Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 2038–2042, April 1987
Medical Sciences

A receptor-binding region in human choriogonadotropin/lutropin β subunit

(peptide synthesis/steroidogenesis/immunoreactivity/conformation/amphipathic sequences)

HENRY T. KEUTMANN*†, M. CRISTINE CHARLESWORTH‡, KATHLEEN A. MASON*, TEOFILA OSTREA*, LESLIE JOHNSON*, AND ROBERT J. RYAN‡

*Endocrine Unit, Massachusetts General Hospital and Department of Medicine, Harvard Medical School, Boston, MA 02114; and ‡Department of Biochemistry and Molecular Biology, Mayo Medical School, Rochester, MN 55905

Communicated by Jerome Gross, November 24, 1986

ABSTRACT **Synthetic fragments have not been widely used thus far to evaluate structure–activity relations in the glycoprotein hormones. We prepared a series of peptides representing the intercysteine "loop" sequence (residues 38–57) in human choriogonadotropin (hCG) and lutropin (hLH) β subunits, anticipating that it might be oriented toward the surface and accessible to receptors. The peptides were characterized chemically and tested for bioactivity by binding to rat ovarian membrane receptor and stimulation of Leydig cell testosterone production. The hCGβ-(38–57) and hLHβ-(38–57) peptides inhibited binding of ^{125}I-labeled hCG half-maximally at 1.51×10^{-4} and 2.03×10^{-5} M, respectively, while other peptide hormones and fragments from elsewhere in the β subunit were inactive. Both peptides stimulated testosterone production, with half-maximal responses at 3.55×10^{-5} M (hCG) and 2.18×10^{-5} M (hLH). By radioimmunoassay with an antibody to thyroglobulin-conjugated hCGβ-(38–57) peptide, native hCG and β subunit were highly reactive, as were the reduced and carboxymethylated subunit and peptide. Helical-wheel projection predicted an amphipathic region in the N-terminal portion of the 38–57 sequence, and circular dichroic measurements showed an increase in ordered structure, especially α-helix, when the 38–57 peptides were transferred from an aqueous to a more lipophilic (90% trifluoroethanol) environment. These results indicate that the 38–57 region of β subunit is exposed on the surface and constitutes a component in the receptor-binding domain for hCG and hLH. A region of amphipathic-helical structure in the 38–57 sequence may promote hormone–receptor interactions in a manner proposed for several other peptide hormones.**

The glycoprotein hormones [lutropin (LH), choriogonadotropin (CG), follitropin (FSH), thyrotropin (TSH)] are structurally the most complex of the peptide hormones. The regions essential for receptor interaction and activation of target-organ responses have not yet been established. Much has been done to demonstrate the importance of certain amino acid residues in both subunits through chemical modifications (see refs. 1–3 for review) and the role of the long-chain asparagine-linked carbohydrate in receptor–effector coupling (reviewed in refs. 3–4). However, prospective sites for additional specific chemical modifications in the native subunit are limited.

In using synthetic peptides replicating continuous sequences, rather than modified individual residues in whole subunits, we have departed somewhat from the more traditional approach to structure–activity relations in these hormones. Sequence regions we consider likely to be available for receptor interaction are those between cysteine residues that might form "loops" toward the subunit surface, as projected in an early model by Ward and co-workers (5). Best known among these regions is the "determinant loop" segment in the β subunit, proposed to be important to receptor interaction and possibly hormonal specificity (6–8). This occurs between residues 93 and 100 in hCGβ and hLHβ (the β subunits of the human hormones).

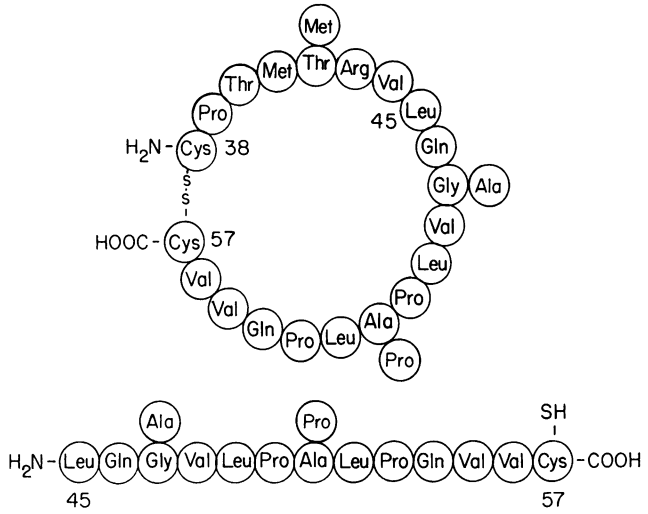

FIG. 1. Synthetic peptides from intercysteine "loop" region 38–57 of hCGβ. The residues in hLH that differ from those in hCG are shown by circles alongside.

