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STRUCTURE OF HUMAN LUTEINIZING HORMONE BETA SUBUNIT: EVIDENCE FOR A RELATED CARBOXYL-TERMINAL SEQUENCE AMONG CERTAIN PEPTIDE HORMONES

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SUMMARY

Amino acid sequence analysis of human luteinizing hormone (lutropin) beta subunit has been carried out in order to reconcile several discrepancies apparent in previously published structures. Our sequence coincides closely with that proposed originally by Shome and Parlow (J.Clin.Endocr.Metab. 36, 618-621, 1973). However, we found a unique solution to the carboxyl-terminal region (--Thr-Cys-Asp-His-Pro-Gln-OH) which is closely homologous with the corresponding segment of hCG and the animal LH beta subunits. The C-terminal three residues appear in closely similar sequences at the C-termini of several other peptide hormones. This suggests a primitive genomic relationship with conservation of this region during evolution of these otherwise divergent polypeptides.

INTRODUCTION

Structural analyses of the human glycoprotein hormones (luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone and chorionic gonadotropin) have all been reported over the past several years. Nevertheless, incomplete sequence segments and areas of discrepancy are still evident among some of the published analyses. Human luteinizing hormone (lutropin)[a] has been studied by numerous laboratories (1-6) and while there is general agreement regarding the majority of the amino acids in the primary sequence, consensus has been lacking in several important regions of both subunits. In an effort to reconcile these differences we recently completed sequence determination of purified hLH alpha (common) subunit (7) and now report analysis of the beta (hormone-specific) subunit. This analysis includes a unique

[a]Abbreviations: LH, luteinizing hormone (lutropin); FSH, follicle stimulating hormone (follitropin); TSH, thyroid stimulating hormone (thyrotropin); CG, chorionic gonadotropin (choriogonadotropin); TLC, thin layer chromatography.

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alignment of the residues at the carboxyl terminus which appears to bear a close resemblance to the carboxyl termini of several other peptide hormones.

METHODS

Hormone Preparations: Purified hLH for these studies was obtained from two separate sources: (a) hormone isolated by the procedure of Bishop and Ryan (8) from acetone-dried pituitary tissue; (b) purified hLH obtained from the National Pituitary Agency. The subunits were separated by countercurrent distribution and gel filtration as described by Ward et al (5). The respective preparations were kept separate throughout the structural analyses.

Carboxymethylation: Reduced, carboxymethylated beta subunit was used in all sequence studies. The separated subunit was treated by dithiothreitol in the presence of 10M guanidine, followed by iodoacetic acid, at pH 8.2 as previously described (9). (^{14}C)-Iodoacetic acid (New England Nuclear) was used to introduce a label at cysteine residues (6,7).

Cleavage Procedures: Cleavage with trypsin, chymotrypsin, cyanogen bromide, and carboxypeptidase A, B and Y was carried out as described previously (7,9). Dilute acid cleavage (9,10) was done by treatment with .03N HCl at 110°C in vacuo for an 8-hour time period. The cleavage product was centrifuged and the supernatant lyophilized for further purification. The quantities used for enzymatic or acid cleavage ranged from 5 to 15 mg subunit. Peptide subfragments were separated by gel filtration on Sephadex G-50 (Pharmacia) and Bio-gel P-30, P-10, P-4 and P-2 (Bio-rad) using .2M ammonium bicarbonate buffer, pH 8.7; by DEAE-cellulose (Whatman) chromatography using an ammonium bicarbonate buffer gradient (7); and by preparative scale thin-layer chromatography and electrophoresis (7).

Sequence Analyses: Automated Edman degradation (11) using the Beckman model 890 Sequencer was carried out on intact subunit and larger peptide fragments. Edman degradation of smaller peptide fragments was done using the three-stage manual procedure (12). Phenylthiohydantoins were identified by TLC (11) and gas-liquid chromatography (13). (^{35}S)-phenylisothiocyanate was used as coupling reagent in most degradations for enhanced sensitivity (6). Amino acid compositions of peptide fragments were determined with the Beckman model 120C or 121M automatic analyzer after hydrolysis in vacuo for 24 hours in 5.7N HCl at 110°C.

RESULTS AND DISCUSSION

The amino acid sequence of hLH beta subunit, and the peptide fragments used in the analysis, are summarized in Figure 1. Analysis of the amino-terminal portion relied principally on automated degradation of intact subunit (6) and the long tryptic fragments T-2 and T-3. There was no evidence for heterogeneity at the amino terminus of the type found in most preparations of alpha subunit (7). As reported previously by Sairam et al (3), we found one or more pre-existing partial cleavages in the region of residues 44-48. This was especially evident on carboxypeptidase digestion, when the enzyme cleaved

