The Amino-terminal Region of the Luteinizing Hormone/Choriogonadotropin Receptor Contacts Both Subunits of Human Choriogonadotropin

II. PHOTOAFFINITY LABELING*

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The luteinizing hormone/choriogonadotropin receptor, a seven-transmembrane receptor, is composed of two equal halves, the N-terminal extracellular exodomain and the C-terminal membrane-associated endodomain. Unlike most seven-transmembrane receptors, the exodomain alone is responsible for high affinity hormone binding, whereas signal is generated in the endodomain. These physical separations of hormone-binding and receptor activation sites are attributed to unique mechanisms for hormone binding and receptor activation of this receptor and its subfamily members. However, the precise hormone contact sites in the exodomain are unclear. In the preceding article (Hong, S., Phang, T., Ji, I., and Ji, T. H. (1998) J. Biol. Chem. 273, 13835–13840), a region immediately downstream of the N terminus of the exodomain was shown to be crucial for hormone binding. To test if the region interacts with the hormone, human choriogonadotropin (hCG) was photoaffinity-labeled with a peptide mimic corresponding to Gly18–Tyr28 of the receptor. This peptide mimic specifically photoaffinity-labeled both the α- and β-subunits of hCG. Interestingly, hCG was preferentially labeled. On the other hand, denatured hCG was not labeled, and a mutant analog of the peptide failed to label hCG. Furthermore, the affinity labeling was UV-dependent and saturable, indicating the specificity of the photoaffinity labeling. Our results indicate that the region of the exodomain interacts with hCG and that the contact points are near both subunits of hCG. Particularly, the alternate residues (Leu20, Cys22, and Gly24) are crucial for hCG binding. In addition, the results underscore the fact that there is a crucial hormone contact site outside of the popularly believed primary hormone-binding site that is composed of Leu-rich repeats and is located in the middle of the exodomain. Our observations are crucial for understanding the molecular mechanism through which the initial high affinity hormone binding leads to receptor activation in the endodomain.

The LH1/CG receptor belongs to a subfamily of glycoprotein hormone receptors within the seven-transmembrane receptor family. Unlike most seven-transmembrane receptors, it is composed of two equal halves, the 341-amino acid long extracellular N-terminal exodomain and the 334-amino acid long membrane-associated C-terminal endodomain, which includes seven transmembrane helices (1, 2). The exodomain binds the hormones with high affinity (3–7) without hormone action (5, 8). The exodomain-hCG complex is thought to make a secondary contact with the endodomain, thus generating a signal (9). Therefore, the high affinity interaction of the exodomain and hCG is the crucial first step leading to signal generation and hormone action. However, only limited information is available regarding the precise hormone contact residues and sites in the exodomain. Three peptide mimics of the exodomain, peptide-(21–38), peptide-(102–115), and peptide-(253–266), attenuated 125I-hCG binding to membranes expressing the LH/CG receptor (10). Receptors lacking the 11 amino-terminal residues or one of the leucine-rich motifs 1–6 were trapped in cells and failed to bind hCG (11).

In this work and the preceding article (12), the exodomain was examined using several independent methods, including serial truncation from the C terminus, Ala scanning, peptide mimics of the receptor, photoaffinity labeling, affinity cross-linking, and immunofluorescence. Our results show that the Leu20–Pro38 sequence contacts both the α- and β-subunits of hCG. In addition, three other sequences near the junctions of exons 3–4, 6–7, and 9–10 are important for hormone binding.

EXPERIMENTAL PROCEDURES

Materials—The N-hydroxysuccinimide (NHS) esters of 4-azidobenzoyltyrglycine (ABG) was synthesized as described (13). The N-hydroxysulfosuccinimide (sulfo-NHS) esters of 4-azidobenzoic acid (AB) and ethylene glycolbis(sulfosuccinimidylglycinate) (SES) were purchased from Pierce. The hCG CR 127 and hCG α- and β-subunits were supplied by the National Hormone and Pituitary Program (NIDDK, National Institute of Health). Denatured hCG was prepared by boiling hCG in 8 M urea for 30 min. Peptide mimics including wild-type and mutant LH/CG receptor peptides, LHR18–36 (see Fig. 1), were synthesized by Biosynthesis (Lewisville, TX) and purified on a Vydac C18 HPLC column using a solvent gradient from 100% of 0.1% trifluoroacetic acid in water to 100% of 80% 1-propanol. The sequence of LHR18–36 corresponds to Gly18–Tyr26 of the LH/CG receptor. In addition, a mutant LRH18–36 was synthesized in which Leu20, Cys22, and Gly24 were substituted with Ala.

