Hormone Interactions to Leu-rich Repeats in the Gonadotropin Receptors

III. PHOTOAFFINITY LABELING OF HUMAN CHORIONIC GONADOTROPIN WITH RECEPTOR LEU-RICH REPEAT 4 PEPTIDE

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Human chorionic gonadotropin (hCG) binds to the extracellular N-terminal domain, exodomain, of its receptor, and the resulting hCG-exodomain complex is thought to modulate the membrane associated domain, endodomain, of the receptor to generate hormone signal. The bulk of the exodomain is speculated to assume a crecent structure consisting of eight to nine Leu-rich repeats (LRRs), which may provide the hormone contact sites. Unfortunately, little experimental evidence is available for the precise hormone contact points in the exodomain and the endodomain. The two preceding articles (Song, Y., Ji, I., Beauchamp, J., Isaacs, N., and Ji, T. (2001) J. Biol. Chem. 276, 3426–3435; Song, Y., Ji, I., Beauchamp, J., Isaacs, N., and Ji, T. (2001) J. Biol. Chem. 276, 3436–3442) show that putative LRR2 and LRR4 are crucial for hormone binding. In particular, the N-terminal region of LRR4 assumes the hydrophobic core of the LRR4 loop, whereas the C-terminal region is crucial for signal generation. However, it is unclear whether LRR4 interacts hCG and the endodomain and how it might be involved in signal generation. In this article, our affinity labeling results present the first evidence that the N-terminal region of LRR4 interacts with hCG, preferentially the hCGα subunit and that the hCG/ LRR4 complex interacts with exoloop 2 of the endodomain. This interaction offers a mechanism to generate hormone signal.

The luteinizing hormone/chorionic gonadotropin receptor (LHR) consists of an extracellular N-terminal half (exodomain) and a membrane-associated C-terminal half (endodomain) (1, 2). The ~350-amino acid-long exodomain has high affinity hormone contact sites (3–5) and shows eight to nine repeats of 22–29 amino acids with several conserved Leu/Ile residues (1, 6–10). These Leu/Ile-rich repeats (LRRs) represent a common structural motif found in a large family of proteins, which includes glycoprotein hormone receptors (11). In the crystal structure of ribonuclease inhibitors, the LRRs assume a helix-strand connected to parallel α helices. The β strands in ribonuclease inhibitors are involved in the interaction with ribonuclease. However, it is unclear whether the putative LRR sequences of LHR and other glycoprotein hormone receptors are indeed LRRs and function as such. In the preceding articles (12, 13), we have shown that some, but not all, LRRs of LHR and the follicle-stimulating hormone receptor are crucial for hormone binding. In particular, LRR2 and LRR4 of LHR are most crucial, but it is unclear whether these LRRs make direct contacts with the hormone. In this article, the evidence is presented for the interaction of the residues around the Leu-Ser-Ile motif, the putative β strand, in LRR4 with hCG, in particular with the hCGα subunit. In addition, our data suggest the interaction of the LRR4-hCG complex with the endodomain, in particular exolop 2, which is likely to modulate signal generation.

