Primary and Secondary Structural Determinants in the Receptor Binding Sequence β-(38–57) from Human Luteinizing Hormone†

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ABSTRACT: The intercysteine "loop" sequence 38–57 in the β subunit has been shown to be a determinant for expression of biological activity in human lutropin (hLH) and choriogonadotropin (hCG) [Keutmann, H. T., Charlesworth, M. C., Mason, K. A., Ostrea, T., Johnson, L., & Ryan, R. J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 2038]. Together with other sequences, the 38–57 region may contribute to a multicomponent receptor binding domain in hLH/hCG. Because the structural features influencing activity in this important region are not easy to evaluate in the full-length subunit, we have used analogues of hLHβ-(38–57) prepared by solid-phase synthesis. The peptides were tested for inhibition of ^{125}I-labeled hCG binding to rat ovarian membrane receptors. Secondary structure was analyzed by circular dichroism (CD) and by reactivity with antibodies to the native 38–57 peptide. An analogue lacking the 38–57 disulfide linkage retained 20% receptor binding and full immunoreactivity. "Far"-ultraviolet CD profiles were essentially identical with those of the disulfide-intact peptide; a transition from 10% to 30% α-helix in 90% trifluoroethanol was characteristic of both. The peptide thus appears not to require the disulfide bridge to retain a looped conformation with amphipathic secondary structure. An essential positive charge at position 43 was shown by complete loss of activity upon substitution of Asp or Ala for the Arg found in all known species of LH. Other analogues showed a requirement for a neutral residue at position 47, also highly conserved. One or more of the prolines at 50, 51, and 53 may confer a bend to help maintain the loop conformation. Substitution of alanine for individual prolines was tolerated, with binding constants comparable to that of the native peptide [(2.5–3.5) × 10^{-5} M]. The double substitution of Ala for Pro at 50 and 51 altered the binding response and changed the physical characteristics toward a more open loop with a marked tendency toward dimerization. These results indicate that the 38–57 sequence is a relatively rigid and structurally autonomous region, not merely a series of residues constrained passively into a loop by a disulfide linkage. It includes segments of ordered structure, probably including both amphipathic helical and turn sequences. Evidence from studies of other hormones suggests that this region may be important to binding and specificity in the glycoprotein hormones as a group.

It is apparent from structure–activity studies of the glycoprotein hormones (lutropin, LH; choriogonadotropin, CG; follitropin, FSH; thyrotropin, TSH)[1] that contact with target-organ receptors involves multiple sites on both α and β subunits [reviewed by Parsons and Pierce (1979), Ward (1978), Gordon and Ward (1985), and Ryan et al. (1987)]. Assays using synthetic fragments have revealed at least four sites in human LH and CG that bind to ovarian membrane receptors at concentrations of 10^{-4}–10^{-6} M: α-(25–41), α-(76–92), β-(38–57), and β-(93–101) (Charlesworth et al., 1987; Keutmann et al., 1987). It may be postulated that these constitute a topographical site composed of the separate sequences from both subunits, assembled to form the receptor-binding domain of the native molecule which has an affinity of 10^{-11} M.

The intercysteine "loop" sequence 38–57 in hLH and hCG β subunit is accessible to antibody probes (Stevens et al., 1986; Keutmann et al., 1987) including those blocked by formation of hormone–receptor complex (Moyle et al., 1988). This region also contains determinants for receptor–effector coupling, since synthetic 38–57 fragment stimulates testosterone production in rat Leydig cells (Keutmann et al., 1987). The sequence also may contain elements of secondary structure that are more prominent than generally attributed to this group of hormones. We have found evidence for a region of amphipathic helical conformation that, together with one or more bends at proline residues, could mediate receptor binding as do amphipathic sequences from other peptide hormones (Kaiser & Kezdy, 1984). Significantly, sequences from the counterpart region 33–53 in hFSHβ have also been found to react with antibodies to whole FSH and to bind testicular membrane FSH receptors (Sluss et al., 1986; Anderson et al., 1987; Schneyer et al., 1988).