Derivatization and Radiodination of Peptides—NHS-ABG was freshly dissolved in dimethyl sulfoxide to a concentration of 50 mM, and sulfo-NHS-AB in 0.1 M sodium phosphate (pH 7.5) to a concentration of 20 mM. These reagent solutions were immediately used to derivatize peptide. In this work and the preceding article (12), the exodomain was examined using several independent methods, including serial truncation from the C terminus, Ala scanning, peptide mimics of the receptor, photoaffinity labeling, affinity cross-linking, and immunofluorescence. Our results show that the Leu20–Pro38 sequence contacts both the α- and β-subunits of hCG. In addition, three other sequences near the junctions of exons 3–4, 6–7, and 9–10 are important for hormone binding.

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The abbreviations used are: LH, luteinizing hormone; LHR, LH receptor; CG, choriogonadotropin; hCG, human CG; NHS, N-hydroxysuccinimide; sulfo-NHS, N-hydroxysulfosuccinimide; ABG, 4-azidobenzoyltyrglycine; AB, 4-azidobenzoic acid; SES, ethylene glycolbis(sulfosuccinimidylglycinate); PBS, phosphate-buffered saline.
7 µl of sodium metabisulfite (2.5 mg/ml) in PBS was introduced to terminate radioiodination. Derivatized and radioiodinated ABG-125I-LHR18–36 or AB-125I-LHR18–36 solution was mixed with 60 µl of 16% sucrose solution in PBS and fractionated on a Sephadex G-10 column (0.6 × 15 cm) using PBS.

Cross-linking of 125I-LHR18–36 to hCG—Disposable glass tubes were siliconized under dimethyldichlorosilane vapor overnight and autoclaved. In each siliconized tube, 20 µl of PBS, hCG (70 ng in 10 µl of PBS), and 125I-LHR18–36 (100 ng in 10 µl of PBS) were mixed and incubated at 37 °C for 90 min. After incubation, 3 µl of 0.1 M SES in Me2SO was added to each tube and further incubated at 25 °C for 20 min. The cross-linking reaction was terminated by adding 3 µl of 5% Gly in PBS. The samples were boiled for 2 min in 2% SDS, 100 mM dithiothreitol, and 8 M urea. The solubilized samples were electrophoresed on 8–12% polyacrylamide gradient gels. Gels were dried and exposed to Eastman Kodak X-Omat x-ray film at −75 °C for 4 days.

Photoaffinity Labeling of hCG—The following solutions were sequentially introduced to siliconized glass tubes: 20 µl of PBS, 10 µl of hCG (10 ng/µl) in PBS, and 10 µl of ABG-125I-LHR18–36 (10 ng/µl) in PBS. For labeling with AB-125I-LHR18–36, 20 µl of PBS, 10 µl of hCG (20 ng/µl) in PBS, and 10 µl of AB-125I-LHR18–36 (15 ng/µl) in PBS were mixed. The mixtures were incubated at 37 °C for 90 min in the dark; irradiated with a Minilamp R-52 UV lamp for 3 min as described previously (13); and solubilized in 2% SDS, 100 mM dithiothreitol, and 8 M urea. The samples were electrophoresed on 8–12% polyacrylamide gradient gels. Gels were dried on filter paper, which was exposed to a molecular imaging screen (Bio-Rad) overnight. The imaging screen was scanned on a Model GS-525 Molecular Image System Scanner (Bio-Rad), and the radioactive band profile was analyzed using Image Analysis Systems (Version 2.1, Bio-Rad). Gels were exposed to X-Omat x-ray film at −75 °C for 4 days.

Competitive Inhibition of Photoaffinity Labeling of hCG—Competitive inhibition experiments were carried out as described for the photoaffinity labeling experiments, except that 10 µl instead of 20 µl of PBS was introduced to each tube, and the mixture was incubated with 10 µl of increasing concentrations of nonradioactive wild-type or mutant LH/CG receptor. In addition, a mutant LHR 18–36 was synthesized in which Leu20, Cys22, and Gly24 were substituted with Ala.