EXPERIMENTAL PROCEDURES

Materials—The N-hydroxysuccinimide (NHS) ester of 4-azidobenzoic acid (AB) was synthesized as described previously (14). The N-hydroxysulfosuccinimide esters of ethylene glycolbis(sulfosuccinimidyl)-sucinate (SES) were purchased from Pierce. The hCG CR 127 and hCG subunits were supplied by the National Hormone and Pituitary Program. Denatured hCG was prepared by boiling hCG in 8 M urea for 30 min. Receptor peptides were synthesized and N-acetylated and C-amidated by Biosynthesis (Lewisville, TX). They were purified on a Vydac C18 high performance liquid chromatography column using a solvent gradient from 100% of 0.1% trifluoroacetic acid in water to 20% of 0.1% trifluoroacetic acid in water and 80% 1-propanol. The peptide mimics include the wild type receptor peptide corresponding to the LHR sequence of Asn96–Asp115 (LHR96–115), a mutant LHR96–115 with Leu103Ala and Ile105Ala mutations (LHR96–115(L103A/I105A)), a mutant LHR96–115 with the Lys101Ala and Ile105Ala mutations (LHR96–115(K101A/I105A)), a mutant LHR96–115 with the Lys101Ala and Ile105Ala mutations (LHR96–115(K101A/I105A)), a mutant LHR96–115 with the Lys101Ala and Ile105Ala mutations (LHR96–115(K101A/I105A)), a mutant LHR96–115 with the Lys101Ala and Ile105Ala mutations (LHR96–115(K101A/I105A)), a wild type peptide encompassing the sequence upstream of LHR96–115 (LHR96–115(LHR96–115)), and a wild type peptide covering the sequence downstream of LHR96–115 (LHR96–115(LHR96–115)).

Derivatization and Radioiodination of Peptides—hCG was freshly dissolved in dimethyl sulfoxide to a concentration of 50 μg in 1.2 ml sodium phosphate (pH 7.5) to a concentration of 20 μg. This reagent solution was immediately used to derivatize receptor peptides. In the dark, 10 μl of NHS-AB was added to 30 μg of LHR96–115 in 40 μl of 0.1 M sodium phosphate (pH 7.5). The mixture was incubated for 30 min for NHS-AB or 60 min for NHS-AB at 25 °C. The following were added to the derivatization mixture: 1 μCi of Na125I in 10 μl of 0.1 M NaOH and 7 μl of chloramine T (1 mg/ml) in 10 mM Na2HPO4 and 0.9% NaCl (pH 7.4) (PBS). After 20 μl of sodium metabisulfite (2.5 mg/ml) in PBS was introduced to terminate radioiodination. Derivatized and radioiodinated AB-125I-LHR96–115 solution was mixed with 60 μl of 16% sucrose solution in PBS and fractionated on Sephadex Superfine G-100 (0.6 × 15 cm) using PBS.

Affinity Cross-linking of 125I-LHR96–115 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 PBS), and 125I-LHR96–115 (100 ng in 10 μl of PBS) were mixed and...
incubated in 37 °C for 90 min. After incubation, 3 μl of 0.1 mM of SES in dimethyl sulfoxide 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 mM Gly in PBS. The samples were boiled for 2 min in 2% sodium dodecyl sulfate, 100 mM diethiothreitol, and 8 μl urea. The solubilized 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 Scanner (Bio-Rad), and radioactive band intensity 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.

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 AB-125I-LHR96–115 (10 ng/μl) in PBS. The mixtures were incubated at 37 °C for 90 min in the dark, irradiated with a Mineralight RT-52 UV lamp for 3 min as described previously (14), and solubilized in 2% SDS, 100 mM diethiothreitol, and 8 μl urea. The samples were electrophoresed on 8–12% polyacrylamide gradient gels. Gels were dried on filter paper and processed as described above.

Competitive Inhibition of Affinity Labeling of hCG—Competitive inhibition experiments were carried out as described for the affinity cross-linking and 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 LHR96–115.

Inhibition of 125I-hCG binding to LHR—A human embryonic kidney cell 293 cell line stably expressing human LHR was incubated with 100,000 cpm of 125I-hCG in the presence of increasing concentrations of nonradioactive wild type or mutant LHR96–115 peptides as described previously (15). After several times washing the cells, the radioactivity associated with the cells was counted, and percent bound 125I-hCG was plotted against the nonradioactive receptor peptides. The results were converted to Scatchard plot by plotting bound/free peptide versus bound peptide. The plot was used to calculate the Kd value following the Scatchard equation (16).

