ISOLATION AND PROPERTIES OF HUMAN LUTEINIZING HORMONE SUBUNITS

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1. Introduction

While ovine and bovine LH are dissociated into subunits, LHα and LHβ, in countercurrent distribution systems at acidic pH [1–4] human LH does not behave similarly [5]. Recently, Hartree et al. [6] have reported the isolation of human LH subunits. However, according to the authors, the procedures employed had a low yield for each subunit. Simultaneously both Saxena and Rathnam [7] and we [8] presented preliminary data on the isolation of human LHα and β subunits.

We wish to describe here a separation method for the subunits of human LH using ion exchange chromatography in dissociating buffers. Our dissociation procedure was found as efficient as in the case of porcine [9] and bovine LH [10]. We wish also to present the characterization of the human chains and to compare our data with those of others [6, 7].

2. Material and methods

Ion exchangers were sulfopropyl (SP) Sephadex C-25 and diethylaminoethyl (DEAE) Sephadex A-25 (Pharmacia).

Desaltings were done by gel filtration on Sephadex G-25 (Pharmacia) in 0.05 M ammonium bicarbonate.

Abbreviations:
LH: luteinizing hormone.
LHα and LHβ: the subunits of LH.

Urea was from Merck, P.A. Grade. The LH isolation procedure was that of Hennen et al. [11]. LH activity was assayed by the ovarian ascorbic acid depletion (OAAD) test of Parlow [15] with NIH-LH-S15 as standard.

2.1. *Dissociation procedures*

2.1.1. Chromatography on SP-Sephadex C-25

Human LH (40 mg) was first equilibrated in 0.02 M sodium acetate 8 M urea, pH 4.8 by gel filtration on Sephadex G-25. The non-retarded fraction was applied on the cation exchanger and the chromatography developed immediately (fig. 1) by applying a first linear gradient of ionic strength, from 0.02 M sodium (sodium acetate) to 0.1 M sodium (0.08 M sodium chloride in 0.02 M sodium acetate pH 4.8) followed by a linear gradient from 0.08 M to 0.3 M sodium chloride in 0.02 M sodium acetate pH 4.8.

2.1.2. Chromatography on DEAE-Sephadex A-25

Human LH (80 mg) was kept for 24 hr in 10 ml 0.05 M HCL, 8 M urea, the final pH being 2.83. Just prior to chromatography, the solution was adjusted to pH 9.5 by addition of 1 N sodium hydroxyde and diluted with 30 ml of 0.01 M sodium glycinate pH 9.5. This solution was immediately applied to the column and a linear gradient of ionic strength was started, from 0.05 M sodium chloride in 0.01 sodium glycinate pH 9.5 to 0.5 M sodium chloride in the same glycine buffer.

The materials from SP- and DEAE-Sephadex were

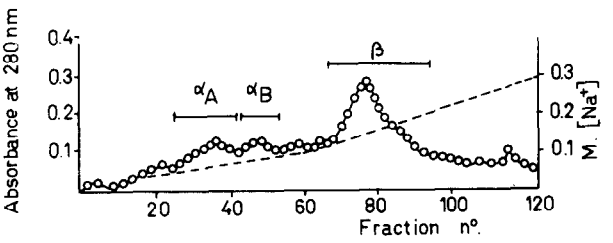

Fig. 1. Chromatography of human LH (40 mg) on SP-Sephadex C-25. Column 0.9 × 10 cm, equilibrated with 0.025 M sodium acetate, 8 M urea, pH 4.8. Fraction size: 2.8 ml. The slope of the linear gradient is indicated by broken line.

tentatively identified as LHα and β, according to the specific composition of those subunits in other species.

The amino acid and sugar compositions were established as previously described [10]. Amino-terminal amino acids and sequence were determined according to Gray [12]. Dansyl-amino acids were identified by thin-layer chromatography on polyamide sheets [13]. Carboxypeptidase A (Worthington, DFP treated, 41 U/mg) and carboxypeptidase B (Worthington, DFP treated, 105 U/mg) were used to analyse carboxy-terminal amino acids [14]. In kinetic experiments, digestions were conducted at 37° during 30 min and 4 hr.

