HUMAN CHORIONIC GONADOTROPIN: AMINO ACID SEQUENCE OF THE α AND β SUBUNITS*

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SUMMARY

The linear amino acid sequences of the α and β subunits of human chorionic gonadotropin have been determined. HCG-α has 92 and HCG-β 139 amino acid residues. There is more homology between HCG-α and ovine LH-α than between HCG-β and ovine LH-β.

Recently, a great deal of progress has been made in the elucidation of the chemistry of glycoprotein hormones such as ovine and bovine luteinizing (LH) (1-3) and bovine thyroid stimulating hormones (TSH) (4). As a result, a fascinating picture of their interrelationships has emerged. Human chorionic gonadotropin (HCG) is a glycoprotein hormone produced by the placenta during pregnancy and is similar to LH in biological action. In early pregnancy HCG prolongs the life of the corpus luteum, but its role during pregnancy is not yet clearly understood. Several pregnancy tests are based on its estimation in urine or serum.

In accordance with the other glycoprotein hormones HCG is made up of two non-covalently bonded subunits, α and β (5). The α-subunit of HCG is interchangeable with the α subunits of the other hormones with no loss of biological or immunological activities (6). The β subunit is hormone specific. The monosaccharide sequence of

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the carbohydrate portion of HCG, which forms about 30% of the molecule, has been reported previously (7). This communication describes the linear amino acid sequences of the α and β subunits and their relationship to the other glycoprotein hormones.

EXPERIMENTAL

The HCG employed in the present studies was purified as previously described and had a potency of 12,000 IU/mg (8). Trypsin and chymotrypsin were products of Worthington Biochemical Corporation, Freehold, N. J. and thermolysin of CalBiochem, La Jolla, California.

Hydrolysis with Proteolytic Enzymes. The α and β subunits of carboxamidomethylated HCG were hydrolyzed with trypsin, chymotrypsin or thermolysin. The amounts of the enzymes used ranged between 2-3% by weight of the substrate and the hydrolyses were carried out in 1% ammonium bicarbonate solution at 37° for periods of 1-2 hours. The hydrolyses of the peptides with these enzymes were performed under similar conditions using 5 to 10% by weight of the enzymes and longer incubation times (4 to 12 hours).

Cleavage of the α-Subunit by Cyanogen Bromide. Incubation of the aminoethyl derivative (9) of HCG-α with an equal weight of cyanogen bromide (50 molar excess over methionine) in 80% formic acid for 24 hours at 4° (10) caused 80% reaction at methionine residues as determined by the amount of homoserine formed. No further reaction occured when additional cyanogen bromide was added to 200 molar excess and incubation continued for another 24 hours.

Purification of Peptides. Peptides were separated by gel filtration on Sephadexes followed by ion exchange chromatography

on AG-1x2 using pyridine acetate and pyridine formate buffers (11) and by paper chromatography and/or paper electrophoresis (12). In the latter part of the work the ion exchange chromatography step was omitted. The cyanogen bromide fragments were subjected to countercurrent distribution (13).

Determination of Amino Acid Sequence. The amino acid sequence was determined by subtractive Edman degradation (12) or by a combined dansyl-Edman technique (14). The dansyl derivatives were identified by chromatography on polyamide sheets (15).