Although evidence for its importance to hormone action is compelling, little information is available concerning the importance of specific residues in this region of any of the glycoprotein hormones. In the 38–57 sequence of hLHB and hCGB, reactive side chains are sparse, and those capable of modification are usually duplicated elsewhere in the subunit. In this paper we describe the evaluation of a series of synthetic 38–57 analogues that document an ordered and stable three-dimensional structure with several sequence positions

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[1] Abbreviations: hCG, human chorionic gonadotropin (choriogonadotropin); LH, luteinizing hormone (lutropin); FSH, follicle-stimulating hormone (follitropin); TSH, thyroid-stimulating hormone (thyrotropin); CD, circular dichroism; TFE, trifluoroethanol.

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SYNTHETIC PEPTIDES:

	38							45					50							57
hLHB (native):	Cys	Pro	Thr	Met	Met	Arg	Val	Leu	Gln	Ala	Val	Leu	Pro	Pro	Leu	Pro	Gln	Val	Val	Cys
38A:	Ala	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
43A:	–	–	–	–	–	Ala	–	–	–	–	–	–	–	–	–	–	–	–	–	–
43D:	–	–	–	–	–	Asp	–	–	–	–	–	–	–	–	–	–	–	–	–	–
45D, 48D:	–	–	–	–	–	–	–	Asp	–	–	Asp	–	–	–	–	–	–	–	–	–
47E:	–	–	–	–	–	–	–	–	–	Glu	–	–	–	–	–	–	–	–	–	–
47V:	–	–	–	–	–	–	–	–	–	Val	–	–	–	–	–	–	–	–	–	–
50A:	–	–	–	–	–	–	–	–	–	–	–	–	Ala	–	–	–	–	–	–	–
50A, 51A:	–	–	–	–	–	–	–	–	–	–	–	–	Ala	Ala	–	–	–	–	–	–
53A:	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Ala	–	–	–	–
hCGB (native):	–	–	–	–	Thr	–	–	–	–	Gly	–	–	–	Ala	–	–	–	–	–	–

NATURAL LH SEQUENCES:

OVINE, BOVINE:	–	–	Ser	–	Lys	–	–	–	Pro	Val	Ile	–	–	–	Met	–	–	Arg	–	–
PORCINE;	–	–	Ser	–	Arg	–	–	–	Pro	–	Ala	–	–	–	Val	–	–	Pro	–	–
RAT, RABBIT:	–	–	Ser	–	Val	–	–	–	Pro	–	Ala	–	–	–	Val	–	–	Pro	–	–
WHALE:	–	–	Ser	–	Val	–	–	–	Pro	–	Ala	–	–	–	Val	–	–	Pro	–	–
EQUINE:	–	–	Ser	–	Val	–	–	Thr	Pro	–	Ala	–	–	Ala	Ile	–	–	Pro	–	–

FIGURE 1: Linear amino acid sequences of synthetic hLHβ-(38–57) fragment and analogues and the corresponding structures from other known species of LH. Dashes indicate residues identical with those of the native human sequence at top. All synthetic fragments except 38A are disulfide-linked between Cys-38 and Cys-57.

critical for binding to hLH/hCG receptors.

Materials and Methods

Peptide Synthesis. The peptides and analogues of hLHβ-(38–57) prepared are shown in Figure 1. Also synthesized were the N-terminal fragments 38–48, and its Ala-38 analogue, and 41–45. hCGβ-(38–57) and the C-terminal fragment 45–57 were prepared as previously (Keutmann et al., 1987). Peptides were synthesized by the solid-phase procedure (Barany & Merrifield, 1979; Stewart & Young, 1984) on the Applied Biosystems Model 430A synthesizer (Applied Biosystems, Inc., Foster City, CA) with methylbenzhydrylamine resin as solid support. Peptides typically commenced with 0.5 mmol of initial coupled residue; the peptide–resin was divided as needed during synthesis to provide products with alternative residues at a given position (e.g., 43A and 43D, 47V and 47E; Figure 1). For full-length 38–57 analogues, single symmetric anhydride couplings were programmed for residues 57–44, and double couplings for residues 43–38. Quantitative ninhydrin tests were used for monitoring coupling efficiency throughout. *tert*-BOC-protected amino acids were purchased from Applied Biosystems and from Bachem Fine Chemicals (Torrance, CA). Side-chain-protecting groups included 4-methylbenzyl (Cys), *O*-benzyl (Asp, Glu, Thr), tosyl (Arg), and *O*-(chlorobenzyl) (Tyr).

