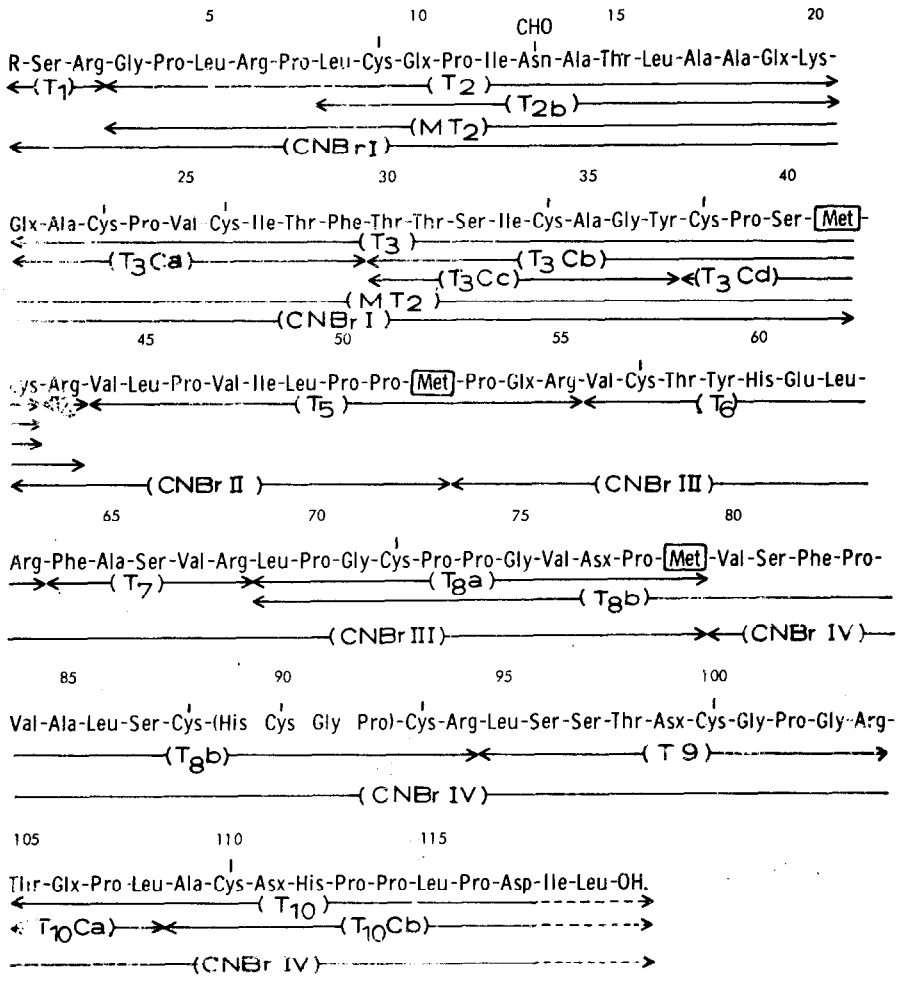

Fig. 1. The amino-acid sequence of bovine LHβ.

amino terminal sequence of the 4 CNBr peptides, together with the characterization of MT2, the largest tryptic peptide resulting from the digestion of the maleylated protein (table 2). The identification of T1 as a blocked amino terminal dipeptide was established after its isolation from a tryptic digest of CNBR 1. The nature of the group blocking the amino terminus was not determined.

Taking into account the modifications of Ward and Liu [5] to their preliminary data [1], a single point of dissimilarity is demonstrated when comparing the ovine and the bovine sequences. Instead of the 50–53 sequence, Pro–Pro–Pro–Met in the ovine chain, the corresponding portion of the bovine molecule is shortened by one proline residue. Noteworthy is that Ward and Liu [5] have assigned all amide or acid forms for the asparaginyl and glutaminyl residues in the ovine sequence, a precision lacking in our study. In addition, the 89–92 sequence has not been determined for the bovine molecule.

Both sequences (ours on B-LHβ and that of Ward and Liu [5] on O-LHβ) differ by 20 positions compared to that proposed by Parkoff et al. [2] for

Table 1.
Tryptic peptide compositions*.

Amino acid	T_1	T_2	T_{2b}**	T_3	T_3C_a	T_3C_b	T_3C_c	T_3C_d	T_4
Lys		0.9 (1)	0.9 (1)	0.9 (1)		1.1 (1)		0.9 (1)	
His									
Arg	1.0 (1)	0.9 (1)							1.0 (1)
CM-Cys		1.1 (1)	0.6 (1)	3.6 (4)	1.6 (2)	2.2*** (2)	1.1 (1)	1.1 (1)	
Asp		1.0 (1)	1.1 (1)						
Thr		0.9 (1)	0.9 (1)	2.6 (3)	1.0 (1)	1.9 (2)	1.9 (2)		
Ser	1.0 (1)			1.6 (2)		1.9 (2)	1.0 (1)	0.7 (1)	
Glu		1.9 (2)	2.0 (2)	1.2 (1)	1.0 (1)				
Pro		2.8 (3)	1.0 (1)	1.6 (2)	1.2 (1)	1.1 (1)		1.1 (1)	
Gly		1.0 (1)		1.0 (1)		1.2 (1)	1.0 (1)		
Ala		2.9 (3)	2.9 (3)	1.8 (2)	1.0 (1)	0.9 (1)	1.0 (1)		
Val				1.0 (1)	1.0 (1)				
Met				0.8 (1)		1.1 (1)		0.8 (1)	
Ile		0.9 (1)	0.9 (1)	1.6 (2)	1.0 (1)	0.9 (1)	0.9 (1)		
Leu		2.7 (3)	1.0 (1)						
Tyr				0.6 (1)		0.6 (1)	0.7 (1)		
Phe				0.9 (1)	0.7 (1)				
Sugars		Present	Present						