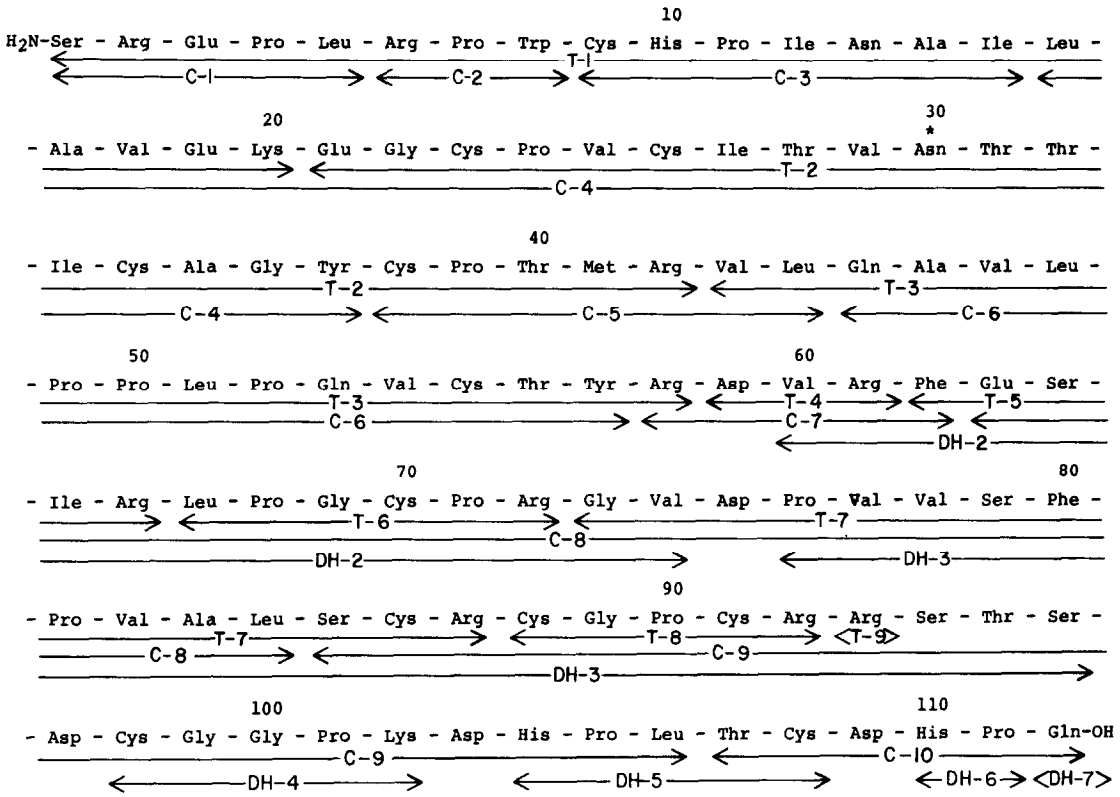

Figure 1. Amino acid sequence of human LH beta subunit. Peptide fragments used in the analysis include: C, chymotrypsin; T, trypsin; DH, dilute acid. Side-chain carbohydrate is indicated by an asterisk.

from this region of the molecule back through the single methionine at position 41. We found the methionine to be resistant to cyanogen bromide cleavage, similar to one methionine (position 44) in the alpha subunit (4,7). The carbohydrate found on compositional analysis of fragments T-2 and C-4 (Fig. 1) were assigned to position 30 on the basis of homology with other beta subunits and presence of the Asn-X-Thr sequence typically coding for carbohydrate attachment.

A larger number of smaller peptides (Fig. 1), many isolated by preparative scale TLC or TLE and degraded by the manual Edman procedure, were used in analysis of the region toward the carboxyl terminus. Our analysis of the far C-terminal region, which differs in several respects from the various earlier proposals, is summarized in Figure 2. The sequence was obtained pri-

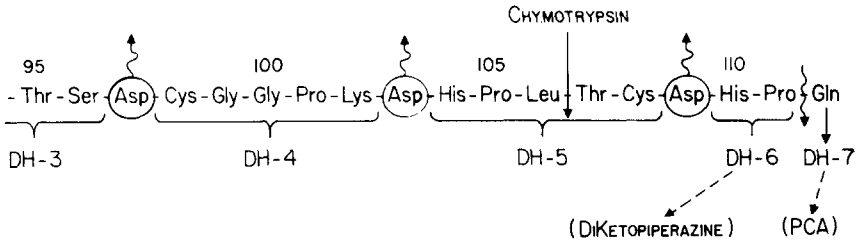

Figure 2. Strategy employed in sequence analysis of carboxyl terminus of hLH beta subunit, showing cleavages obtained with chymotrypsin, and with dilute acid (DH) treatment which cleaves aspartic acid from the peptide chain.

marily from chymotryptic digests, from which fragment C-10 (Fig. 1) was isolated by Sephadex G-50 or Biogel P-30 chromatography and preparative-scale TLC. This peptide (-Thr-Cys-Asp-His-Pro-Gln) was obtained from separate digests representing both types of hormone preparation (see Methods). As a second approach, carboxypeptidase digestion was complicated by cleavage within the central portion of the molecule (see above) and presence of the penultimate proline which appeared to have limited the extent of digestion at the C-terminus itself.