Completed peptides were cleaved from the solid support with anhydrous HF containing 10% anisole and 5% dimethyl sulfide, reacted 1 h at 0 °C in a Kel-F distillation apparatus (Peptide Institute, Osaka, Japan). After evaporation of reagents, the resin was treated with diethyl ether to remove residual scavenger, and the peptide was extracted into 10% acetic acid, diluted with water to a volume of 150–200 mL, and lyophilized. The peptide was redissolved in 0.01 N acetic acid (100 μg/mL), the pH was adjusted to pH 6.5 with ammonium hydroxide, and the solution was stirred 4–6 h at 20 °C to close the disulfide bond.

The peptides were purified by gel filtration on Sephadex G-25 (Pharmacia) eluted with 1.0 M acetic acid, followed by ion-exchange chromatography on (carboxymethyl)cellulose (Whatman CM-52) eluted with a linear gradient of ammonium acetate, pH 4.5, from 0.1 to 2.0 M. For derivatives with anionic substitutions (Glu, Asp) or those lacking arginine (e.g., 43A, 43D; Figure 1), a shallower gradient of 0.1–1.0 M ammonium acetate was used. For these preparations a final step of reversed-phase HPLC was added, using a Bio-Rad RP-300 Hi-Pore C_{18} semipreparative column eluted with a gradient of acetonitrile (typically 10%–60%) in 0.1% trifluoroacetic acid. Homogeneity of the peptides was confirmed by amino acid analysis on the Beckman Model 6300 instrument after acid hydrolysis (6 M HCl, 24 h, 110 °C, in vacuo) and by sequencing and "preview" analysis (Tregear et al., 1977) using the Beckman System 890 sequencer with quantitation of phenylthiohydantoin derivatives by HPLC.

Circular Dichroic Measurements. Circular dichroism was measured at a peptide concentration of 100 μg/mL in either 5 mM sodium phosphate buffer (pH 7.5) or the helicogenic solvent 90% trifluoroethanol (TFE)/5 mM sodium phosphate, pH 7.5, on the Jasco Model J-500A spectropolarimeter with a cell path length of 1 mm. The wavelength range 250–180 nm was analyzed over a minimum of eight replicate scans, at a time constant setting of 8. Mean residue content of ordered structure (helix, β-sheet, and "random" which included β-turns) was computed by the method of Chen et al. (1974).

Receptor Binding Assays. Receptor activity was assayed by inhibition of ^{125}I-labeled hCG binding to rat ovarian membrane receptors (Lee & Ryan, 1973). Native hCG (preparation K-881) for preparation of radioligand and for use as reference standard was prepared as described previously (Keutmann et al., 1983). Primary dilutions of peptides for assay (usually 10 mg/mL) were prepared in 0.01 N acetic acid

Table I: Secondary Structure of hLHβ-(38–57) Peptides

peptide	5 mM phosphate[a] helix	5 mM phosphate[a] β-sheet	90% TFE[a] helix	90% TFE[a] β-sheet
native sequences				
38–57	14	22	31	12
38–48	14	12	48	4
45–57	9	26	22	11
38–57 analogues				
Ala-38 (open disulfide)	11	20	31	8
Ala-43	12	23	28	12
Asp-43	11	22	25	13
Val-47	14	27	24	14
Glu-47	13	21	31	13
Asp-45,48	11	23	19	15
proline substitutions				
Ala-50	15	20	36	10
Ala-53	15	16	38	8
Ala-50,51 (monomer)	35	15	41	8
Ala-50,51 (dimer)	44	11	33	18

[a] Percent content of ordered structure in aqueous and lipophilic solvents, computed from circular dichroic profiles by the method of Chen et al. (1974).

and then diluted to final concentrations with assay buffer. Potencies were determined by calculating the peptide concentrations effecting half-maximal inhibition of labeled hCG binding from the logit-transformed response curves based on four dose levels. Mass of all peptides was corrected for peptide content by amino acid analysis of an aliquot (30–50 μg) taken from the primary dilution at the time of assay.