The longest of the intercysteine regions in the β subunit is in the midregion, between residues 38 and 57 in hLH and hCG. Certain residues in this segment appear to be accessible to solvents, and changes in their charge result in loss or alteration of the activity of the whole hormone (9–11). To evaluate this region more directly, we have prepared a series of synthetic peptides comprising the 38–57 sequence from hCG and hLH β subunits (Fig. 1). Their chemical and biological properties, described in this report, are consistent with a significant role for this region in the process of receptor binding and receptor–effector coupling. We also present evidence for amphipathic-helical conformational properties that suggest a previously unrecognized contribution of secondary structure to glycoprotein hormone action.

METHODS

Peptide Synthesis. The following synthetic fragments were prepared: hCGβ-(38–57) and hCGβ-(45–57), hLHβ-(38–57)

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CG, choriogonadotropin; LH, luteinizing hormone (lutropin); CRF, corticotropin-releasing factor (corticoliberin); FSH, follicle-stimulating hormone (follitropin); h-, human; Cm, carboxymethyl; F_3EtOH, trifluoroethanol.

†To whom reprint requests should be addressed.

and hLHβ-(45–57), and the analog [Asp^{45,48}]hLHβ-(38–57). Synthesis was carried out by the solid-phase procedure (12, 13) using benzhydrylamine resin (Beckman) and *t*Boc amino acids purchased from Peninsula Laboratories (Belmont, CA) or Bachem Fine Chemicals (Torrance, CA). Peptides were cleaved from the resin by using hydrogen fluoride (0°C, 1 hr) with 10% (vol/vol) anisole and 3% (vol/vol) dimethyl sulfide. The 38–57 peptides were incubated at pH 8.5 (20°C, 4 hr, 100 μg/ml) to effect closure of the disulfide bond.

The peptides were purified by gel filtration on Sephadex G-25 (Pharmacia) with 1.0 M acetic acid eluant, followed by ion-exchange chromatography on carboxymethylcellulose (Whatman CM-52) with elution by a linear gradient of ammonium acetate (pH 4.5) from 0.1 to 2.0 M. The [Asp^{45,48}]-hLHβ-(38–57) analog was purified on diethylaminoethylcellulose (Whatman DE-52) by using a similar gradient of ammonium bicarbonate (pH 8.2). Peptide homogeneity was determined by amino acid analysis after acid hydrolysis (6 M HCl, 110°C, 24 hr under reduced pressure) or total enzymatic digestion with papain and aminopeptidase M, and by sequencing and "preview" analysis (14) in the Beckman System 890 Sequencer. Content of free sulfhydryl groups was evaluated by Ellman's reagent [5,5'-dithiobis(2-nitrobenzoic acid)] (15). Peptides and subunits were reduced and carboxymethylated with dithiothreitol/guanidine hydrochloride followed by iodoacetic acid (7). These modified peptides are indicated by the prefix Cm-. Native hCG (preparation K-881) and β subunit were purified as described previously (7).

Circular Dichroic Measurements. Circular dichroism was measured in either 5 mM sodium phosphate buffer (pH 7.5) or 90% (vol/vol) trifluoroethanol (F_3EtOH)/5 mM sodium phosphate (pH 7.5) at 100 μg/ml in the Jasco model J-500A spectropolarimeter with a cell path length of 1 mm. A minimum of eight scans per sample were performed over the wavelength range 250–185 nm at a time constant setting of 8. Mean residue content of ordered structure was computed by the method of Chen *et al.* (16) on the basis of spectra for proteins of known ordered structure.