The aspartic acid rich nature of the carboxyl-terminal portion suggested dilute acid cleavage as a feasible alternative procedure; this method had been used successfully in our earlier analysis of the C-terminus of hCG-beta (9). The results of dilute acid cleavage, while unorthodox in one respect, were consistent with our proposed sequence (Fig. 2). The pentapeptides DH-4 and DH-5 were isolated by gel filtration on Sephadex G-50 and Bio-gel P-2 followed by TLC. However, instead of a terminal tripeptide, two fragments (DH-6 and DH-7), both ninhydrin-negative, were recovered from TLC. These appeared to result from acid cleavage of the terminal glutamine, which cyclized into pyrollidone-carboxylic acid (DH-7). The remaining His-Pro dipeptide (DH-6) also cyclized under the acidic reaction conditions to form the diketopiperazine. We have previously encountered the cleavage of C-terminal glutamine, and diketopiperazine formation from a proline-containing dipeptide, in dilute acid hydrolyzates of parathyroid hormone (14) and calcitonin (15) respectively.

hLH:	
Reference (1)	-thr-cys-asp-glx-pro-his(ser,lys,gly)
" (2)	-thr-cys(asx,glx,ser,lys)gly
" (3)	-thr-cys-asp-pro-gln-his-ser-gly
Present Study	-THR-CYS-ASP-HIS-PRO-GLN
oLH, bLH:	-ala-cys-asp-his-pro-pro-leu-pro-asp-ile-leu
pLH:	-ala-cys-asx-arg-pro-pro-leu-pro-gly-leu-leu
hCG:	-thr-cys-asp-asp-pro-arg-phe-gln-asp-ser-ser-

Figure 3. Comparison of C-terminal sequence of hLH beta from current study with previously published sequences of corresponding region from various species. The complete sequences of the different species are reviewed in reference (16).

Most of the structure agrees closely with that reported originally by Shome and Parlow (1). However, the extreme carboxyl terminus differs from all previous proposals (Fig. 3), two of which included only tentative assignments based on compositional studies alone. Although our sequence is shorter (112 residues) than the other proposals, we did not locate any other fragments that might have represented longer variants of the carboxyl terminal sequence. In light of previous reports (1,3), however, C-terminal heterogeneity of the hLH beta subunit remains a consideration despite our finding of an identical C-terminal sequence in separate hormone preparations extracted by distinctly different procedures.

The carboxyl terminal sequence reported here is closely homologous with the corresponding regions of ovine, bovine and porcine beta subunits, as well as hCG beta subunit (Fig. 3)(16). The importance of sequence information in this region is emphasized by recent evidence for crossreactivity of pituitary tissue with antisera derived from the 30-residue C-terminal extension of hCG beta (17). If, as these observations suggest, a similar "extension peptide" for LH beta does exist intracellularly, the sequence differences between hLH and hCG in this region could explain its eventual removal from the LH subunit and retention in the hCG subunit. The C-terminal structure is also important in development of radioimmunoassay systems to distinguish between hCG and hLH (17-19). Comparison of the length and homology of the beta subunits is es-

Hormone	Species	Length (Residues)	C-Terminal Sequence
LH-beta	Human	112	-His-Pro-Gln
CG-beta	Human	145	-Leu-Pro-Gln
Parathyroid Hormone	Bovine, Porcine	84	-Lys-Pro-Gln
	Human	84	-Lys-Ser-Gln
Motilin	Porcine	22	-Lys-Gly-Gln
β-Lipotropin	Human, Bovine, Ovine, Porcine	91	-Lys-Gly-Gln

Figure 4. Summary of peptide hormones found to contain "related" carboxyl-terminal sequences. The complete sequences may be found in reference (16).

sential to the preparation of synthetic fragments for developing specific C-terminal hCG antisera used clinically in detection of pregnancy and certain neoplastic states, and potentially in the control of fertility.

There is a similarity between the final -His-Pro-Gln sequence of our HLH sequence and the C-terminus of several other hormones of neural-crest origin (Figure 4). The sequence of the final three residues typically comprises a basic residue followed by proline (or a common proline substitution) and finally a C-terminal glutamine. Among other glycoprotein beta subunits, this sequence is found at the C-terminus of hCG (Fig. 4) but appears not to be present in human FSH. It does, however, appear as an internal Lys-Pro-Gln segment just inside the C-terminus of human and bovine TSH (16).

The significance of this homology among the C-terminal sequences of these diverse hormones is not as yet apparent. Initially it suggested to us a common mode of intracellular processing of the peptide chain from a putative C-terminal extended precursor form. However, in two of these hormones --beta lipotropin and parathyroid hormone--the recently completed nucleotide sequences (20,21) have shown the terminal glutamine to be followed by a "stop" codon. Thus, although they are unlikely to represent processing "signals", the sequences perhaps imply a primitive genomic relationship among these hormones with strong tendency toward conservation in this region during otherwise marked divergence of structure and function.

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