Human chorionic gonadotropin (hCG) consists of an α subunit and a β subunit. The existing evidence from various studies using truncation, substitution, synthetic hormone peptides, and hCG crystals suggests that the C-terminal region of the α subunit contacts the luteinizing hormone/chorionic gonadotropin (LH/CG) receptor and is involved in receptor activation. Despite a deluge of the speculation and the important role of the α C-terminal region, direct evidence for its interaction with the receptor has been elusive. Because of the significant biological activity, it is imperative to prove the interaction of the α C-terminal region. For this purpose, decamer peptides corresponding to the α subunit sequence from His83 to Ser92 (α83–92) were derivatized with the N-hydroxysuccinimide ester of 4-azidobenzoylglycine (ABG) and radioiodinated. The resulting ABG-125I-α83–92 was capable of binding and activating the LH/CG receptor. Furthermore, UV-sensitive ABG-125I-α83–92 exclusively photoaffinity-labeled an ~86-kDa molecule. This labeled molecule was shown to be the LH/CG receptor by various methods including immunoprecipitation by anti-LH/CG receptor antiserum. In addition, evidence is presented that the amino group of α Lys91 of α83–92 is in such close proximity to a carbonyl group of the receptor that this pair is cross-linked to form an amide, a zero-length cross-link. This low affinity contact of α83–92 and the receptor is sufficient for receptor activation and is crucial for the full understanding of the mechanistics of the receptor activation steps.

hCG is a placental hormone and is involved in maintenance of the corpus luteum during pregnancy in human females. It is a member of the glycoprotein hormone family which includes LH, FSH, TSH and equine chorionic gonadotropin. These hormones consist of a common β subunit and a distinct α subunit, which are noncovalently associated (1).

Photoaffinity labeling studies demonstrated that both α and β subunits affinity-labeled the LH/CG receptor (2). The truncation or substitution of hCG C-terminal amino acid residues reduces the receptor-binding affinity and abolishes cAMP induction (3–6). This is consistent with the observation that a dodecamer peptide corresponding to the hCG α C-terminal region, α81–92, inhibited 125I-hCG binding to the receptor (7) and that a decamer peptide, α83–92, was capable of binding to cells possessing the LH/CG receptor and inducing cAMP synthesis (6). Also, the crystal structure of HF treated hCG suggests the α C-terminal region as part of the potential receptor binding site (8, 9). Recently, a study using a set of reciprocal mutants of the NH2 and the LH/CG receptor, hCGα81–92Asp and LH/CG-Rα81–92Lys, indicated that the two amino acids were complementary in receptor activation (10). These results predict that the C-terminal region of hCGα interacts with the receptor and is crucial for receptor-activation to induce cAMP synthesis. Despite the increasing number in speculation, direct proof has been elusive for the crucial interaction of the C-terminal region of hCGα with the receptor. For this reason, it is necessary to demonstrate the direct interaction of the C-terminal region of hCGα with the receptor. In this communication, we present the first unequivocal evidence for this interaction. Furthermore, evidence is presented that α Lys91 of hCG is near to a carbonyl group of the receptor and that this ion pair is susceptible to cross-linking with EDC to form an amide bond.

EXPERIMENTAL PROCEDURES

Materials—hCG CR127 was supplied by the National Hormone and Pituitary Program. Peptides were synthesized by Multiple Peptide Systems (San Diego, CA) and purified as described previously (7). The N-hydroxysuccinimide ester of 4-azidobenzoylglycine (NHS-ABG) was synthesized, α83–92 was derivatized with NHS-ABG, and it was radioiodinated as described previously (11). The resulting ABG-125I-α83–92 was fractionated on a Sephade G-10 column. It is important to carefully moniodinate the peptide and preserve its biological activity during the harsh iodination reaction steps.