Biological Assays. Binding of peptides to rat ovarian membrane receptors was measured as described by Lee and Ryan (17). Radioiodinated hCG ligand (^{125}I-hCG) was prepared as described previously (17). Assays for testosterone production were based on the procedure of Dufau *et al.* (18), using Leydig cells from 200- to 250-g rats, incubated 3 hr at 37°C in 0.5 ml of medium 199/0.1% ovalbumin/25 mM Hepes buffer, pH 7.2 (GIBCO). Immunoassays for testosterone were done as described (18).

Radioimmunoassays. Immunoreactivity of peptides and hCG was measured in a double-antibody assay (19) using a polyclonal antibody prepared against thyroglobulin-conjugated Ala-Pro$_6$-hCGβ-(38–57) (20). hCGβ, labeled as noted above, was used as radioligand. Potencies for all assays were determined as the molar concentration effecting half-maximal inhibition of ligand binding (8). Concentrations of added peptides were fixed by amino acid analysis of aliquots from the primary dilutions.

RESULTS

Synthetic Peptides. The hCGβ-(38–57) product from HF cleavage eluted in Sephadex G-25 chromatography at a K_d of 0.30, a position identical to the monomeric reduced and carboxymethylated peptide. Chromatography on CM-52 yielded a principal peptide component at conductivity 8–9 mS, identified as 38–57 by composition and sequence analysis. Additional purification steps, involving either rechromatography over CM-52 with a different gradient or reversed-phase HPLC, yielded products that did not differ significantly from the initial CM-52 product by sequence analysis or receptor binding. The shorter C-terminal 45–57 peptide was obtained in monomeric homogeneous form directly from Sephadex G-25 gel filtration. Similar profiles and results were obtained in purification of the peptides from hLH, including the analog [Asp^{45,48}]hLHβ-(38–57) from DE-52 chromatography. Theoretical recoveries of all amino acids were obtained by compositional analysis of the purified products, and sequence analyses revealed cumulative "preview" levels of less than 10% for 10 cycles, an acceptable level as noted by us previously (14).

Circular Dichroic Measurements. Circular dichroic plots of hCGβ-(38–57) in the far-ultraviolet region (Fig. 2) indicated a shift in the profile with 90% F_3EtOH, with development of a region of negative ellipticity in the range 220–230 nm and a positive shift in the region below 200 nm. Similar, but less marked, changes were observed in the profiles for native hCGβ. The data in Table 1 derived from the circular dichroic profiles indicate that in F_3 EtOH both hCGβ and hLHβ-(38–57) underwent an increase in ordered structure, including content of α helix. Comparable profiles were observed with the reduced and carboxymethylated 38–57 peptides.

Membrane Receptor Assays. Inhibition of labeled hCG binding to ovarian receptors by the 38–57 peptides took place with response curves parallel to the curve for the hCG standard, as shown in Fig. 3. Binding affinity of the hLHβ-(38–57) peptide was 8-fold higher than that of the hCG peptide. The shorter 45–57 peptide from hCG retained binding activity, but hLHβ-(45–57) was nearly devoid of

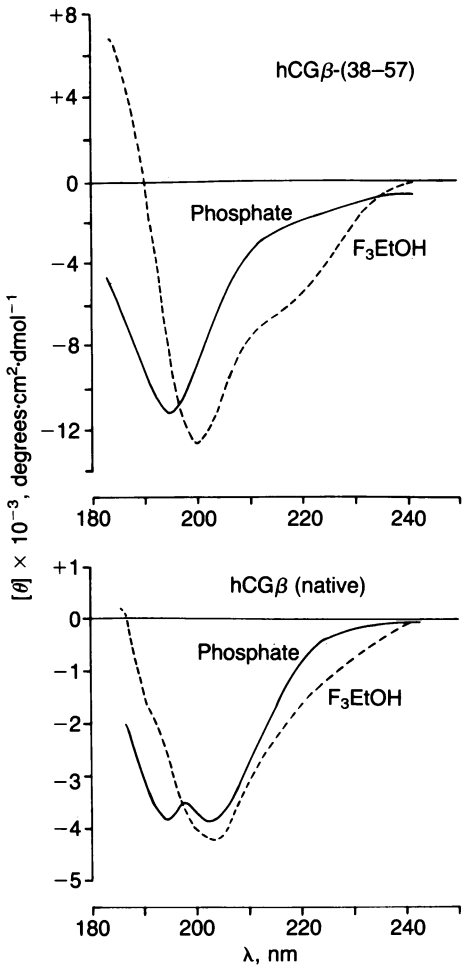

FIG. 2. Circular dichroic profiles of synthetic hCGβ-(38–57) and native hCGβ in aqueous (phosphate buffer) and organic (90% F_3EtOH) medium. [θ], Mean residue ellipticity. Analysis of ordered structure appears in Table 1.

