Cloning and sequence analysis of the cDNA for the precursor of the beta subunit of ovine luteinizing hormone

Gisèle d'Angelo-Bernard, Mohieddine Moumni, Marian Jutisz and Raymond Counis* Laboratoire des Hormones Polypeptidiques, CNRS, 91198 Gif sur Yvette Cedex, France

Submitted March 19, 1990

EMBL accession no. X52488

Clones containing cDNA for the beta-subunit of ovine luteinizing hormone (LHbeta) were isolated from an ovine pituitary pBR322 cDNA library using a rat LHbeta cDNA as a probe. The complete DNA sequence for the LHbeta subunit, presented in Figure 1, was obtained by sequencing three overlapping recombinant inserts using both the chemical cleavage (1) and chain terminator (2) methods. The 533 base-pair cDNA included the entire coding region for a 141 amino acid LHbeta subunit precursor preceeded by a short 5' non coding region as for other mammals (3-5), and followed by a 103 nucleotide long 3' region. Comparison of the amino acid sequence deduced from the open reading frame of the cDNA with that previously established by direct polypeptide sequencing (7) implies a 20 amino acid leader peptide, and a 121 amino acid mature subunit in which we noted four conservative substitutions and a two amino acid extension to the carboxyl terminus giving the ovine LHbeta a size identical to that of the other mammals studied (3-6). In addition, residues 106 and 111 were identified as Gln and Asp, respectively.

Comparison of either the entire cDNA or the coding region of the ovine LHbeta with that of the corresponding bovine sequence (5) gave an overall homology of 98%. Interestingly, although it has long been thought that ovine and bovine LHbeta subunits share complete homology in their primary structures, DNA sequencing of the different clones has revealed two amino acid differences. In addition to the 98% homology with bovine LHbeta, the amino acid sequence of the ovine LHbeta determined in the present study displays an overall homology of 84% with porcine (6), 81% with rat (4), 74% with equine (8), 69% with human (3) and 43% with the chicken (9) or eel (10) sequences.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Institut National de la Santé et de la Recherche Médicale (CRE no. 864 005).

REFERENCES

- 1. Maxam, A.M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560-564.
- Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
- 3. Talmadge, K., Vamvakopoulos, N.C. and Fiddes, J.C. (1984) Nature 307, 37-40.
- Jameson, L., Chin, W.W., Hollenberg, A.N., Chang, A.S. and Habener, J.F. (1984) J. Biol. Chem. 259, 15474-15480.
- Virgin, J.B., Silver, B.J., Thomason, A.R. and Nilson, J.N. (1985) J. Biol. Chem. 260, 7072-7077.
- 6. Kato, Y. and Hirai, T. (1989) Mol. Cell. Endocrinol. 62, 47-53.
- Liu, W.K., Nahm, H.S., Sweeney, C.M., Holcomb, G.N. and Ward, D.N. (1972) J. Biol. Chem. 247, 4365-4381.
- Bousfield,G.R., Liu,W.K., Sugino,H. and Ward,D. (1987) J. Biol. Chem. 262, 8610-8620.
- Noce, T., Ando, H., Ueda, T., Kubokawa, K., Higashinakagawa, T. and Ishii, S. (1989) J. Mol. Endocrinol. 3, 129-137.
- Quérat, B., Moumni, M., Jutisz, M., Fontaine, Y.A. and Counis, R. (1990) J. Mol. Endocrinol. 4, (in press).

		м	Е	м	L	Q	G	L	L	L	w	L	L	L	G	v	A	G	v	W	A	s	R	G	P	L	R	P	L	
ACC	ACCAAGGATGGAGATGCTCCAGGGGACTGCTGCTGTGGCTGCTGCTGGGCGTGGGGGTGTGGGGCTTCCAGGGGGCCACTGCGGCCGCC															9 0														
С	Q	P	I	ŶN	A	т	L	A	A	E	к	Е	A	с	P	v	С	I	т	F	т	т	s	I	с	A	G	Y	с	
GTG	TGCCAGCCCATCAAUGCCACCCTGGCGGCTGAGAAGGAGGCCTGCCCTGTCTGTATCACTTTCACCACCAGCATCTGCGCCGGCTACTG															180														
L^{T} S M K Q^{T} V L P V I L P P M P Q R V C T Y H E L R F A S V R CTCAGGATGAAGGAGGTGCTGCCGTGTGCATCCATGCCGCCCAGGGGGGTGTGGCACCAGGAGCTGCGCGTCGGCCTCGGCTCGGCTCGGCTGCGCGGGGGG															270															
,	ъ	c	~	п	в	c	.,	n	п	м	v	c	F	ъ	v		Ŧ	c	c		~	~	Б	~	ъ	,			т	
	L P G C P P G V D P M V S F P V A L S C H C G P C R L S S T GCTCCCCGGCTGCCCACCTGGCGTGGACCCAATGGTCTCCTTCCCCGTGGCCCTGAGCTGTCACTGTGGGCCCTGCCGCCTCAGCAGCAC															360														
D	с	G	_G 7	P	R	т	q 7	P	L	A	С	$_{\mathrm{D}} \tau$	H	Р	Р	L	P	D	I	L	_F 7	' ı7	*							
TGA	CTGC	CGGG	GCT	ccc	AGA	ACC	CAA	ccc	CTG	CCC	TGT	GAC	CAC	ccc	CCG	СТС	CCA	GAT	ATC	СТС	TTC	сто	TAA	GGA	TGC	ccc	ACT	TCA	ΤA	450
СТС	CAT	rgco	CAC	сст	'AAC	тсс	CAA	AGC	CAG	CAG	ACG	стс	стс	ссс	тсс	CTT	ccc	<u>AAT</u>	AAA	GAC	TTC	TC	AGO	TGC	AAA	AAA	A			533
pre	FIGURE 1. Nucleotide cDNA sequence and predicted amino acid sequence of the ovine LHbeta-subunit precursor. The amino acids differing from the published peptide sequence [7] are indicated by τ . The asterisk indicates the stop codon and the losange, the position of the single N-linked																													

carbohydrate chain. The polyadenylation signal is underlined.

* To whom correspondence should be addressed