Binding, cAMP Induction, and Photoaffinity Labeling—Human embryonic kidney 293 cells were transfected with the LH/CG receptor construct as described previously (10). Cells transiently expressing the LH/CG receptor (293*) were incubated with 125I-α83–92, ABG-125I-α83–92 or 125I-NaCONHα83–92 in the presence of increasing concentrations of α83–92. After washing cells three times until nonspecific binding was insignificant, the radioactivity of bound 125I-α83–92, ABG-125I-α83–92 or 125I-NaCONHα83–92 was determined. Heat denatured fetal calf serum and bovine serum albumin, 15 and 3%, respectively, were used to reduce nonspecific binding. For cAMP assay, 293* cells were incubated with increasing concentrations of α83–92, ABG-α83–92 or NaCONH-α83–92 and intracellular cAMP was determined as described previously (10). For photoaffinity labeling, 293* cells were incubated with ABG-α83–92 and washed three times. The cells were irradiated with an ACME-LITE model 228A xenon flash lamp (12). UV light from the flashes is capable of activating UV sensitive groups such as the arylazide of ABG. Each flash lasts ~0.2 ms and minimizes random colli-
RESULTS AND DISCUSSION

As shown in Fig. 1, 125I-α83–92, ABG-125I-α83–92 and 125I-NAC/CONH2-α83–92 bound to the LH/CG receptor on 293 cells with $K_d$ values of 126 μM ($p < 0.05$), 264 μM ($p < 0.05$) and 253 μM ($p < 0.05$), respectively. They were capable of inducing cAMP synthesis with $EC_{50}$ values of 86 μM ($p < 0.05$), 135 μM ($p < 0.05$) and 100 μM, respectively. These results demonstrate the bioactivity of the peptide and its derivatives. To determine the identity of their binding molecule, increasing numbers of 293 cells were incubated with 125I-ABG-α83–92, irradiated with xenon flashes, and electrophoresed. The gel was exposed to x-ray film. The lower band represents 125I-ABG-α83–92 which was originally bound to 293 cells but dissociated during solubilization in sodium dodecyl sulfate. The ~86-kDa band represents the photoaffinity labeled band material.

![Binding of hCGα C Terminus to the LH/CG Receptor](https://example.com/image)

**Fig. 1. Biological activities of α83–92, ABG-α83–92 and NAC/CONH2-α83–92.** For the receptor binding assay, cells expressing the LH/CG receptor (293) were incubated with a constant amount of 125I-ABG-α83–92, 125I-NAC/CONH2-α83–92 in the presence of increasing concentrations of α83–92. After washing, the cell-bound radioactivity was measured. The data were used in Scatchard plots to determine $K_d$ values. For cAMP induction, cells expressing the LH/CG receptor were incubated with increasing concentrations of α83–92, ABG-α83–92 or NAC/CONH2-α83–92 and intracellular cAMP concentrations were determined. These experiments were repeated three times, each in triplicate. The data were analyzed using Student’s t-test.

![Photoaffinity labeling](https://example.com/image)

**Fig. 2. Photoaffinity labeling.** Increasing concentrations of 293 cells were incubated with 125I-ABG-α83–92 and irradiated with xenon flashes. Cells were solubilized and electrophoresed. The gel was exposed to x-ray film. The lower band represents 125I-ABG-α83–92 which was originally bound to 293 cells but dissociated during solubilization in sodium dodecyl sulfate. The ~86-kDa band represents the photoaffinity labeled band material.