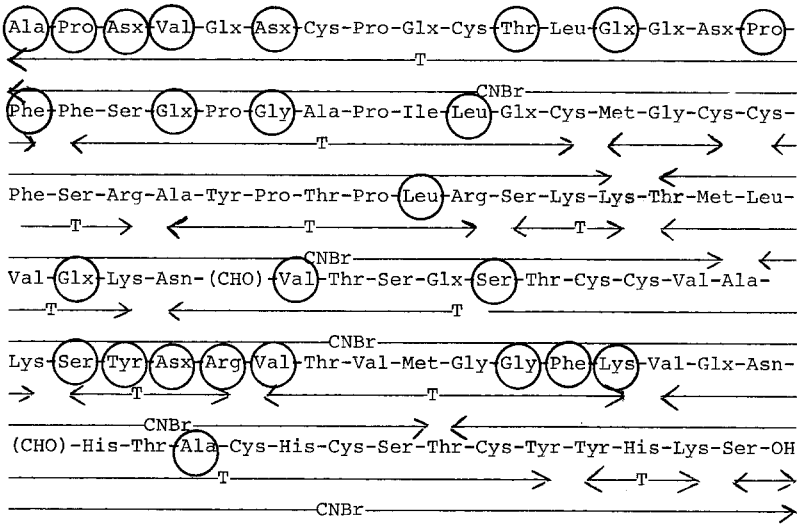
RESULTS AND DISCUSSION

Structure of the α-Subunit. The sequence of the α-subunit (Fig. 1) was derived from cyanogen bromide fragments and tryptic peptides. HCG-α has three methionine residues and cleavage with cyanogen bromide yielded four fragments (Fig. 1). The order of the cyanogen bromide fragments was established from the overlapping tryptic peptides as indicated in Fig. 1. The sequence of the N-terminal 35 residues of human LH-α starting with valine has recently been reported (16). It is identical to the N-terminal residues of HCG-α except that HCG-α has three additional residues at the N-terminus. This may reflect microheterogeneity such as has been observed at both the amino and carboxy termini of the α and β subunits of ovine LH (1,2). Bovine TSH-α and the α subunit of ovine LH each contain 96 residues and have identical amino acid sequences (1-4). HCG-α has about 25 amino acid substitutions relative to ovine LH-α, indicated by circles in Fig. 1, and has four fewer residues at the N-terminus.

The composition of the carbohydrate units in HCG-α is quite different from that of ovine or bovine LH-α or bovine TSH-α. The

Fig. 1

The Amino Acid Sequence of the α-Subunit
of Carboxamidomethylated HCG

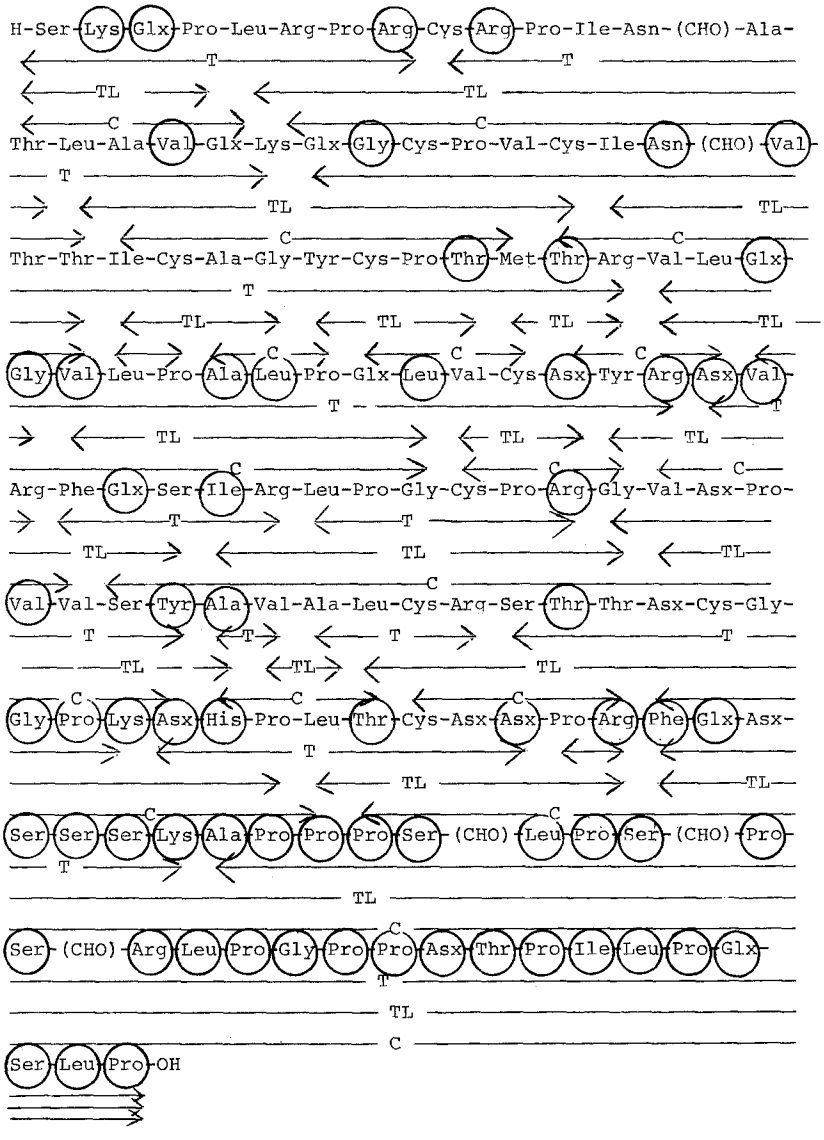


striking difference is the absence of N-acetylgalactosamine and presence of sialic acid and galactose in HCG-α. This suggests at least a partially different monosaccharide sequence. It is interesting that, despite these differences in amino acid sequence and carbohydrate composition, the human, ovine and bovine α subunits are biologically interchangeable.