Radioimmunoassay. Immunoreactivity of hLHβ-(38–57) peptide and its subfragments was determined by a double-antibody assay (Keutmann et al., 1978). Polyclonal antibodies 35 and 36 to hLHβ-(38–57) were prepared by multisite immunizations of New Zealand rabbits with thyroglobulin-conjugated extended (Ala–Pro_6) hLHβ-(38–57) peptides as described by Stevens et al. (1986). An N-terminal tyrosine-extended 38–57 peptide, synthesized and purified as described above, was used as radioligand. Labeling was carried out by the procedure of Hunter and Greenwood (1962). Potencies and peptide concentrations were determined as described above.

Results

Peptide Synthesis. Coupling of the residues in the 45–57 region proceeded without difficulty. However, preview analyses of single-coupled final products generally showed incomplete coupling of one or more of the later cycles, notably Pro-39 and Thr-40, despite consistently negative ninhydrin reactions. This difficulty was also encountered in the shorter 38–48 peptide. It is likely that this represented restricted accessibility of reagents to the α-amino group within a region of highly ordered structure in organic medium (Mutter et al., 1985). We adopted a double-coupling strategy, with extended coupling times, for the later residues that reduced deletion peptides to a level (10% or less) that could be effectively removed during subsequent chromatography.

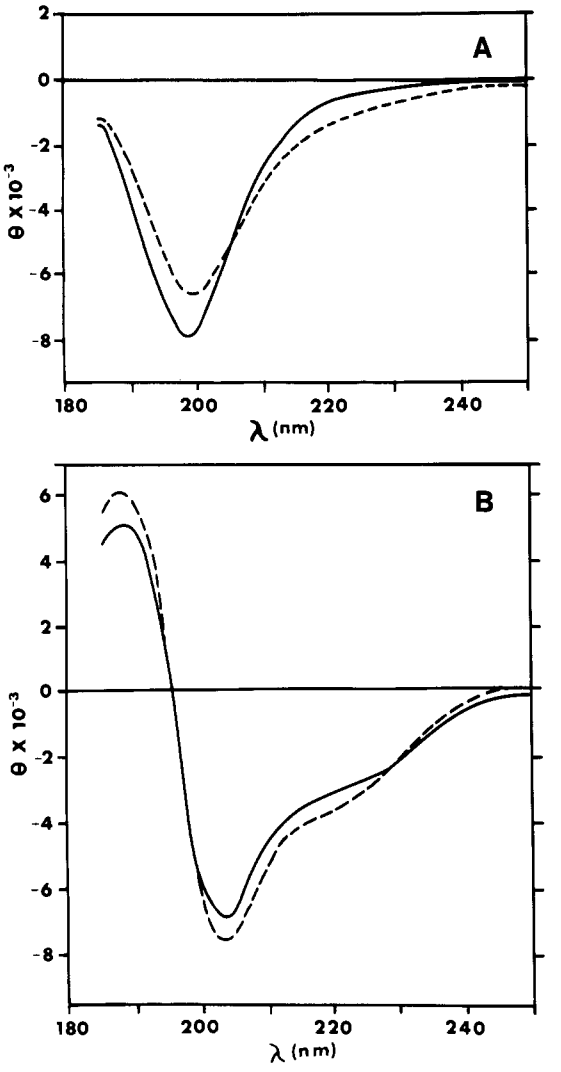

FIGURE 2: Circular dichroic profiles of disulfide-linked (– –) and open-disulfide (Ala-38) (—) analogues of hLHβ-(38–57) sequence, measured in aqueous (5 mM phosphate buffer) (A) and lipophilic (90% TFE) (B) solvents. [θ], mean residue ellipticity in deg·cm²/dmol. Analysis of ordered structure from these plots appears in Table II.