3. Results and discussion

3.1. *Chromatography on SP-Sephadex*

As shown in fig. 1., no complete separation between the various peaks was achieved by chromatography on this exchanger. The amino acid compositions (table 1) of LHαA, LHαB and LHβ (fig. 1) show clearly that a dissociation of the hormone had occurred, but the poor resolution of the various peaks did not guaranty complete separation of its subunits.

3.2. *Chromatography on DEAE-Sephadex*

In this system, 2 well separated peaks were found (fig. 2), the first one being non-adsorbed (28 mg), the second being readily eluted by the gradient (30 mg). The composition of both peaks is given in table 2, the materials being easily identified as LHα and LHβ. Our data demonstrate significant differences between the

Table 1
Amino acid compositions* of human luteinizing hormone (LH) subunits isolated by chromatography on sulfopropyl Sephadex.

Amino acid	LHαA	LHαB	LHβ
Lysine	7.9	5.4	3.5
Histidine	3.1	3.2	3.1
Arginine	3.8	4.6	8.8
Aspartic acid	6.8	6.9	7.0
Threonine	8.3	8.7	7.3
Serine	7.0	7.3	6.2
Glutamic acid	10.3	10.2	7.3
Proline	7.1	6.9	10.1
Glycine	9.7	9.9	6.8
Alanine	6.2	5.9	5.5
½ Cystine	6.7	6.9	7.5
Valine	6.8	6.9	9.0
Methionine	2.7	2.8	2.1
Isoleucine	1.9	1.8	3.4
Leucine	4.8	4.8	7.7
Tyrosine	3.3	3.4	2.1
Phenylalanine	3.8	3.8	2.5

* Amino acid compositions expressed as residue per 100 residues.

compositions of the human chains and those of their counterpart in other animal species [1–4, 9]. Our results agree well with the amino acid compositions of human LH and its subunits as described by Hartree et al. [6].

The biological potencies of the LH and its subunits are given in table 3 where our data are compared with those of others [6, 7]. In all cases, the individual subunits exhibit a significantly lower activity than that of the native hormone.

Valine was identified as amino-terminal amino acid for human LHα while no amino-terminal amino acid was clearly evidenced for LHβ if we except traces of leucine. Compared to the bovine chain (table 4) the amino-terminal sequence of human LHα starts at a position corresponding to the 8th amino acid in the most elongated bovine amino-terminal sequence. Our data agree with those presented by Nurredin et al. [16] except that they reported Pro in position 5.

As previously described [16], internal homology was found between amino and carboxy-terminal portion in the bovine LHα sequence. Homology was thus expected and indeed found between those portions of

Table 2
Compositions of human luteinizing hormone and its subunits.

Amino acid*	LH			LHα			LHβ		
	Present data	Hartree et al. [6]	Saxena and Rathman [7]	Present data	Hartree et al. [6]	Saxena and Rathman [7]	Present data	Hartree et al. [6]	Saxena and Rathman [7]
Lysine	4.1	4.0	3.9	6.1	6.0	4.6	2.5	2.3	3.3
Histidine	2.5	2.6	2.9	3.3	2.9	2.7	2.7	n.d.	3.1
Arginine	6.3	6.3	4.7	3.6	4.5	3.8	8.7	8.2	5.6
Aspartic acid	7.0	6.5	7.2	5.2	6.1	7.9	6.2	6.6	6.5
Threonine	6.5	7.5	7.0	8.2	8.2	8.0	6.1	6.0	6.1
Serine	6.8	7.2	6.9	8.2	7.8	7.0	6.0	5.7	6.7
Glutamic acid	8.8	9.5	8.0	10.2	10.2	8.9	7.2	7.3	7.1
Proline	13.4	10.2	13.0	7.4	9.2	9.4	14.8	13.5	16.6
Glycine	6.5	6.5	5.5	6.9	5.3	5.5	8.3	7.0	5.5
Alanine	6.1	4.8	6.0	5.0	4.6	5.7	5.0	4.7	6.3
½ Cystine	7.9	8.9	7.6	8.3	9.9	8.9	7.2	9.9	6.7
Valine	8.7	8.7	8.7	7.7	7.9	8.7	9.1	9.75	8.7
Methionine	1.7	1.9	1.8	3.8	2.6	1.9	2.0	1.5	1.7
Isoleucine	3.5	3.0	5.0	1.4	1.7	5.0	3.9	3.8	5.0
Leucine	5.8	6.3	5.6	5.2	5.1	5.0	7.1	7.4	6.2
Tyrosine	2.9	2.8	2.4	4.4	3.6	2.7	2.0	2.0	2.1
Phenylalanine	2.1	2.8	3.6	4.6	4.4	4.1	2.1	1.9	3.2
Fucose**	0.053	0.060		0.045	0.036		0.060	0.044	
Mannose	0.280	0.250		0.335	0.307		0.140	0.143	
Galactose	0.127	0.107		0.121	0.157		0.081	0.064	
Glucose	0.040	-		0.029	-		0.057	-	
Glucosamine	0.284	0.310		0.400	0.514		0.120	0.237	
Galactosamine	0.042	0.057		0.092	0.080		0.00	0.035	
Sialic acid	0.091	0.150		0.140	0.014		0.057	0.00	