Table 1. Circular dichroism of subunits and peptides

Preparation	Phosphate		90% F_3EtOH	
	% helix	% β-structure	% helix	% β-structure
hCGβ-(38–57)	9	25	23	16
hLHβ-(38–57)	11	25	25	15
Cm-hCGβ-(38–57)	10	22	24	14
Cm-hLHβ-(38–57)	11	23	25	14
hCGβ (native)	8	36	15	28
hCGβ (deglycosylated)*	8	36	15	28

Data are percent content of ordered structure in 5 mM sodium phosphate, pH 7.4, or 90% (vol/vol) F_3EtOH/5 mM phosphate, pH 7.4.
*Deglycosylated with HF (see ref. 38).

activity. No binding was observed with the hLHβ-(38–57) analog in which the hydrophobic residues at positions 45 and 48 had been replaced by aspartic acid. Table 2 compares the binding constants for these peptides with those of fragments from other regions of the β subunit and for two peptides unrelated to LH/hCG. Only the fragment 93–101 replicating the determinant loop region of hCGβ showed binding activity comparable to that of the 38–57 peptides.

Steroidogenic Assays. In rat Leydig cells (Table 3), both 38–57 peptides showed weak, but complete, agonism as measured by stimulation of testosterone production over a concentration range similar to that observed for binding of the peptide from hLH. A control peptide from parathyroid hormone was inactive, as was the hCGβ-(93–101) peptide noted above.

Radioimmunoassays. Fig. 4 shows the displacement curves from an immunoassay with the polyclonal antibody against thyroglobulin-hCGβ-(38–57) conjugate. Immunopotency of the 38–57 peptide was 3.2×10^{-11} M. Native hCGβ was 2.4-fold more reactive, while the relative potencies of Cm-hCGβ-(38–57) and Cm-hCGβ were 0.6 and 0.8 compared with the unmodified peptide. In a separate assay, relative potency of whole native hCG was 0.75. The hLH peptide and subunit were markedly less reactive (relative potencies 0.03 and 0.13), and neither of the shortened 45–57 peptides effected significant displacement.

DISCUSSION

The receptor binding and agonist activity of the synthetic 38–57 peptides from hCGβ and hLHβ provide direct evidence that a continuous sequence from a glycoprotein hormone is capable of interacting with a target cell. The specificity of the process is affirmed by the absence of binding by other hormonal peptides, by subunit regions known not to be essential for activity, and by the 38–57 analog with two hydrophobic residues replaced by aspartic acid. Binding by a single sequence from β subunit implies that the subunit itself should do so. Although such binding was the subject of earlier controversy, there is now substantial evidence for inherent binding and steroidogenic activity in the separate subunits (21–23).

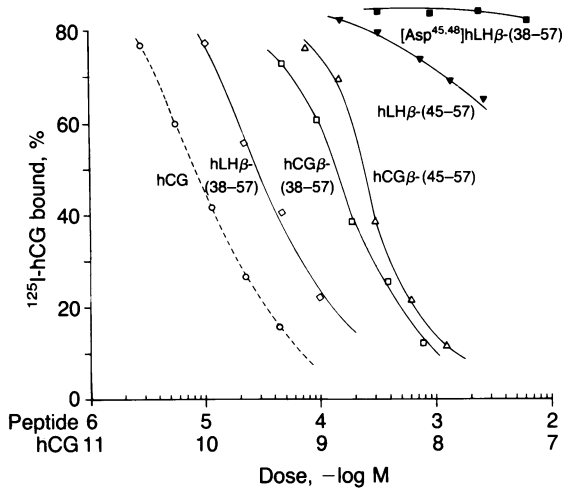

FIG. 3. Inhibition curves for hCGβ and hLHβ fragments and analogs in ovarian membrane receptor assay, measured against standard hCG preparation K-881. Potencies are summarized in Table 2.