Effect of Elimination of the Disulfide Linkage. The open disulfide analogue [Ala-38]-hLHβ-(38–57) (Figure 1, peptide 38A) was prepared to assess the influence of the disulfide bond on the conformational and biological properties of the peptide. By gel filtration (data not shown) we confirmed that this analogue remained in the monomeric form for the incubation times and concentrations required for CD and bioassay measurements. "Far"-ultraviolet CD profiles of the disulfide-linked and open-chain peptides were nearly identical (Figure 2). Both underwent an increase in ordered structure (predominantly α-helix) when transferred into 90% TFE, as summarized in Table I.

Table II: Immunoreactivity of Synthetic Peptides from 38–57 Region of Human LH β Subunit

peptide	antibody 35 ID_{50} (pmol)[a]	antibody 35 potency (%)	antibody 35 slope	antibody 36 ID_{50} (pmol)[a]	antibody 36 potency (%)	antibody 36 slope
hLHβ-(38–57)	3.9	100	−1.47	11.9	100	−1.43
[Ala-38]-hLHβ-(38–57)	4.8	81	−1.54	10.1	118	−1.50
hLHβ-(45–57)	>190.0	<2		>230.0	<5	
hLHβ-(38–48)	>1000.0	<0.5		>1000.0	<1	
[Glu-47]-hLHβ-(38–57)	>1000.0	<0.5		>1000.0	<1	
hCGβ-(38–57)	>1000.0	<0.5		>1000.0	<1	

[a] Half-maximal inhibition of binding by [Tyr]-hLHβ-(38–57) radioligand.

Table III: Ovarian Receptor Binding Activity of 38–57 Peptides and Analogues from hLH and hCG β Subunits

peptide/analogue	assays	binding constant ($M \times 10^{-5}$)[a]	relative potency (%)
native sequences			
hLHβ-(38–57)	19	3.23 (2.77–3.69)	1.0
hLHβ-(38–48)	4	61.6 (46.5–76.7)	0.05
hLHβ-(45–57)[b]	4	>400.0	<0.01
hCGβ-(38–57)[b]	10	15.1	0.21
hLHβ-(38–57) analogues			
Ala-38	6	15.9 (12.6–19.2)	0.20
Ala-43	7	>400.0	<0.01
Asp-43	3	>400.0	<0.01
Asp-45, Asp-49	5	>400.0	<0.01
Glu-47	4	>400.0	<0.01
Val-47	5	3.75 (2.98–4.52)	0.87
proline substitutions			
Ala-50	5	2.50 (1.91–3.09)	1.29
Ala-53	5	2.74 (2.00–3.48)	1.17
Ala-50,51	5	(4.03)[c]	(0.80)

[a] Concentration effecting half-maximal displacement of labeled hCG tracer (mean ± SE). [b] From Keutmann et al. (1987). [c] Estimated from incomplete displacement curve.

The open disulfide analogue also remained immunoreactive with both polyclonal anti-hLHβ-(38–57) antibodies 35 and 36 (Table II), with displacement curves parallel to that of the closed-loop peptide. Both antibodies required the full-length peptide for recognition; the N-terminal (38–48) and C-terminal (45–57) fragments were essentially nonreactive as were peptides with substitutions in the midregion of the molecule (Table II). These results suggest that the 38–57 peptide is a rigid structure, retaining its shape and conformation in either aqueous or lipophilic solvents in the absence of the disulfide linkage.

Ovarian receptor binding activity was also retained by the Ala-38 analogue, although affinity was reduced to 20% of that of the closed-disulfide peptide (Table III). This decrease was more than expected from the minimal changes in CD profiles and immunoreactivity and may represent an effect of altered primary structure at residue 38. Carboxymethylation of hCGβ-(38–57) was previously found (H. T. Keutmann, K. Kitzmann, and R. J. Ryan, unpublished results) to virtually eliminate binding, despite retention of immunoreactivity, suggesting a deleterious effect of the negative carboxymethyl groups in the closely proximal 38 and 57 positions.