Inhibition of 125I-hCG Binding to the LH/CG Receptor—A human embryonic kidney 293 cell line stably expressing the rat LH/CG receptor was incubated with 100,000 cpm of 125I-hCG in the presence of increasing concentrations of nonradioactive wild-type or mutant LH/CG receptor. In addition, a constant concentration of hCG was incubated with increasing concentrations of unlabeled LHR18–36 in PBS. The samples were electrophoresed on 8–12% polyacrylamide gradient gels. Gels were dried on filter paper, which was exposed to a molecular imaging screen (Bio-Rad) overnight. The imaging screen was scanned on a Model GS-525 Molecular Image System Scanner (Bio-Rad), and the radioactive band profile was analyzed using Image Analysis Systems (Version 2.1, Bio-Rad). Gels were exposed to X-Omat x-ray film at −75 °C for 4 days.

Fig. 1. Sequences of LHR peptide mimics. LHR18–36 was synthesized, with its sequence corresponding to Gly18–Tyr36 of the LH/CG receptor. In addition, a mutant LHR18–36 was synthesized in which Leu20, Cys22, and Gly24 were substituted with Ala.

Fig. 2. Autoradiography of cross-linking of 125I-LHR18–36 to hCG. After incubating 125I-LHR18–36 with hCG, the mixture was treated with increasing concentrations of SES, solubilized under reducing conditions, and electrophoresed (C). Gels were dried and autoradiographed (B) as described under “Experimental Procedures.” In addition, 125I-hCGα, 125I-hCGβ, and cross-linked 125I-hCGαβ were electrophoresed as standards (A).

Fig. 3. 125I-LHR18–36 and hCG concentration-dependent cross-linking. A constant concentration of hCG was incubated with increasing concentrations of 125I-LHR18–36 and treated with SES (A and D). Conversely, a constant concentration of 125I-LHR18–36 was incubated with increasing concentrations of hCG and treated with SES (B and E). Wild-type (WT) and mutant (Mut) LHR18–36 were incubated with hCG and treated with SES (C and F).
RESULTS

In the preceding article (12), we showed that the amino acid sequence near the N terminus of the LH/CG receptor, particularly Leu20-Arg21-Cys22-Pro23-Gly24, is crucial for hCG binding. This raises the question as to whether the peptide sequence directly interacts with the hormone or indirectly influences the hormone/receptor interaction by impacting on the global structure of the receptor. To examine these possibilities, a peptide mimic corresponding to the receptor sequence from Gly18 to Tyr36, LHR18–36 (Fig. 1), was synthesized and tested for its ability to bind and to affinity label hCG. The sequence of LHR18–36 corresponds to Gly18–Tyr36 of the LH/CG receptor. In addition, a mutant LHR18–36 was synthesized in which Leu20, Cys22, and Gly24 were substituted with Ala.

Cross-linking of 125I-LHR18–36 to hCG—125I-LHR18–36 was incubated with hCG in the presence of hCG in the presence of increasing concentrations of wild-type (WT) LHR18–36 (A) or mutant LHR18–36 (B) and treated with SES. Samples were processed as described in the legend to Fig. 2. The intensities of the α and β bands were plotted against increasing concentrations of unlabeled wild type (WT) and mutant peptides (C).

The affinity of LHR18–36 binding to hCG was determined using two independent methods. 125I-LHR18–36 was incubated with hCG in the presence of increasing concentrations of unlabeled LHR18–36 (A) as described under “Experimental Procedures.” Alternatively, 125I-hCG was incubated with 293 cells stably expressing the LH/CG receptor in the presence of increasing concentrations of unlabeled LHR18–36 (B). WT, wild-type; NS, not significant.

Specificity of Cross-linking of LHR18–36 to hCG—To determine whether the cross-links are specific between the receptor peptide and hCG, cross-linking was performed under increasing concentrations of 125I-LHR18–36 to hCG required SES, hCG, and LHR18–36 since it did not occur in the absence of any of the three. The extent of cross-linking was dependent on the SES concentration (Fig. 2, B and C), reaching the maximum level at 0.3–1 mM SES. Under this condition, ~20% of 125I-LHR18–36 was cross-linked to each hCGα and hCGβ. At a higher SES concentration, e.g. 3 mM, the extent of cross-linking decreased. This decrease was due to a non-cross-linking, monofunctional reaction (only one of the two NHS groups reacting with a target amino group while leaving the other NHS group unused) of excess SES with 125I-LHR18–36, hCG, and its subunits (16). In conclusion, our results indicate that 125I-LHR18–36 was covalently cross-linked to hCGα and hCGβ. Furthermore, the N-terminal amino group of 125I-LHR18–36, the only amino group of the peptide, was cross-linked to an amino group of either hCGα or hCGβ. The distance between the pair of two cross-linked amino groups is expected to be ~13 Å.