Table 2. Ovarian receptor assay of β subunit peptides

Peptide	No. of assays	Binding constant,* M
Native hCG	13	1.03×10^{-10}
β-(38–57) peptides		
hCGβ-(38–57)	10	1.51×10^{-4}
hLHβ-(38–57)	10	2.03×10^{-5}
hCGβ-(45–57)	4	4.21×10^{-4}
hLHβ-(45–57)	4	2.38×10^{-2}
[Asp^{45,48}]hLHβ-(38–57)	2	No inhibition
Other β peptides		
hCGβ-(100–110)	2	No inhibition
hCGβ-(111–118)	2	No inhibition
hCGβ-(115–140)	1	No inhibition
hCGβ-(93–101)	8	1.66×10^{-4}
Nongonadotropin peptides		
AcChoR-(185–199)	2	No inhibition
CRF-(1–41)	2	No inhibition

AcChoR, acetylcholine receptor; CRF, corticotropin-releasing factor.
*Concentration effecting half-maximal inhibition of labeled hCG binding. "No inhibition" indicates there was no inhibition of tracer binding at 10^{-2} M or higher.

The complete domain for full expression of biological activity clearly includes multiple sites, some probably "topographical" in nature, involving both subunits (1–3). The lower binding affinities of the 38–57 fragments (binding constants 10^{-4} to 10^{-5} M) compared to native hCG (10^{-10} to 10^{-11} M) are thus not unexpected. Specific binding of this order has been used to define binding domains and design successful inhibitors for parathyroid hormone (13) and oxytocin (24), among other peptides.

The information available from chemical modifications to the whole subunit is limited, but it lends support to the importance of the 38–57 region. Porcine and bovine LH contain methionine instead of leucine at position 52. This residue could be modified through oxidation to the sulfoxide (10) or carboxymethylation (9), resulting in unimpaired subunit association but markedly diminished receptor binding after recombination with native α subunit. Thus, although substitution of another hydrophobic residue is tolerated, replacement by a negative charge is not. It has also been suggested that methionine-41 in bovine LH may be "exposed" and important to receptor binding (1), and the

Table 3. Leydig cell bioassay for testosterone production

Compound	EC_{50},* M	Maximum testosterone produced,† pmol
hCG	2.81×10^{-12}	7.05
hCGβ-(38–57)	3.55×10^{-5}	6.65
hLHβ-(38–57)	2.18×10^{-5}	9.02
hCGβ-(93–101)	No activity	—
hPTH-(53–84)	No activity	—

hPTH, human parathyroid hormone.
*Concentration effecting half-maximal testosterone production.
†Based on 10^7 viable cells.

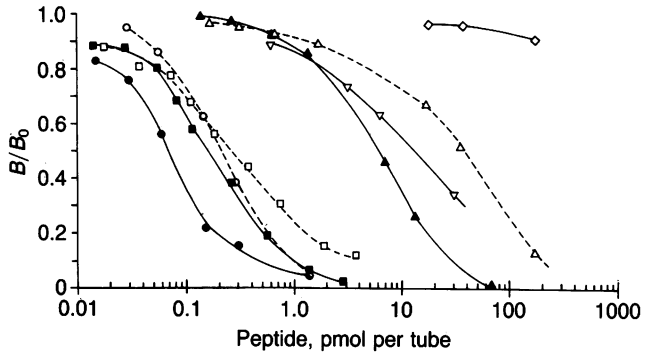

FIG. 4. Displacement curves for peptides in a radioimmunoassay based on antiserum to thyroglobulin-conjugated hCGβ-(38–57). *B* and B_0, ^{125}I-hCGβ bound in the presence and absence of peptide, respectively. ■, hCGβ-(38–57); ●, hCGβ; □, Cm-hCGβ-(38–57); ○, Cm-hCGβ; ▲, hLHβ-(38–57); ▽, hLHβ; △, Cm-hLHβ-(38–57); ◇, hCGβ-(45–57).