* Amigo acid composition expressed as residue per 100 residues.
** Sugar composition expressed as μmole per mg.
N.D: Not determined or not reported.

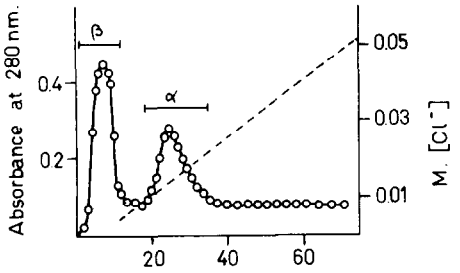

Fig. 2. Chromatography of human LH (80 mg) on DEAE-Sephadex A-25. Column (1.9 × 10 cm), equilibrated with 0.01 M sodium glycinate, pH 9.5. Fraction size 2.8 ml. Slope of gradient is indicated by broken line.

Table 3
Biological potencies of human luteinizing hormone (LH) and its subunits.

	Present* data	Hartree** et al. [6]	Saxena and*** Rathnam [7]
	Specific activity (U/mg)		
Native human LH	3.8	3.45	8.82
Human LHα	0.3	0.53	0.75
Human LHβ	0.7	0.13	2.08

Potencies are expressed respectively in terms of: NIH-LH-S15*; NIH-LH-S12**; and NIH-LH-S1***.

Table 4

Amino-terminal sequence of human LHα	Val–Glx–Asx–Cys–Val–Glx–Cys
Amino-terminal sequence of bovine LHα	Phe–Pro–Asp–Gly–Glu–Phe–Thr–Met–GlN–Gly–Cys–$\frac{\text{Pro}}{\text{Thr}}$–Glu–Cys
Amino-terminal sequence of the carboxy-terminal cyanogen bromide fragment of bovine LHα	Gly–Asx–Val–Arg–Val–Glx–AsN–$\frac{\text{His}}{\text{Ser}}$–Thr–Glx–Cys

bovine LHα and the amino-terminal sequence of human LHα(table 4). It is nevertheless striking to note that positions 8 (Met) and 10 (Gly) in the bovine amino-terminal sequence correspond to Val and Asx, respectively, in the human sequence. Those are indeed the residues found in that portion of bovine carboxy-terminal sequence which show homology with the amino-terminus.

As for bovine LHα [14], heterogeneity was found at the carboxy-terminus of human LHα, the most elongated sequence being Tyr–His–Lys–Ser–COOH, while at least half of the chains are terminated at Tyr.

4. Conclusion

Two procedures were investigated for the isolation of the subunits of human LH. The most efficient method resulted from incubation of native LH in acidic medium containing 8 M urea, followed by chromatography on DEAE-Sephadex A-25 in alkaline buffer. In those conditions, the hormone subunits were eluted as well resolved peaks. The yields of both LHα and LHβ subunits were high and about equal.

Sequence homology of the amino-terminal portion of human LHα was found with both amino and carboxy-terminal regions of bovine LHα chain.

Heterogeneity was demonstrated at the carboxy-terminus of human LHα as in the case of the bovine chain.

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