RESULTS

In the preceding articles (12, 13), we showed the crucial roles of LRRs of LHR in hormone binding, particularly LRR4. This raises the question as to whether the LRRs directly interact with the hormone or indirectly influence the hormone/receptor interaction by impacting the global structure of the receptor exodomain. To examine these possibilities a peptide mimic corresponding to the receptor sequence encompassing the β-stranded Leu103-Ile105, LHR96–115, was synthesized and tested for its ability to bind and affinity label hCG. For affinity labeling, we employed two complementary affinity labeling methods. In the first approach, 125I-LHR96–115 incubated with hCG, and the resulting 125I-LHR96–115-hCG complexes were cross-linked using SES, a homobifunctional reagent that is capable of cross-linking two amino groups up to 13 Å apart (17). In the second approach, 125I-LHR96–115 was derivatized with AB, an UV-activatable reagent, to produce AB-125I-LHR96–115 and incubated with hCG. The resulting 125I-LHR96–115-hCG complex was irradiated with UV to photoaffinity label hCG with AB-125I-LHR96–115. The advantages and disadvantages of both methods will be discussed later.

To determine whether AB-125I-LHR96–115 and 125I-LHR96–115 would bind and label hCG, they were incubated with hCG and treated with UV or SES, respectively. The samples were solubilized in SDS under the reducing condition and electrophoresed, as described under “Experimental Procedures.” The autoradiographic phosphoimage of the gel shows that both AB-125I-LHR96–115 and 125I-LHR96–115 labeled both the α and β subunits in hCG (Fig. 1). In addition, the hCG αβ dimer was cross-linked and labeled with 125I-LHR96–115 when the 125I-LHR96–115-hCG complex was treated with SES. The positions of hCGα, hCGβ, and the hCGαβ dimer were determined by comparing the respective positions of 125I-hCGα, 125I-hCGβ, and the cross-linked 125I-hCG αβ dimer on the autoradiograph (Fig. 1, lanes 1 and 5).
Labeling Specificity—Specific labeling should be displaced by wild type peptide but not by a peptide that could not bind hCG. We have shown in the previous reports (12), (13) that the Leu103 → Ala or Ile105 → Ala substitution in LHR abrogated hormone binding. Therefore, Leu103 and Ile105 were substituted with Ala in LHR96–115 to produce a mutant peptide, LHR96–115(L103A/I105A). To test whether the wild type and mutant LHR peptides could inhibit affinity labeling, hCG was incubated with AB-125I-LHR96–115 in the presence of increasing concentrations of nonderivatized wild type peptide (Fig. 3A) and nonderivatized mutant peptide (Fig. 3C). Increasing concentrations of LHR96–115 inhibited photoaffinity labeling in a dose-dependent manner and eventually, completely blocked it. These results indicate the labeling specificity of AB-125I-LHR96–115 for the photoaffinity labeling. In contrast, the inhibition by mutant LHR96–115(L103A/I105A) was significantly less effective (Fig. 3C).