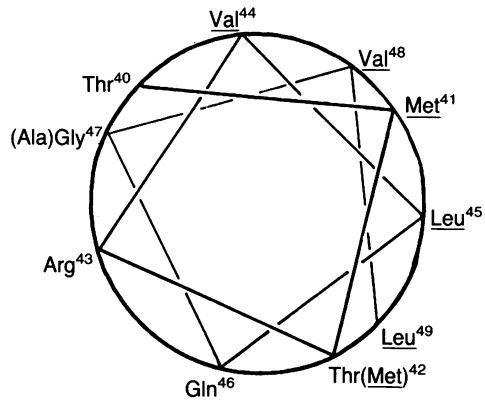

FIG. 5. Helical-wheel projection of 40–49 sequence from hCGβ. Residues forming a hydrophobic "face" are underlined. Residues in hLHβ are shown in parentheses where they differ from those in hCGβ.

influence of residue 42 on hormonal specificity is described by Goverman and Pierce (11). A recent report (22) describes (Thr-Arg-Asp-Leu) representing residues 34–37 in human FSHβ, which corresponds to the sequence 40–43 in hCGβ and hLHβ.

Our radioimmunoassay results support the contention that the 38–57 loop sequence is exposed to the surface in native hCG. An antibody against the hCGβ-(38–57) peptide recognized both the native hormone and the β subunit. Since the antibody did not bind the shorter 45–57 fragment, the epitope may be somewhat different from the receptor-binding site. Stevens *et al.* (20) have described a monoclonal antibody to hCGβ that reacts specifically with a 40–52 peptide from this region. The markedly lower reactivity of hLHβ and hLHβ-(38–57) peptide with the antibody used in the current study is consistent with earlier findings (20) showing specificity for hCGβ radioligand. These results suggest an approach to preparation of hCG-specific antisera through further selective modifications of the peptide.

With the possible exception of FSH, secondary structure has not been considered to be a prominent feature of native gonadotropin molecules—estimates of helix ranging from 0% to 11%, with β structure up to 25–30% (25). However, one study (26) noted that helicogenic solvents could enhance helix formation in LH subunits. This suggested that distinctive secondary-structural features might be induced in approximation to hydrophobic surfaces such as a cell membrane. Helical sequences with amphipathic properties were described originally for apolipoproteins in conjunction with phospholipid binding (27) and have been found in several hormones and bioactive peptides, including mellitin, endorphin, calcitonin, and CRF (28–31).

Fig. 5 shows a two-dimensional vertical-axis projection (32) of the sequence between residues 40 and 49 of hCG and hLH β subunits. Alignment of multiple hydrophobic residues along one face, with more polar residues opposite, indicates the potential for formation of an amphipathic helix. The circular dichroic measurements lend support to this prediction. In aqueous solution, the ordered structure of hCGβ-(38–57) appears to be primarily β-sheet, consistent with predictions by the Chou–Fasman method (33). In the lipophilic solvent F_3EtOH there is an increase in ordered structure with a shift toward helical conformation. The 40–49 segment would represent about three turns, close to the average length predicted for helical regions within a number of proteins (16), including those with looped sequences such as insulin. The hydrophobic face may be critical to the activity of the 38–57 fragment, as shown by the complete loss of binding activity when the surface is disrupted by substitution of charged aspartic acid residues at positions 45 and 48. In the sequence from hLH, substitution of methionine for threonine at position 42 appears to extend the hydrophobic face toward the N terminus, and alanine provides a strong helix former at position 47. These may contribute to the increased binding affinity compared to the hCG peptide. On the other hand, the highly amphipathic but otherwise unrelated hormone CRF is devoid of binding activity (Table 2).

In peptide hormones, amphipathic sequences have been postulated to promote partitioning between an aqueous and a membrane microenvironment to facilitate access to receptors or induce additional conformational changes that ultimately favor receptor binding (28, 34). The general mechanisms proposed by Schwyzer (34) and by Ross and Subramanian (35) comprise an initial low-affinity hydrophobic interaction followed by a more stable association with the receptor based on charge and hydrophilicity.

The midportion of the 38–57 region includes proline residues, predicted to break the helical sequence and induce one or more bends in the peptide chain (33). The higher binding affinity of the peptide from hLHβ may be promoted by an extra proline at position 51, as well as by the differences at positions 42 and 47. This region could represent a structural basis for previously observed differences between LH and hCG in binding characteristics and efficacy of postreceptor activation (36, 37).