The affinity of LHR18–36 binding to hCG and of 125I-hCG binding to the LH/CG receptor. The affinity of LHR18–36 binding to hCG was determined using two independent methods. 125I-LHR18–36 was incubated with hCG in the presence of increasing concentrations of unlabeled LHR18–36 (A) as described under “Experimental Procedures.” Alternatively, 125I-hCG was incubated with 293 cells stably expressing the LH/CG receptor in the presence of increasing concentrations of unlabeled LHR18–36 (B). WT, wild-type; NS, not significant.

FIG. 5. Inhibition of 125I-LHR18–36 binding to hCG and of 125I-hCG binding to the LH/CG receptor. The affinity of LHR18–36 binding to hCG was determined using two independent methods. 125I-LHR18–36 was incubated with hCG in the presence of increasing concentrations of unlabeled LHR18–36 (A) as described under “Experimental Procedures.” Alternatively, 125I-hCG was incubated with 293 cells stably expressing the LH/CG receptor in the presence of increasing concentrations of unlabeled LHR18–36 (B). WT, wild-type; NS, not significant.

Specifity of Cross-linking of LHR18–36 to hCG—To determine whether the cross-links are specific between the receptor peptide and hCG, cross-linking was performed under increasing concentrations of 125I-LHR18–36 while maintaining hCG at a constant concentration (Fig. 3A). Conversely, 125I-LHR18–36 and hCG were cross-linked at increasing concentrations of hCG and a constant concentration of 125I-LHR18–36 (Fig. 3B). If
cross-links are specific, they should reach saturation under both conditions. The results (Fig. 3, D and E) indeed show plateaus under both conditions, an indication of saturable and specific cross-linking. This specific cross-linking is not expected to occur with peptides that do not recognize hCG. In the preceding article (12), Ala substitution for Leu20, Cys22, or Gly24 of the receptor resulted in the loss of hCG binding by the receptor. Therefore, a mutant LHR18–36 was synthesized in which Leu20, Cys22, and Gly24 were substituted with Ala (Fig. 1). As expected, mutant LHR18–36 was not cross-linked to either subunit of hCG (Fig. 3, C and F). It is not clear whether the lack of observed cross-linking of mutant LHR18–36 was caused by a lack of mutant LHR18–36 binding to hCG or by a lack of a cross-linking reaction by SES due to the putative steric hindrance even though mutant LHR18–36 successfully bound to hCG. To distinguish these possibilities, 125I-LHR18–36 was cross-linked to hCG in the presence of increasing concentrations of unlabeled wild-type LHR18–36 (Fig. 4A) and mutant LHR18–36 (Fig. 4B). Wild-type LHR18–36, but not mutant LHR18–36, competitively attenuated the cross-linking, but mutant LHR18–36 was significantly less effective (Fig. 4C), an indication of the less efficient binding of mutant LHR18–36 to hCG.