Taken together these results clearly indicate 125I-ABG-α83–92 binding to the LH/CG receptor. Therefore, they suggest that the ~86-kDa band represents photoaffinity labeled LH/CG receptors. To obtain more direct evidence for this conclusion, the photoaffinity labeled band material was immunoprecipitated using rabbit anti-LH/CG receptor antiserum (Fig. 4). When 293 cells were incubated with 125I-ABG-α83–92, photolyzed, solubilized in Triton X-100, and immunoprecipitated with anti-LH/CG receptor antiserum, the ~86-kDa band was precipitated (Fig. 4). However, it was not precipitated when normal rabbit sera was used, when 293 cells were processed in the...
absence of $125^\text{I}$-ABG-$\alpha^{83–92}$, or when 293$^-$ cells were used instead of 293$^+$ cells. These results, along with the photoaffinity labeling data in Figs. 1–3, positively identify the photoaffinity-labeled 86-kDa band as the LH/CG receptor. $125^\text{I}$-ABG-$\alpha^{83–92}$ has two amino groups which could have been derivatized with ABG. They are the amino group of Lys$^{\text{91}}$ and the N-terminal amine. Our data cannot specify whether or not the amino group of Lys$^{\text{91}}$ was involved in the photoaffinity labeling of the receptor. Furthermore, it is difficult to predict the chemical group(s) of the receptor which was labeled as the phenyl nitrene reacts nonspecifically with a variety of functionalities (16). Since this information is necessary to define the cross-linked point of the peptide and the receptor, we have synthesized $125^\text{I}$-NaC/CONH$_2$$\alpha^{83–92}$ of which the N-terminal amine of $\alpha^{83–92}$ was acetylated and the C-terminal carboxylate was amidated. This modified peptide was used for affinity labeling the receptor. Transiently transfected 293$^+$ cells were incubated with $125^\text{I}$-NaC/CONH$_2$$\alpha^{83–92}$ and washed to remove unbound peptide. The cells complexed with $125^\text{I}$-NaC/CONH$_2$$\alpha^{83–92}$ were treated with EDC and solubilized for gel electrophoresis. The sample treated with EDC showed the 86-kDa band of the receptor which was labeled with $125^\text{I}$-NaC/CONH$_2$$\alpha^{83–92}$ and the N-terminal amine of Lys$^{\text{91}}$ was in the proximity of a loop in the hCG crystal and Met$^{\text{47}}$, the residue closest to Lys$^{\text{91}}$. Therefore, it is not expected for $\alpha^{83–92}$ to cross-link to a carbohydrate group to form an amide. They suggest that the amino group and side chain of hCG $\alpha$-Lys$^{\text{51}}$ is in the proximity of a carbohydrate group of the receptor in the hormone receptor complex. EDC induced a zero length cross-link between these two counter ions suggests the existence of a salt bridge between them. This conclusion is consistent with the complementarity of hCG $\alpha$-Lys$^{\text{51}}$ and LH/CG receptor's Asp$^{\text{97}}$ (10). The binding and labeling site of $125^\text{I}$-ABG-$\alpha^{83–92}$ appears to be specific since it was blocked by $\alpha^{38–57}$, but not by two other peptides, $\alpha^{26–46}$ and $\alpha^{38–57}$ which are known to inhibit $125^\text{I}$-hCG binding to the receptor. This is consistent with the x-ray crystal structure of HF treated hCG (8, 9). In the crystal, the $\alpha$C terminus shows a structure up to $\alpha$Ty$^{\text{99}}$. The last three residues of the $\alpha$C terminus, His$^{\text{90}}$-Lys$^{\text{91}}$-Ser$^{\text{92}}$ does not show a structure, despite their presence in an open $\sim 5$ nm solvent channel. This result suggests that $\alpha$His$^{\text{90}}$-Lys$^{\text{91}}$-Ser$^{\text{92}}$ are flexible. It is interesting to speculate the $\alpha$C-terminal positions up to $\alpha$Ty$^{\text{99}}$, in relationship with $\alpha^{26–46}$ and $\beta^{38–57}$. $\alpha^{26–46}$ is part of a loop in the hCG crystal and $\alpha$Me$^{\text{47}}$, the residue closest to $\alpha$Ty$^{\text{99}}$ and the $\alpha$C-terminal peptide backbone, is 16.5 Å away from $\alpha$Ty$^{\text{99}}$. Therefore, it is not expected for $\alpha^{26–46}$ to compete with $125^\text{I}$-ABG-$\alpha^{83–92}$ for receptor binding, consistent with the data in Fig. 3. On the other hand, $\beta^{38–57}$ is closer to $\alpha$Ty$^{\text{99}}$, $\beta$Gln$^{\text{54}}$ being 8.6 Å away from $\alpha$Ty$^{\text{99}}$. Therefore, it may be possible for $\beta^{38–57}$ to weakly interfere with, although not completely block, binding of $125^\text{I}$-ABG-$\alpha^{83–92}$ to the receptor. In fact, the intensity of the photoaffinity labeled 86-kDa band is somewhat reduced in the presence of $\beta^{38–57}$ in Fig. 3. In this study we demonstrate that $\alpha^{83–92}$ specifically interacts with the LH/CG receptor. In this complex of $\alpha^{83–92}$
and the receptor, the amino group of hCG αLys91 is near to a carboxyl group of the receptor to form an ion pair. This interaction between the peptide and the receptor is sufficient to activate the receptor to induce cAMP synthesis as does the hormone itself.

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Binding of hCGα C Terminus to the LH/CG Receptor

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Photoaffinity Labeling of the Lutropin Receptor with Synthetic Peptide for Carboxyl Terminus of the Human Choriogonadotropin Subunit
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