The sequence of HCG-β (Fig. 2) was deduced from the tryptic, chymotryptic and thermolysin peptides. The order of the tryptic peptides was established by the overlapping chymotryptic and thermolysin peptides. The first 110 residues of HCG-β show considerable homology with ovine LH-β with about 40 amino acid substitutions, shown by circles in Fig. 2. With respect to ovine LH-β, there are two single residue deletions in HCG-β corresponding to LH-β positions 32 and 95 and a six residue deletion at LH-β positions 87-92. The C-terminal 28 residues of HCG-β are not present in LH-β. HCG-β has 139 residues and LH-β 119.

Fig. 2

The Amino Acid Sequence of the β-Subunit
of Carboxamidomethylated HCG



The carbohydrate portion of HCG-β is quite different from that of ovine or bovine LH-β. LH-β has only one carbohydrate unit whereas HCG-β has five. Furthermore, HCG-β has two different types of carbohydrate units, asparagine linked and serine linked. The

asparagine linked carbohydrate units are complex and bulky (7) and are located at positions 13 and 28. There are three short serine linked oligosaccharide chains (7) at positions 118, 121 and 123.

On the basis of studies on several glycoproteins (17) the invariant sequence around the asparagine has been found to be -Asn-X-Thr/Ser and this has been postulated as the recognition site for the enzyme which attaches N-acetylglucosamine to the amide group of asparagine in an N-glycosidic linkage. The sequence in the vicinity of the asparagine linked carbohydrate units in HCG-β is consistent with the above generalization.

Little information is available on the sequence of amino acids around serine linked carbohydrate units. There are three such units in HCG-β. The invariant sequence around all three carbohydrate linked serine residues in HCG-β is -Pro-Ser(CHO)-. Rabbit immunoglobulin IgG has been found to contain a single residue of N-acetylgalactosamine linked to threonine in the hinge region with a similar sequence of -Pro-Thr(CHO)- (18). Although the data are limited, it appears highly likely that a -Pro-Ser/Thr- sequence may be the recognition site for the N-acetylgalactosaminyl transferase.

The amino acid sequence reported here should facilitate the studies on human LH, which is available in only very limited quantity. Furthermore, with a knowledge of the monosaccharide and amino acid sequences of HCG, the work on structure-function relationships is expected to gain momentum.

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REFERENCES

1. Papkoff, H., Sairam, M. R. and Li, C. H., J. Am. Chem. Soc., 93, 1531 (1971).

2. Ward, D. N. and Liu, W. K., in Structure-Activity Relationships of Protein and Polypeptide Hormones, M. Margoulies and F. C. Greenwood (Editors), 1971, p. 80.

3. Maghuin-Rogister, G. C. and Hennen, G. P., Eur. J. Biochem., 21, 489 (1971).

4. Liao, T. -H. and Pierce, J. G., J. Biol. Chem., 246, 850 (1971).

5. Swaminathan, N. and Bahl, O. P., Biochem. Biophys. Res. Commun., 40, 422 (1971).

6. Pierce, J. G., Bahl, O. P., Cornell, J. S. and Swaminathan, N., J. Biol. Chem., 246, 2321 (1971).

7. Bahl, O. P., J. Biol. Chem., 244, 575 (1969).

8. Bahl, O. P., J. Biol. Chem., 244, 567 (1969).

9. Cole, R. D., in C. H. W. Hirs (Editor), Methods in Enzymology, Vol. XI, Academic Press, New York, 1967, p. 315.

10. McMenamy, R. H., Dintzis, H. M., and Watson, F., J. Biol. Chem., 246, 4744 (1971).

11. Schroeder, W. A., Jones, R. T., Cormick, J. and McCalla, K., Analyt. Chem., 34, 1570 (1962).

12. Bahl, O. P. and Smith, E. L., J. Biol. Chem., 240, 3585 (1965).

13. Liao, T. -H., Hennen, G., Howard, S. M., Shome, B., and Pierce, J. G., J. Biol. Chem., 244, 6458 (1969).

14. Gray, W. R., in C. H. W. Hirs (Editor), Methods in Enzymology, Vol. XI, Academic Press, New York, 1967, pp. 139, 469.

15. Woods, K. R., and Wang, K. T., Biochim. Biophys. Acta., 133, 369 (1967).

16. Inagami, T., Murakami, K., Puett, D., Hartree, A. S., and Nureddin, A., Biochem. J., 126, 441 (1972).

17. Spiro, R. G., Ann. Review of Biochem., 39, 599 (1970).

18. Smyth, D. S., Utsumi, S., Nature, 216, 332 (1967).