Similar results were obtained with affinity cross-linking of AB-125I-LHR96–115 to hCG (Fig. 3, B, D, and F). Although these results indicate the labeling specificity of AB-125I-LHR96–115 and LHR96–115, the futile inhibition could be interpreted as the mutant peptide binding to a site in hCG different from the AB-125I-LHR96–115 binding site. To test this hypothesis and test whether the mutant peptide could label hCG, LHR96–115(L103A/I105A) was radiiodinated or derivatized and then radiiodinated to prepare 125I-LHR96–115(L103A/I105A) or AB-125I-LHR96–115(L103A/I105A), respectively. As shown in Fig. 4, AB-125I-LHR96–115(L103A/I105A) and 125I-LHR96–115(L103A/I105A) labeled the hCG subunits significantly less. Only trace amounts of labeling were detected, indicating the labeling af-
Fig. 3. Competitive inhibition of affinity labeling by unlabeled wild type peptide and mutant peptide. AB-125I-LHR96–115 was incubated with hCG in the presence of increasing concentrations of wild type LHR96–115 (A) or mutant LHR96–115(K101A) (B) and irradiated with UV for 30 s. Samples were processed as described in the legend to Fig. 2. In addition, 125I-LHR96–115 was incubated with hCG in the presence of increasing concentrations of wild type LHR96–115 (C) or mutant LHR96–115(K112A) (D) and treated with 0.3 mM SES. The samples were electrophoresed and processed to determine the percent labeling of the hCG α and β subunits. The percent intensities of the labeled α and β bands were determined and plotted against increasing concentrations of unlabeled wild type and mutant peptide or mutant LHR96–115(L103A/I105A) (lanes 4 and 8) or 125I-LHR96–115(L103A/I105A) (lanes 7 and 8) and treated with UV for 1 min (lane 4) or 0.3 mM SES (lane 8), respectively. These samples were processed as described in the legend to Fig. 1. The autoradiograph shows no affinity labeling of hCG as compared with successful labeling of hCG by wild type LHR96–115 (lanes 2 and 6). Lanes 1 and 5 show the control hCG samples that were incubated with the wild type peptide but without UV or SES treatment.

Fig. 4. Futile affinity labeling of hCG by mutant LHR96–115. hCG was incubated with mutant AB-125I-LHR96–115(K101A) (lanes 3 and 4) or 125I-LHR96–115(L103A/I105A) (lanes 7 and 8) and treated with UV for 1 min (lane 4) or 0.3 mM SES (lane 8), respectively. These samples were processed as described in the legend to Fig. 1. The autoradiograph shows no affinity labeling of hCG as compared with successful labeling of hCG by wild type LHR96–115 (lanes 2 and 6). Lanes 1 and 5 show the control hCG samples that were incubated with the wild type peptide but without UV or SES treatment.

Affinity for a peptide (20, 21). In contrast, the \( K_d \) value of mutant LHR96–115(K101A) was 5 mM, which is insignificant. This result, taken together with the futile labeling of denatured hCG (Fig. 5), shows the biological specificity of the binding and labeling of LHR96–115 to hCG. Furthermore, the results show that the interaction between hCG and LHR96–115 simulates the interaction between hCG and the receptor. Biological Specificity of Affinity Labeling—Although the affinity labeling is specific, our data do not show the biological significance of the affinity labeling. To test this concern, two different experiments were performed. In the first test, denatured hCG was tested for affinity labeling, and in the second the peptides were examined whether they could inhibit 125I-hCG binding to the receptor on intact cells. For the first test, denatured hCG was incubated with increasing concentrations of AB-125I-LHR96–115 or 125I-LHR96–115, and treated with UV or SES, respectively (Fig. 5). Denatured hCG was not labeled at all by either of the LHR peptide derivatives, despite high concentrations of the peptide probes. The results suggest the specificity of the affinity labeling for biologically active hCG. Since SES failed to cross-link 125I-LHR96–115 to denatured hCG, 125I-LHR96–115 appears to have a difficulty to recognize denatured hCG. To test this possibility, 125I-hCG was incubated with intact cells expressing LHR in the presence of increasing concentrations of the wild type or mutant peptide, LHR96–115 or LHR96–115(K101A) (Fig. 6). The wild type LHR96–115 inhibited 125I-hCG binding to the receptor with a \( K_d \) value of 43.4 \( \mu M \), suggesting its binding to the receptor with a reasonable 

Unlabeled Peptides (ng)

| 0 | 20 | 40 | 80 | 160 | 320 | 640 | 1280 | 2560 | 5120 |
|---|---|---|---|---|---|---|---|---|---|
| α | α | α | α | α | α | α | α | α | α |
| UV (min) | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| SES (mM) | 0 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |

Lanes 1–8: AB-125I-LHR96–115 or 125I-LHR96–115 treated with UV or SES for 1 min. Lanes 1–3: AB-125I-LHR96–115(K101A) and lanes 4–6: 125I-LHR96–115(K101A). Lanes 7–9: AB-125I-LHR96–115(K112A) and lanes 10–12: 125I-LHR96–115(K112A). Lanes 1 and 4: hCG alone; lanes 2 and 5: hCG treated with UV or SES; lanes 3 and 6: hCG treated with UV or SES and incubated with unlabeled wild type or mutant peptide; lanes 7 and 10: hCG treated with 0.3 mM SES; lanes 8 and 11: hCG treated with 0.3 mM SES and incubated with unlabeled wild type or mutant peptide; lanes 9 and 12: hCG treated with 0.3 mM SES and incubated with unlabeled wild type or mutant peptide. The autoradiograph shows no affinity labeling of hCG as compared with successful labeling of hCG by wild type LHR96–115 (lanes 2 and 6). Lanes 1 and 5 show the control hCG samples that were incubated with the wild type peptide but without UV or SES treatment.

Unlabeled Peptides (ng)

| 0 | 20 | 40 | 80 | 160 | 320 | 640 | 1280 | 2560 | 5120 |
|---|---|---|---|---|---|---|---|---|---|
| α | α | α | α | α | α | α | α | α | α |
| UV (min) | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| SES (mM) | 0 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |

Lanes 1–8: AB-125I-LHR96–115 or 125I-LHR96–115 treated with UV or SES for 1 min. Lanes 1–3: AB-125I-LHR96–115(K101A) and lanes 4–6: 125I-LHR96–115(K101A). Lanes 7–9: AB-125I-LHR96–115(K112A) and lanes 10–12: 125I-LHR96–115(K112A). Lanes 1 and 4: hCG alone; lanes 2 and 5: hCG treated with UV or SES; lanes 3 and 6: hCG treated with UV or SES and incubated with unlabeled wild type or mutant peptide; lanes 7 and 10: hCG treated with 0.3 mM SES; lanes 8 and 11: hCG treated with 0.3 mM SES and incubated with unlabeled wild type or mutant peptide; lanes 9 and 12: hCG treated with 0.3 mM SES and incubated with unlabeled wild type or mutant peptide. The autoradiograph shows no affinity labeling of hCG as compared with successful labeling of hCG by wild type LHR96–115 (lanes 2 and 6). Lanes 1 and 5 show the control hCG samples that were incubated with the wild type peptide but without UV or SES treatment.

Photoaffinity labeling of hCG with AB-125I-LHR96–115

The results show the biological specificity of the binding and labeling of LHR96–115 to hCG. Furthermore, the results show that the interaction between hCG and LHR96–115 simulates the interaction between hCG and the receptor.
LHR96–115(K112A) was cross-linked to hCG with SES significantly better than 125I-LHR96–115(K101A) (Fig. 9B). However, neither of the derivatized peptides labeled denatured hCG, indicating a specificity of affinity labeling of hCG by AB-125I-LHR96–115 and 125I-LHR96–115 (data not shown). Taken together, these results indicate that Lys 101 is more suitable for affinity labeling hCG than Lys 112 is. They also suggest that Lys 101 is at or near the hCG contact point as suggested by the computer model that the short β strand is a ligand contact site, and the Lys 112 is projected toward ligand (Fig. 7). In contrast, Lys 112 is located near the α helix as part of the outer lining of the donut structure, at the opposite side from the ligand binding site. Since Lys 101 is in the N-terminal area of LHR96–115, whereas Lys 112 is in the C-terminal region, one way to verify the conclusion is to use peptide mimics covering the sequences upstream and downstream of LHR96–115. To this end, we synthesized two peptide mimics, LHR84–104 and LHR113–132, and tested them for their ability to inhibit photoaffinity labeling of hCG by AB-125I-LHR96–115 and affinity cross-linking of 125I-LHR96–115 to hCG (Fig. 10). LHR84–104 and LHR113–132 inhibited the affinity labeling of hCG, but their potency was less than that of LHR96–115. LHR84–104 was more effective in inhibiting hCGα than LHR113–132 was. On the other hand, LHR85–104 was similar to LHR113–132 in inhibiting the labeling of hCGβ.