Affinity of LHR18–36 Binding to hCG—To determine the binding affinity, 125I-LHR18–36 was incubated with increasing concentrations of unlabeled LHR18–36 (Fig. 5A). Unlabeled LHR18–36 inhibited 125I-LHR18–36 binding to hCG, with a $K_d$ value of $29\pm1$ nM. It is not clear whether this inhibition was caused by competitive binding of LHR18–36 and the receptor to hCG or by a putative allosteric effect of LHR18–36 binding to hCG. To examine these possibilities and the relevance of the inhibition of hCG binding to the LH/CG receptor, 125I-hCG was incubated with a 293 cell line stably expressing the receptor (14) in the presence of increasing concentrations of unlabeled wild-type LHR18–36 (Fig. 4A) and mutant LHR18–36 (Fig. 4B). Wild-type LHR18–36 and mutant LHR18–36 attenuated the cross-linking, but mutant LHR18–36 was significantly less effective (Fig. 4C), an indication of the less efficient binding of mutant LHR18–36 to hCG.
ity labeling either hCG wild-type or mutant LHR 18–36. Peptide was derivatized with either AB or ABG (13) to produce more preferentially labeled than hCG b. Interestingly, AB-125I-LHR18–36 labeled them more efficiently than ABG-125I-LHR18–36 (Fig. 6, C–E). The cross-linking reaction by SES. As shown in Fig. 6, AB-125I-LHR18–36, the labeling reaction by AB and ABG is more restricted than the maximum cross-linkable 13 Å of SES, and therefore, ABG can reach and label target molecules up to 7 and 10 Å, not be able to cross-link one hCG subunit to another. AB and ABG can reach and label target molecules up to 7 and 10 Å, respectively (17). These distances are considerably shorter than the maximum cross-linkable 15 Å of SES, and therefore, the labeling reaction by AB and ABG is more restricted than the cross-linking reaction by SES. As shown in Fig. 6, AB-125I-LHR18–36 and ABG-125I-LHR18–36 were capable of photoaffinity labeling either hCG or hCGβ, but not both subunits at the same time to produce the labeled hCGγ complex. Interestingly, AB-125I-LHR18–36 labeled them more efficiently than ABG-125I-LHR18–36 (Fig. 6, C and D). In addition, hCGγ was more preferentially labeled than hCGβ. This result is consistent with the SES cross-linking results. One possible explanation is that the N terminus of the LHR18–36 derivatives is <7 Å from the hCG α- and β-subunits and that the peptide derivatives are bound closer to α than β. The labeling required UV irradiation and was dependent on the irradiation time, reaching the maximum labeling after ~1 and 0.5 min of irradiation of the AB-125I-LHR18–36-hCG and AB-125I-LHR18–36-hCG complexes, respectively. Unlike SES cross-links, the maximum levels were sustained after longer UV exposure. This UV dependence clearly indicates photoaffinity labeling. In addition, the sustained maximum levels and the preferential labeling of hCGα without simultaneous labeling of both subunits suggest a labeling specificity. To further examine the specificity of photoaffinity labeling, the concentration of either hCG or the peptide derivatives was changed. Concentration Effect of hCG and Peptide Derivatives—When a constant amount of hCG was incubated with increasing concentrations of AB-125I-LHR18–36 or ABG-125I-LHR18–36, the intensity of labeled hCG α- and β-bands gradually increased and plateaued (Fig. 7). A similar result was obtained in a converse experiment when a constant concentration of AB-125I-LHR18–36 or ABG-125I-LHR18–36 was incubated with increasing concentrations of hCG (Fig. 8). These results indicate that the photoaffinity labeling is dependent on both of the derivatized peptides and hCG as they are limiting factors. In both cases, the derivatized peptides labeled hCGα more than hCGβ, an indication of a labeling specificity. Competitive Inhibition of Photoaffinity Labeling by Nondervatized Peptides—A nondervatized peptide is expected to displace specific labeling. Therefore, hCG was incubated with AB-125I-LHR18–36 or ABG-125I-LHR18–36 in the presence of increasing concentrations of nondervatized peptide (Fig. 9, A and D). Increasing concentrations of LHR18–36 inhibited photoaffinity labeling in a dose-dependent manner and eventually completely blocked it. These results indicate the specificity of LHR18–36 for the photoaffinity labeling. However, this specific labeling should not be blocked by a peptide that does not recognize hCG. Increasing concentrations of mutant LHR18–36 failed to significantly block the photoaffinity labeling of hCG by AB-125I-LHR18–36 or ABG-125I-LHR18–36 (Fig. 9, B and E). Only at the highest concentrations of the mutant peptide was label-
ing by AB-\(^{125}\text{I}-\text{LHR}_{18-36}\) slightly reduced. Although these results indicate the labeling specificity of AB-\(^{125}\text{I}-\text{LHR}_{18-36}\) and ABG-\(^{125}\text{I}-\text{LHR}_{18-36}\), the futile inhibition could be interpreted as the mutant peptide binding to a site in hCG different from the AB-\(^{125}\text{I}-\text{LHR}_{18-36}\), or ABG-\(^{125}\text{I}-\text{LHR}_{18-36}\)-binding site. To test this hypothesis, mutant LHR\(_{18-36}\) was derivatized and radioiodinated to prepare AB-\(^{125}\text{I}-\text{mutant LHR}_{18-36}\) and ABG-\(^{125}\text{I}-\text{mutant LHR}_{18-36}\).

**Photoaffinity Labeling by Mutant LHR\(_{18-36}\)**—As shown in Fig. 10, AB-\(^{125}\text{I}-\text{mutant LHR}_{18-36}\) and ABG-\(^{125}\text{I}-\text{mutant LHR}_{18-36}\) failed to conspicuously label the hCG subunits. Only trace amounts of labeling were detected, indicating that the labeling affinities were significantly low. These results are consistent with the observation that the highest concentrations of nonderivatized mutant LHR\(_{18-36}\) slightly attenuated the labeling by AB-\(^{125}\text{I}-\text{LHR}_{18-36}\). In addition, AB-\(^{125}\text{I}-\text{LHR}_{18-36}\) and ABG-\(^{125}\text{I}-\text{LHR}_{18-36}\) did not photoaffinity label denatured hCG that was boiled in 8 M urea and did not bind to the receptor (data not shown).