Table 1 (Cont'd)

Amino acid	T_5	T_6	T_7	T_{8a}**	T_{8b}	T_9	T_{10}	$T_{10}C_a$	$T_{10}C_b$
Lys									
His		0.8 (1)			1.3		0.9 (1)		0.9 (1)
Arg	1.0 (1)	0.9 (1)	1.0 (1)		0.9	0.8 (1)			
CM-Cys		1.0 (1)		0.8 (1)	2.6	1.0 (1)	0.7 (1)		1.0 (1)
Asp				1.0 (1)	1.3	1.0 (1)	1.9 (1)		1.9 (2)
Thr		0.9 (1)				0.9 (1)	0.8 (1)	0.9 (1)	
Ser			0.9 (1)		1.6	1.7 (2)			
Glu	1.0 (1)	1.1 (1)					1.0 (1)	1.0 (1)	
Pro	3.6 (4)			3.5 (4)	6.7	1.1 (1)	4.0 (4)	1.0 (1)	3.0 (3)
Gly				1.6 (2)	2.9	1.8 (2)			
Ala			1.1 (1)		1.3		1.0 (1)		1.0 (1)
Val	1.6 (2)	1.0 (1)	1.1 (1)	0.8 (1)	2.6				
Met	0.8 (1)			0.9 (1)	1.0				
Ile	0.6 (1)						0.2		0.2
Leu	1.9 (2)	1.0 (1)		0.7 (1)	2.3	1.0 (1)	2.0	1.0 (1)	1.1
Tyr		0.9 (1)							
Phe			1.0 (1)		0.8				
Sugars									

* Data expressed as molar ratios of the various amino acids. The numbers in parentheses are theoretical values. Hydrolysis time was 24 hr. No correction for destruction during hydrolysis was made.

** Peptide resulting from a non-specific cleavage.

*** Mercaptoacetic acid added for the hydrolysis [7].

Table 2

Amino acid	MT_2 composition*	Cyanogen bromide peptide compositions*							
		CNBr I		CNBr II		CNBr III		CNBr IV	
Lys	2.0	0.9	(1)	0.8	(1)				
His						0.4	(1)	1.2	(2)
Arg	1.3**	2.1	(2)	1.4	(1)	1.4	(3)	1.9	(2)
CM-Cys	5.1***	3.6	(5)			1.8	(2)	4.7	(5)
Asp	1.0	1.6	(1)			1.2	(1)	3.2	(3)
Thr	3.8	3.4	(4)			0.6	(1)	2.3	(2)
Ser	1.9	2.8	(3)			1.3	(1)	3.4	(4)
Glu	3.1	3.2	(3)			1.8	(2)	1.3	(1)
Pro	4.7**	5.0	(5)	2.9	(3)	5.0	(5)	5.7	(7)
Gly	1.5**	2.0	(2)			2.1	(2)	3.1	(3)
Ala	4.9	4.5	(5)			0.7	(1)	2.2	(2)
Val	1.2	1.3	(1)	1.9	(2)	2.7	(3)	2.2	(2)
Met	0.9	0.8	(1)	1.0	(1)	0.9	(1)		
Ile	2.6	2.4	(3)	0.6	(1)			0.4	(0–1)
Leu	2.6**	3.4	(3)	2.1	(2)	2.0	(2)	4.13	(4–5)
Tyr	0.6	0.9	(1)			0.4	(1)		
Phe	1.0	0.9	(1)			0.5	(1)	0.9	(1)
Sugars	Present	Present							

* Data expressed as molar ratios of the various amino acids. The numbers in parentheses are theoretical values. Hydrolysis time was 24 hr. No correction for destruction during hydrolysis was made.

** Non-integrality due to partial cleavage between 5 and 6 positions.

*** Mercaptoacetic acid added for the hydrolysis [7].

Table 3
Sugar compositions.

Residue	B-LHβ	T_2	T_{2b}	O-LH*β
Fucose	0.9	1.0	1.0	0.9
Mannose	3.5	3.5	3.8	2.0
Galactose	0	0	0	0.5
Glucosamine	2.8	2.6	2.9	2.9
Galactosamine	1.8	1.5	1.7	0.9

Composition of B-LHβ is expressed after correction of the results for 100% recovery and for a molecular weight of 15,000 for the subunit. The values for the sugars of the glycopeptides are expressed as residues per residue of aspartic acid.

* from reference [6].

O-LHβ. Ward and Liu [5] have described a sequence heterogeneity at the carboxy terminus of O-LHβ. In our hands, a 4 hr digestion with carboxypeptidase A of the carboxy-terminal bovine peptide, T_{10}, yielded 0.17 μmole/mg of isoleucine and 0.1 μmole of leucine. The kinetic study of this digestion together with the amino acid composition of T_{10} also suggest a variability in the length of B-LHβ with a major portion of the chain population ending with Asx-OH, but the sequences Asx–Ile-OH and Asx–Ile–Leu-OH also being present.

It seems striking that while our studies on B-LHα demonstrated sequence variability, no such phenomenon was encountered during the elucidation of B-LHβ structure. Particularly, no amino acid sequence variability was observed around the carbohydrate attachment site. Both tryptic glycopeptides T_2 and T_{2b}, resulting from specific and non-specific cleavages, have agreeing sequences with a sugar composition identical in both cases to that of the entire B-LHβ molecule. As seen in table 3, the polysaccharide unit of B-LHβ differs from that of O-LHβ [6]. This appears to be the major specific difference in the structures of the ovine and bovine LHβ subunits.

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