Although the 38–57 peptides were prepared as a disulfide loop, the constraint at residues 38 and 57 may not be a rigid one. There is as yet no confirmation that these cysteines are linked to each other in the native subunit. Indeed, disulfide linkages may not be necessary to maintain the overall shape and conformation of the loop. Ordered structure of the 38–57 peptides is unchanged after reduction and carboxymethylation (Table 1) and Cm-hCGβ subunit and peptide remain immunoreactive (Fig. 4). By circular dichroic analysis we have observed an increase in ordered structure of glycoprotein subunits upon reduction (M.C.C., H.T.K., and R.J.R., unpublished data). Hence, although the disulfide bridges may actually restrain secondary structure while conferring the tertiary structure unique to these glycoproteins, the 38–57 region appears able to retain significant ordered structure even in the disulfide-linked native subunit.

The ability of the 38–57 peptides to promote testosterone production, in concentration ranges similar to those required for binding, suggests that this sequence is also part of a determinant for receptor–effector coupling. In one regard this is surprising, since carbohydrate appears to be a requirement for the coupling process (3, 4). However, recombinants involving deglycosylated β subunit may show a steroidogenic or adenylate cyclase response at high doses (38, 39). Conse-

quently, potential applications of the 38–57 peptides as competitive inhibitors will probably require preparation of analogs with lower inherent agonism.

The disulfide-loop peptide 93–101 from hCGβ bound to receptors with an affinity similar to 38–57 (Table 2). This fragment includes the determinant loop sequence found to be important in hormone–receptor interaction by disulfide reduction (6) and enzymatic modification (8). Structurally different from 38–57, the 93–101 peptide is highly hydrophilic and lacks significant ordered structure; significantly, it also lacks agonist activity by testosterone assay. In preliminary experiments, the two peptides appear to be additive in binding affinity. Charlesworth *et al.* have also evaluated a series of linear peptides from α subunit and found at least one with hCG receptor-binding activity.[§] While definition of the three-dimensional structure of the whole molecule awaits crystallographic analysis, efforts to show synergism through coupling of one or more of these active peptides to the complementary subunit, and to oligosaccharides and glycopeptides, represent a next step in analysis of the site or sites ultimately responsible for binding and activation.

[§]Charlesworth, M. C., McCormick, D. J. & Ryan, R. J. (1986) Proceedings of the 68th Annual Meeting of the U.S. Endocrine Society, Anaheim, CA, June 25–27, 1986, abstr. 614.

The authors gratefully acknowledge the expert technical assistance of Beth Turner, Kathleen Kitzmann, and Ken Peters, and thank Michelle Chilante for preparing the manuscript. We thank Dr. Vernon C. Stevens (Ohio State University) for generously providing us with the hCGβ-(38–57) antiserum and Dr. Franklyn Prendergast (Mayo Medical School) for helpful discussions and assistance in computing ordered structure. This work was supported by Grants HD 12851 and HD 09140 from the National Institutes of Health.