**Interaction of LHR96–115-hCG Complex with Exoloops—**In the preceding article (13), we pointed out the absolute homology in the 8 residues (boldface) in the sequence among cloned LHR, follicle-stimulating hormone receptor, and thyroid-stimulating hormone receptor.

![Fig. 5](https://example.com/fig5.png)

**Fig. 5.** Denatured hCG is not affinity-labeled. Denatured hCG (200 ng) was incubated with increasing concentrations of AB-125I-LHR96–115 (A) or 125I-LHR96–115 and treated with UV for 60 s or 0.3 mM SES (B). The samples were processed as described in the legend to Fig. 1.

![Fig. 6](https://example.com/fig6.png)

**Fig. 6.** Inhibition of 125I-hCG binding to the receptor by LHR peptides. 125I-hCG was incubated with intact 293 cells expressing LHR in the presence of increasing concentrations of unlabeled wild type and mutant LHR96–115 peptides. After washing cells several times to remove unbound 125I-hCG, cells were counted for the bound 125I-hCG as described under “Experimental Procedures.” The results were plotted against the concentrations of unlabeled peptides (left panel) and converted to Scatchard plots (right panel). The $K_d$ values of individual peptides in the table were determined with standard deviations based on the bound/free and bound peptide values as described previously (16).

![Fig. 7](https://example.com/fig7.png)

**Fig. 7.** Model of LRR4. Lys101 and Lys112 in LRR4 are projected on the opposite side of LRR4 (13).
ulating hormone receptor of various species. Furthermore, we showed that the tandem three conserved residues, Asn\(^{107}\)-Thr\(^{108}\)-Gly\(^{109}\), were more important for cAMP induction than hormone binding. This is unique because the exodomain is responsible for high affinity hormone binding and mutations in the exodomain impact hormone binding, which in turn affected cAMP induction, not the other way around. Therefore, we have raised the possibility that this region may be involved in the interaction with the endodomain and, thus, in signal generation. This is a crucial issue, because the exodomain and endodomain are known to interact (22–25), and this interaction regulates the generation of hormone signals (22), (23). However, the exact contact points in the exodomain and endodomain are unknown. Since the three exoloops in the endodomain are a logical candidate for the exodomain/endodomain interaction, we have synthesized peptide mimics for the exoloops 1, 2, and 3 of LHR (LHRexo1, LHRexo2, and LHRexo3) and tested whether they could inhibit the photoaffinity labeling of hCG by AB-125I-LHR\(^{96–115}\) (Fig. 11). LHRexo2 effectively inhibited the photoaffinity labeling, whereas the inhibition by LHR exo1 was less. In contrast, LHRexo3 did not inhibit the labeling. These differential effects suggest the specificity of the inhibition.

**DISCUSSION**

Our results show that AB-125I-LHR\(^{96–115}\) photoaffinity labels hCG. Ample evidence is presented to support the specificity of the photoaffinity labeling under rigorous conditions. The labeling is saturable and dependent on the hCG concentration, derivatized 125I-LHR\(^{96–115}\) concentration, and UV activation. AB-125I-LHR\(^{96–115}\) photoaffinity labels bioactive hCG but not denatured hCG. This labeling is blocked by nonderivatized wild type LHR\(^{96–115}\) but not by nonderivatized mutant LHR\(^{96–115}\)(L103A/I105A). The same Ala mutations in LHR abolish the hCG binding activity of LHR. Furthermore, AB-125I-LHR\(^{96–115}\)(L103A/I105A) does not photoaffinity label bioactive hCG and denatured hCG. LHR\(^{96–115}\) inhibits 125I-hCG binding to the receptor expressed on intact cells but LHR\(^{96–115}\)(L103A/I105A) is not capable of inhibiting 125I-hCG binding to the receptor. To avoid the potential interference of the photoactivable group on binding of AB-125I-LHR\(^{96–115}\) to the receptor and the subsequent labeling, 125I-LHR\(^{96–115}\) was affinity-cross-linked to hCG with SES. This affinity labeling is equally successful with similar specificity.

