COMPARISON OF CHORIONIC GONADOTROPIN AND LUTEINIZING HORMONE: A NOTE ON A PROPOSED SIGNIFICANT STRUCTURAL DIFFERENCE IN THE BETA SUBUNIT*

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1. Introduction

The marked degree of conservation of structure among the glycoprotein tropic hormones, TSH, LH, FSH, and hCG, was first clearly noted by Pierce and his colleagues [1]. The alpha subunits of these hormones have very similar primary structures; the beta subunits, although they differ in details of amino acid sequence, also possess recognizable common features. Structural studies on LH in various species have confirmed these views [2–8]. The amino acid sequence of the hCG alpha subunit also fits this pattern [9].

However, when we compare the proposed structures for hLH beta [8] and hCG beta [9] we find at least two striking differences. hCG beta appears to contain a deletion of seven residues, including two half-cystine residues, in the region 83–94 of hLH beta; and it also possesses an additional twenty amino acids at the COOH terminal end. The absence of the two half-cystine residues would suggest that hCG beta has one less disulfide bridge than the beta subunits of the other glycoprotein hormones, whose beta subunits all possess 12 half-cystine residues. Such differences would have important implications in regard to the evolution of these hormones and could well explain the biological and immunological differences found between hCG and hLH.

We wish to present evidence derived from our study of the amino acid sequence of hCG that one of these differences may not exist. We have isolated from a tryptic digest of hCG-beta a peptide that contains a region corresponding to 83–94 in hLH as proposed by Closset et al. [8] and which includes the two half-cystine residues in question. hCG beta appears then to contain twelve half-cystine residues in positions analogous to those of the beta subunits of the other glycoprotein tropic hormones.

2. Materials and methods

hCG and its subunits were prepared from crude urinary hCG (Organon, Oss, Netherlands) as previously described [10–12]. Asialo, S-carboxymethylated hCG beta [10], 200 mg (approx. 8 μmoles), was digested with TPCK-treated trypsin (2% by weight) for 1.5 hr at room temp. in 0.1 M NH_4HCO_3. Glycopeptides were separated from other tryptic peptides by chromatography on Sephadex G-50 (2.5 × 200 cm) in 0.08 M ammonium acetate pH 5.9 [13]. Tryptic peptides were further purified on Dowex 50 X 4 (2.2 × 50 cm) at 50° using a linear gradient from 0.05 M ammonium acetate, pH 4.1 to 0.5 M ammonium acetate, pH 5.8. Peptide-containing fractions obtained from the ion exchange chromatography were assessed for purity by one dimensional high voltage paper electrophoresis in pyridinium acetate, pH 3.55 [14]. Amino acid analysis was performed after hydrolysis *in vacuo* in 6 N HCl at 110°. Amino acid sequence was determined by the

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Abbreviations:

TSH: thyrotropin; LH: luteinizing hormone; FSH: follicle stimulating hormone; CG: chorionic gonadotropin; h: human; b: bovine; PITC: phenylisothiocyanate; PTH: 3-phenyl-2-thiohydantoin; TPCK: L-(1-tosylamido-2-phenyl) ethyl chloromethyl ketone; SCM: S-carboxymethyl.

PITC degradation [15] on 0.2–0.3 μmole of peptide. PTH-amino acids were directly identified by thin-layer chromatography [15] and by gas chromatography [16]. Radioactivity, introduced into the protein by use of iodo-[^{14}C]acetic acid for alkylation, served to monitor the position of half-cystine-containing peptides during chromatography, and of PTH-S-carboxymethylcysteine during amino acid sequence analysis.