Importance of Arginine-43. The arginine at residue 43 is invariant among known species of LH/CG (Figure 1). Its essential nature was established by assays of analogues with the charge reversed, [Asp-43]-hLHβ-(38–57), or neutralized, [Ala-43]-hLHβ-(38–57) (Figure 1, 43D and 43A). Neither analogue was capable of displacing labeled hCG from ovarian membrane receptors (Table III). The content and distribution of ordered structure was not affected by these modifications (Table I). These data imply a primary structural requirement for a positive charge at position 43.

Effect of Charge at Residue 47. Position 47 contains Ala in human LHβ and Gly in hCGβ. Another neutral substitution (Val) is found in ovine and bovine LH β subunits (Figure 1). The analogue [Val-47]-hLHβ-(38–57) was found to be fully active by receptor binding assay (Table III), but substitution of a negative charge (Glu) eliminated activity. This glutamic acid is favorable to helix formation (Chou & Fasman, 1978), and the peptide appeared amphipathic by CD (Table I) and helical wheel projection. Thus, there is a primary structural requirement for a neutral residue at this position.

Substitutions for Proline Residues. Because of the prevalence of proline in "turn" sequences (Chou & Fasman, 1978; London et al., 1982), we studied the effects on activity and secondary structure after successive replacement of prolines at 50, 51, and 53. As summarized in Table III, replacement of individual prolines by alanine was tolerated with retention of partial or complete biological activity. The 50A and 53A analogues were essentially fully active (Figure 3). Replacement of Pro-51 by Ala occurs in the natural hCGβ fragment, which is about 20% as active as the 38–57 peptide from hLHβ.

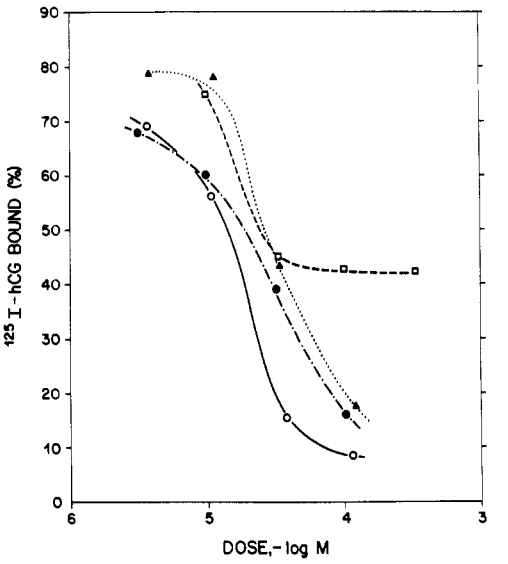

FIGURE 3: Dose–response curves for inhibition of labeled hCG binding by proline-substituted analogues of hLHβ-(38–57) in ovarian membrane receptor assay: native sequence (●); Ala-51 (▲); Ala-53 (○); Ala-50,51 (□). Summary of potencies from multiple assays appears in Table III.

Double replacement of prolines-50 and -51 affected both physical–chemical properties and biological response. After being allowed to stand 5 h at pH 6.5 to effect disulfide formation, 65% of the 50A, 51A analogue eluted as a dimer from Sephadex G-25 chromatography. The native structure (and the analogues replacing single prolines at 50 or 53) eluted predominantly in the monomeric form, only 25% appearing as dimer. This suggested an alteration of the loop structure, favoring dimer formation, after replacement of the prolines at 50 and 51. By analysis of tryptic fragments of the dimerized analogue, we found a mixture of head–head and head–tail configurations. Circular dichroic profiles of the 50A, 51A analogues (monomer and dimer) also differed from those of the other peptides, showing a high content of α-helix in aqueous solution as well as TFE (Table I).

The 50A, 51A analogue was active by receptor assay, but the displacement curve was qualitatively different from those of the other peptides, typically failing to displace completely the labeled hCG tracer (Figure 3). Although the dimeric peptide was weakly active in the receptor binding assay, it was too insoluble to permit assays over a full dose range.