**DISCUSSION**

Our results show that AB-\(^{125}\text{I}-\text{LHR}_{18-36}\) and ABG-\(^{125}\text{I}-\text{LHR}_{18-36}\) photoaffinity label hCG. The \(\alpha\)-subunit is preferentially labeled. Ample evidence was presented to support the specificity of the photoaffinity labeling of hCG. The labeling is saturable and dependent on the hCG concentration, derivatized \(^{125}\text{I}-\text{LHR}_{18-36}\) concentration, and UV exposure. AB-\(^{125}\text{I}-\text{LHR}_{18-36}\) and ABG-\(^{125}\text{I}-\text{LHR}_{18-36}\) photoaffinity label bioactive hCG, but not denatured hCG. This labeling is blocked by nonderivatized wild-type LHR\(_{18-36}\), but not by nonderivatized mutant LHR\(_{18-36}\). Furthermore, AB-\(^{125}\text{I}-\text{mutant LHR}_{18-36}\) and ABG-\(^{125}\text{I}-\text{mutant LHR}_{18-36}\) do not photoaffinity label bioactive hCG and denatured hCG.

Both subunits of hCG are labeled, indicating that the UV-activable group coupled to LHR\(_{18-36}\) can reach them. This is consistent with other studies (18–20) and not surprising since the two subunits are closely intertwined in the crystal structure (21, 22). Interestingly, hCGs was preferentially labeled. Since only one photosensitive group is attached to the N terminus of each derivatized peptide, AB-\(^{125}\text{I}-\text{LHR}_{18-36}\) and ABG-\(^{125}\text{I}-\text{LHR}_{18-36}\) bound to hCG can photoaffinity label only one, but not both, of the subunits. Obviously, the reagent more readily reaches and labels the \(\alpha\)-subunit than the \(\beta\)-subunit.

Since the maximum labeling distances of AB and ABG are 7 and 10 Å, respectively (17), and AB-\(^{125}\text{I}-\text{LHR}_{18-36}\) labels hCG more efficiently than ABG-\(^{125}\text{I}-\text{LHR}_{18-36}\), the N terminus of the LHR\(_{18-36}\) derivatives is \(<7\) Å from both subunits. Therefore, both subunits of hCG are likely to contact the LHR\(_{18-36}\) derivatives. Our results are not consistent with the unlikely possibility that the peptide associates with hCG at sites other than the receptor contact site, impacts on the global structure of hCG, and interferes with the hormone/receptor interaction. Clearly, LHR\(_{18-36}\) interacts with hCG at or near a contact site of hCG and the LH/CG receptor.

The recent crystallization of Leu-rich repeats (23, 24) and their presence in the middle of the exodomain of all glycoprotein hormone receptors (1) generated a deluge of the popular and probable thoughts (11, 25–27) that eight to nine Leu-rich repeats compose the primary contact site for the ligand. They compose the bulk of the exodomain at its center and are computer-modeled to show a crescent structure (Fig. 11). The inner surface of the crescent consists of \(\beta\)-sheets of the repeats and is thought to be the ligand contact site (24–26), perhaps interacting with the putative receptor-binding C-terminal and seat belt side of hCG (21). Our results in this work and the preceding article (12) indicate that there is a crucial hormone contact site outside of this Leu-rich crescent. It will be interesting to see if the Gly\(^{18-46}\) sequence of the receptor reaches the opposite face of the seat belt side (Fig. 11). In the Gly\(^{18-46}\) sequence, the alternate Leu\(^{20}\), Cys\(^{27}\), and Gly\(^{24}\) residues are crucial for hormone binding (12). These three residues appear to be at one side of a \(\beta\)-like structure and could face the hormone and provide a direct contact site.

Our results are consistent with the observations of others indicating multiple contact sites for the hCG/receptor interaction (10, 11, 28, 29). Each contact site is likely to contribute to the overall interaction and affinity. For complete understanding of the interaction, it is necessary to know whether the multiple contact sites are independent or related and whether they interact with hCG simultaneously or sequentially. This information will be useful for designing agonists and antagonists of hCG.

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