Both subunits of hCG are labeled, indicating that the UV-activatable group coupled to AB-125I-LHR\(^{96–115}\) can reach them. This is consistent with other studies (26–28) and not surprising, since the two subunits are closely intertwined in the crystal structure (29, 30). Interestingly, hCGs was preferentially labeled. Obviously, the reagent more readily reaches and labels the \(\alpha\) subunit than the \(\beta\) subunit. Since the maximum labeling distances of AB is 7 Å (19), hCG\(^{a}\) is likely to contact AB-125I-LHR\(^{96–115}\). Our results are inconsistent with the unlikely possibility that the peptide associates with hCG at sites other than the receptor contact site, impacts the global structure of hCG, and interferes with the hormone/receptor interaction. Since
LHR96–115 inhibits hCG binding to the receptor, AB-125I-LHR96–115 interacts with hCG at or near a contact site of hCG and the LH/CG receptor. It is significant that only one of the hCG subunits, but not both, is labeled, although two AB could be attached to the two Lys residues of LHR96–115. This suggests that only one of the Lys residues is close to hCG. Indeed, photoaffinity labeling using mutant peptides lacking one of the Lys residues shows that the AB coupled to Lys101 is capable of labeling hCG, whereas the AB attached to Lys112 is less effective. This is strong evidence to support the orientation of Lys101 and Lys112 in the LRR4 loop model (Fig. 7) and implicates the N-terminal region of LHR96–113, including the putative β strand of LRR4, in the interaction with hCG.

The crystallization of Leu-rich repeats (11, 31) and their presence in the middle of the exodomain of all glycoprotein hormone receptors (1) generated much speculation (8, 32–34) that the eight to nine LRRs provide the primary contact site for the cognate ligands, LH/CG, FSH, and TSH. They comprise the bulk of the exodomain at its center and are computer-modeled to show a crescent structure. The inner surface of the crescent consists of β sheets of the repeats and is thought to be the ligand contact site (8, 31, 32), perhaps interacting with the putative receptor binding αC terminus and seat belt side of hCG (29). However, little experimental evidence has been available to support these popular views. Our results of this and the preceding articles (12, 13) are the first experimental evidence supporting the LRR structure of LHR and the direct interaction of the LRR4 β strand with hCG. Our studies have laid the groundwork to determine the contact residues of the receptor and the hormone.

It has been known that LHR interacts with hCG initially at the exodomain, and the exodomain-hCG complex impacts the endodomain. This secondary contact is thought to generate the hormone signals (22, 23). There is evidence that the exodomain and endodomain are intimately associated before and after hormone binding (24, 25). This association is crucial because it affects the hormone binding affinity and provides a mechanism for the signal generation (24, 25). Unfortunately, there are few clues to the site of the interaction between the exodomain and endodomain except the recent reports implicating exoloops 2 and 3 (24, 25). The observations described in this and preceding articles (12, 13) show the involvement of LRR4 in the signal generation, implicating exoloop 2 and, perhaps, exoloop 1 as contact points of the exodomain/hCG complex. In fact, our computer modeling shows that the exoloop 2 projects straight up from the connecting the transmembrane 4 and 5, like a hairpin, toward the exodomain. It will be interesting to see whether the hairpin structure of exoloop 2 interacts with the crescent LRR structure of the exodomain, in particular LRR4. Such an exodomain/endodomain interaction could provide a...
mechanism for the mutual modulation of the two distinct domains (24, 25) and signal generation.

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