Discussion

Synthetic analogues have not been used extensively in the study of structure–function relations among glycoprotein hormones. This stems primarily from the long-held contention that binding sites are determined exclusively by discontinuous determinants formed by folding about disulfides, with little contribution from linear sequences within the primary peptide backbone. Our own studies (Charlesworth et al., 1987; Keutmann et al., 1987) and those of others (Ramakrishnan et al., 1978; Sluss et al., 1986; Anderson et al., 1987; Schneyer

et al., 1988) have demonstrated that certain linear sequence segments and "loops" are exposed on the surface and independently capable of binding to receptors. The current results affirm the value of synthetic analogues in documenting the contributions of both primary and secondary structure to receptor interaction by the 38–57 region of hLH/hCG β subunit.

Requirements for activity suggested by interspecies conservation of a positive charge (arginine) at postion 43 and neutral residues at 47 are substantiated. Loss of activity by Ala or Asp substitution for Arg-43 was essentially complete. An earlier study (Sairam, 1976) showed a substantial decrease in activity of ovine LH after modification of multiple arginines in the β subunit by reaction with cyclohexanedione. Our findings imply residue 43 may have been among these. Other requirements implicated by structure–activity studies using the whole subunit include a neutral charge at 52 (leucine in hLH/hCG) and an intact methionine at 41 (Ward, 1978). Both of these positions are also highly conserved (Figure 1).

CD measurements, immunoreactivity, and receptor binding assays of the Ala-38 analogue showed the retention of essential conformational features in the absence of the disulfide bridge. The results suggest that the 38–57 loop is a structurally autonomous region, not simply a segment thrown into a loop by constraints in the surrounding molecule. This is important since the cysteines at 38 and 57, while evidently in close proximity, have not been proven to be linked to one another in the native subunit.

In this regard the 38–57 sequence resembles the "Ω loop" proposed by Lesczyznski and Rose (1986) to be a functionally important structural unit in enzymes and other globular proteins. These loops are rigid and compact with at least some side chains oriented into the center and one or more "turn" sequences to effect changes in direction. They differ from our sequence, however, in specifically excluding other ordered structure and tend not to favor hydrophobic residues. On the contrary, our studies suggest that secondary structure is a critical factor in maintaining the orientation and integrity of the 38–57 sequence. This is a novel feature for the gonadotropins, since secondary structure otherwise appears to be suppressed by the numerous disulfide linkages. Marked increases in ordered structure of gonadotropin subunits have been observed after disulfide reduction (Birken et al., 1986; M. C. Charlesworth and R. J. Ryan, unpublished observations).

Evidence for amphipathic helical structure through the N-terminal segment of hLH/hCGβ-(38–57) has been described by us previously (Keutmann et al., 1987). Most of the analogues with single substitutions retained this property (Table I). We designed the Asp-45, Ap-49 analogue to disrupt the hydrophobic surface of the helix; inactive by receptor assay, it also lost much of its helical potential in trifluoroethanol. Computer modeling using the CHARMM program has shown that a helix about three turns in length can form within the 20-residue looped native sequence at a favorable energy minimum (R. P. Milius et al., unpublished results). The hydrophobic "face" appears oriented predominantly into the core of the loop, with the more hydrophilic residues (e.g., Arg-43) toward the outside.

CD profiles of fragments (Table I) confirm the strong helical potential in the N-terminal half of the 38–57 peptide. However, the N-terminal segment alone appears insufficient for receptor binding activity; the fragment hLHβ-(38–48) and its Ala-38 analogue are very weakly active (Table III). Similarly, the C-terminal fragments 45–57 show low or minimal activity (Keutmann et al., 1987). The requisite complete loop sequence includes three proline residues (50, 51, and 53) that favor one or more "turns" in the C-terminal half of the peptide.

Although Pro-50 and Pro-53 are each highly conserved (Figure 1), their individual replacement by Ala did not impair receptor binding activity. Alanine may introduce a short helical segment that adequately duplicates the bend conferred by proline in the native sequence. Alternatively, the alanine residue may be sufficiently flexible to permit the peptide to assume the native shape in presence of the receptor (Marshall et al., 1984). The importance of reverse-turn formation as peptides assume a more compact conformation on interaction with receptors has been proposed (Hruby & Mosberg, 1982).