3. Results

A radioactive fraction emerged from the Dowex-50 column at a position corresponding to pH 4.18 and an ammonium acetate concentration of 0.12 M. On paper electrophoresis at pH 3.55, the fraction contained one major peptide (β6T4) with a mobility of 0.23 with respect to arginine. This peptide was further purified by preparative paper electrophoresis in the same system. Amino acid analysis after 24 hr of hydrolysis gave the following composition based on one arginine residue: alanine 3.1, arginine 1.0, S-carboxymethylcysteine 2.7, glutamic acid 1.2, leucine 2.2, serine 0.9, valine 1.1. The yield from the Dowex-50 column was 3.6 μmoles, approx. 50% of the theoretical value. The following amino acid sequence was determined by a continuous manual PITC degradation:

Ala–Val–Ala–Leu–Ser–SCMCys–Gln–SCMCys–Ala–Leu–SCMCys–Arg.

Steps 1–11 were identified as PTH-amino acids by thin-layer chromatography, and confirmed in the case of steps 1, 2, 3, 4, 5, 9, and 10 by gas chromatography; steps 6, 8 and 11 were confirmed as SCMCys by the presence of significant radioactivity in these cycles of the degradation (table 1). The identity of step 12 was confirmed by the finding of free arginine in the reaction mixture after eleven cycles of the degradation.

Table 1
Relative quantitation of ^{14}C in PTH-amino acid obtained in sequential degradation.

Edman degradation step	Radioactivity (cpm)
1	0
2	20
3	40
4	40
5	60
6	500
7	50
8	410
9	0
10	50
11	280

The radioactivity of equal portions of the PTH-amino acid obtained at each step of the degradation was determined with a low background planchet counter. As step 12 was identified as free arginine, no PTH amino acid was obtained at this step. Steps 6, 8, and 11, were also identified as PTH-SCMCys by chromatography.

4. Conclusion

The amino acid sequence of the peptide described above is clearly homologous with the region 83–94 of hLH beta as proposed by Closset et al. [8], table 2. It is in disagreement with the same region as earlier proposed for hCG beta by Bahl et al. [9], where a deletion of seven residues including two half-cystine residues has been suggested [8] to maximize homologies; nor is this peptide accounted for by any other region of the proposed hCG beta sequence [9]. The other major features of the structure have been accounted for by the remaining tryptic peptides and these results will be published shortly. We have previously reported a half cystine content of 12.7 residues per 146 residues in the beta subunit [11], and an uncorrected half-cystine content of 10.8 was reported by Swaminathan and Bahl [17]. It seems likely on the basis of our results that a deletion in hCG beta in this region does not exist and that hCG conforms even more closely to LH than has been proposed. It is reassuring to find that hCG beta contains the same number of half-cystine residues as do the other glycoprotein hormones, since a change in this feature which is commonly preserved in homologous series [18] would be a remarkable finding in the presence of such otherwise marked conservation of structural features.

The fact that tissue specificity for these hormones appears to reside in the β subunit makes any difference in β subunit structure potentially highly significant. With this in mind, we should also note that in the region of carbohydrate attachment at position 30, we

Table 2
Amino acid sequences in hCG and hLH.

Hormone	Amino acid sequence	Reference
hCG	–Ala–Val–Ala–Leu–Ser–Cys–Gln–Cys–Ala–Leu–Cys–Arg–	(This paper)
hCG	–Ala–Val–Ala–Leu– X –Cys–Arg– X – X – X – X – X –	[9]
hLH	–Pro–Val–Ala–Leu–Ser–Cys–Arg–Cys–Gly–Pro –Cys–Arg–	[8]
	83 84 85 86 87 88 89 90 91 92 93 94	

The alignment and numbering is that proposed in [8].

find a sequence Cys–Ile–Thr–Val–Asn (CHO)–Thr–Thr–Ile–Cys in disagreement with the earlier report of Bahl et al. [9] Cys–Ile–Asn (CHO)–Val–Thr–Thr–Ile–Cys, but in complete agreement with the proposal for hLH [8] and homologous with the carbohydrate containing region in bTSH beta [1]. Here again the suggestion of a deletion between these half-cystine residues and the variation in position of carbohydrate attachment is not substantiated. It may be wise then to exercise caution in interpreting evolutionary and structure–function relationships among the glycoprotein hormones until firm evidence is available to substantiate structural proposals.

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