1. Ward, D. N. (1978) in *Structure and Function of the Gonadotropins*, ed. McKerns, K. W. (Plenum, New York), pp. 31–46.
2. Pierce, J. G. & Parsons, T. F. (1981) *Annu. Rev. Biochem.* **50**, 465–495.
3. Gordon, W. L. & Ward, D. N. (1985) in *Luteinizing Hormone Receptors and Action*, ed. Ascoli, M. (CRC, Paris), pp. 173–198.
4. Sairam, M. R. (1983) in *Hormonal Proteins and Peptides*, ed. Li, C. H. (Academic, New York), Vol. 11, pp. 2–32.
5. Ward, D. N., Berchert, L. E., Liu, W.-K., Nahm, H. S., Hsia, J., Lambert, W. M. & Jones, N. S. (1973) *Recent Prog. Horm. Res.* **29**, 533–559.
6. Ward, D. N. & Moore, W. T., Jr. (1979) in *Animal Models for Research on Contraception and Fertility*, ed. Alexander, N. J. (Harper & Row, New York), pp. 151–163.
7. Keutmann, H. T., Ratanabanangkoon, K., Pierce, M. W., Kitzmann, K. & Ryan, R. J. (1983) *J. Biol. Chem.* **258**, 14521–14526.
8. Ratanabanangkoon, K., Keutmann, H. T., Kitzmann, K. & Ryan, R. J. (1983) *J. Biol. Chem.* **258**, 14527–14531.
9. Cheng, K.-W. (1976) *Biochem. J.* **159**, 79–87.
10. De la Llosa, P., El Abed, A. & Roy, M. (1980) *Can. J. Biochem.* **58**, 745–748.
11. Goverman, J. M. & Pierce, J. G. (1981) *J. Biol. Chem.* **256**, 9431–9435.
12. Barany, G. & Merrifield, R. B. (1979) in *The Peptides*, ed. Gross, E. & Meienhofer, J. (Academic, New York), Vol. 2, pp. 1–284.
13. Rosenblatt, M., Segre, G. V., Tyler, G. A., Shepard, G. L., Nussbaum, S. R. & Potts, J. T., Jr. (1980) *Endocrinology* **107**, 545–550.
14. Tregear, G. W., van Reitschoten, J., Sauer, R., Niall, H. D., Keutmann, H. T. & Potts, J. T., Jr. (1977) *Biochemistry* **16**, 2817–2823.
15. Habeeb, A. F. S. A. (1971) *Methods Enzymol.* **25**, 457–464.
16. Chen, Y.-H., Yang, J. T. & Chau, K. H. (1974) *Biochemistry* **13**, 3350–3359.
17. Lee, C. Y. & Ryan, R. J. (1973) *Biochemistry* **12**, 4609–4619.
18. Dufau, M. D., Pock, R., Neubauer, A. & Catt, K. J. (1976) *J. Clin. Endocrinol. Metab.* **42**, 958–969.
19. Faiman, C. & Ryan, R. J. (1967) *J. Clin. Endocrinol. Metab.* **27**, 444–447.
20. Stevens, V. C., Chou, W.-S., Powell, J. E., Lee, A. C. & Smoot, J. (1986) *Immunol. Lett.* **12**, 11–18.
21. Moudgal, N. R. & Li, C. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2500–2503.
22. Sluss, P. M., Krystek, S. R., Jr., Anderson, T. T., Melsen, B. E., Huston, J. S., Ridge, R. & Reichert, L. E., Jr. (1986) *Biochemistry* **25**, 2644–2649.
23. Grasso, P. & Crisp, T. M. (1985) *Endocrinology* **116**, 319–327.
24. Hruby, V. J. & Mosberg, H. I. (1982) *Peptides* **3**, 329–336.
25. Garnier, J. (1978) in *Structure and Function of the Gonadotropins*, ed. McKerns, K. W. (Plenum, New York), pp. 381–414.
26. Hilgenfeldt, H. H., Merz, W. E. & Brossmer, S. F. (1974) *Hoppe-Seylers Z. Physiol. Chem.* **355**, 1051–1057.
27. Segrest, J. P., Jackson, R. L., Morrisett, J. D. & Gotto, A. M. (1974) *FEBS Lett.* **38**, 247–253.
28. Kaiser, E. T. & Kezdy, F. J. (1984) *Science* **223**, 249–255.
29. deGrado, W. F., Musso, G. F., Lieber, M., Kaiser, E. T. & Kezdy, F. J. (1982) *Biophys. J.* **37**, 329–338.
30. Hammonds, R. G., Jr., Hammonds, A. S., Ling, N. & Puett, D. (1982) *J. Biol. Chem.* **257**, 2990–2995.
31. Pallai, P. V., Mabilia, M., Goodman, M., Vale, W. & Rivier, J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6770–6774.
32. Schiffer, M. & Edmundson, A. B. (1967) *Biophys. J.* **7**, 121–135.
33. Chou, P. Y. & Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47**, 251–276.
34. Schwyzer, R. (1985) in *Peptides: Structure and Function*, Proc. Ninth Am. Peptide Symp., eds. Deber, C. M., Hruby, V. J. & Kopple, K. J. (Pierce, Rockford, IL), pp. 3–12.
35. Ross, P. D. & Subramanian, S. (1981) *Biochemistry* **20**, 3096–3102.
36. Buettner, K. & Ascoli, M. (1984) *J. Biol. Chem.* **259**, 15078–15084.
37. Mock, E. J. & Niswender, G. D. (1983) *Endocrinology* **113**, 259–264.
38. Keutmann, H. T., McIlroy, P. J., Bergert, E. R. & Ryan, R. J. (1983) *Biochemistry* **22**, 3067–3072.
39. Kalyan, N. K. & Bahl, O. P. (1983) *J. Biol. Chem.* **258**, 67–74.