The analogue substituting Ala for Pro at both 50 and 51 was unique among the peptides studied in showing high helical content in aqueous solution. This may be attributable to extension of helical potential from the N-terminal region through residue 51 or even 52 (leucine). We believe that the striking propensity of this fragment to form the dimer results from separation of the approximated cysteines characteistic of the native sequence. The peptide could be either more flexible in solution or rigidly extended, for example, through intercession of an extra or extended helical segment as suggested by the CD measurements. At least some binding activity remains in the monomeric form, although we cannot explain kinetically the incomplete dose–response curve shown in Figure 3. It is possible that conversion to dimer attenuated the response at higher doses.

Experience with bradykinin (Vavrek & Stewart, 1980; London et al., 1982) provides a potentially useful prototype for more detailed analysis of conformation about the proline residues in hLHβ-(38–57). The segment (Leu-Pro-Pro-Leu) (residues 49–52) is a candidate for a β-turn. Although its predicted occurrence (0.46) is not particularly high (Chou & Fasman, 1978), it is similar in hydrophobicity and predictive parameters to the sequence Phe-Ser-Pro-Phe proposed to be a β-turn in bradykinin (London et al., 1982). Replacement of the Pro by Ala in bradykinin diminished bioactivity, perhaps analogous to the effect of similar substitution of Ala for Pro at position 51 in the less active 38–57 peptide from hCG. A second sequence in bradykinin (Arg-Pro-Pro-Arg) also tolerates alanine replacement of one, but not both, of the prolines. This may not represent a β-turn, however, since the prolines appear not to be in the turn-favoring cis configuration (London et al., 1982). Studies with analogues to determine the cis or trans configuration of the prolines in the 38–57 peptide are in progress.

Reports of binding activity by the corresponding "loop" sequence in hFSHβ extend its importance to another glycoprotein hormone. Inhibition of labeled FSH binding to calf testis membranes by the fragment hFSHβ-(33–53) occurred at concentrations similar to those for hCGβ- or hLHβ-(38–57) (Anderson et al., 1987). hFSH subfragments as short as four residues (i.e., TRDL and KTCT from the N- and C-terminal ends, respectively) also inhibited FSH binding, although at substantially higher concentrations (Sluss et al., 1986; Schneyer et al., 1988). We did not observe binding by the analogous short sequences 41–45 and 38–48 from hLH at concentrations up to 4×10^{-3} M. The hFSHβ-(33–53) sequence differs extensively from that of hLHβ- or hCGβ-(38–57), identities appearing at only four residues other than cysteine, and it does not appear amphipathic by helical wheel projection. These differences suggest that the nature and location of the binding determinants and perhaps the mechanism for receptor interactions by this region differ between FSH and LH/hCG.

The region is represented in hTSHβ by the slightly longer sequence 31–52. It again differs from LH/hCGβ but retains some similarity to FSH in the N-terminal portion. Synthetic fragments from TSHβ have not been evaluated, although the potential influence on TSH specificity of at least one residue from this region has been noted (Goverman & Pierce, 1981). It may thus be anticipated that the contribution of this intercysteine sequence to binding and specificity will prove to be a general characteristic of the glycoprotein hormones.

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Registry No. LH, 9002-67-9; hLBβ-(38–57), 116954-02-0; hLHβ-(38–48), 116954-03-1; hLHβ-(45–57), 108303-19-1; hCGβ-(38–57), 116954-04-2; (Ala-38)-hLHβ-(38–57), 116970-40-2; (Ala-43)-hLHβ-(38–57), 116954-05-3; (Asp-43)-hLHβ-(38–57), 116954-06-4; (Asp-45,Asp-49)-hLHβ-(38–57), 116970-41-3; (Glu-47)-hLHβ-(38–57), 116954-07-5; (Val-47)-hLHβ-(38–57), 116954-08-6; (Ala-50)-hLHβ-(38–57), 116954-09-7; (Ala-53)-hLHβ-(38–57), 116954-10-0; (Ala-50,51)-hLHβ-(38–57), 116954-11-1; CG, 9002-61-3.

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