Spatial relationships of the human chorionic gonadotropin (hCG) subunits in the assembly of the hCG–receptor complex in the luteinized rat ovary

(affinity cross-linking/corpus luteum)

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ABSTRACT In an attempt to examine the spatial relationships of the human chorionic gonadotropin (hCG) subunits in the assembly of the hCG–receptor complex, the recombinant ^{125}I -labeled hCG, with label in either the α subunit or the β subunit, was cross-linked to the luteinizing hormone (LH)/hCG receptor. The efficacy of the cross-linking of the ^{125}I - α subunit or the ^{125}I - β subunit of hCG to the LH/hCG receptor was then examined. The autoradiographic profile of ^{125}I -hCG–receptor complex containing the label in the α subunit of hCG showed that the α subunit can cross-link with all four subunits of the LH/hCG receptor. However, only one faint labeled band, corresponding to $M_r = 68,000$, was detected when the ^{125}I -hCG–receptor complex with label in the β subunit was subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis under reducing conditions. When the electrophoresis was performed under nonreducing conditions, the $M_r 68,000$ band disappeared concomitantly with the accumulation of radioactivity in the high molecular weight region. These results indicated that the β subunit of hCG, unlike the α subunit, can cross-link only weakly with the smallest subunit of the LH/hCG receptor. A comparison of the differential effectiveness of the cross-linking of ^{125}I - α subunit with ^{125}I - β subunit of hCG to the LH/hCG receptor suggests that both α and β subunits of hCG are intimately associated with the receptor, but the bulk of the β subunit of hCG is buried in between the receptor and the α subunit of hCG. On the basis of our data, a model for the spatial arrangement of hCG subunits in the hCG–receptor complex is proposed.

Human chorionic gonadotropin (hCG), a glycoprotein hormone, is composed of two dissimilar noncovalently bound subunits designated as α ($M_r = 16,000$) and β ($M_r = 22,000$) (1). The α subunit is common to all glycoprotein hormones and the β subunit confers the biological activity of the hormone. However, binding to the receptor occurs only when the hormone is in the $\alpha\beta$ form. Since neither subunit alone can effectively bind to the receptor with high affinity or exert biological effect (2, 3), the direct interaction of the subunits in the hormone–receptor complex has been difficult to assess. Investigators have approached this problem by chemical modification of either α or β subunit in the intact hCG, followed by examining the binding and biological activity of the modified hCG (4–14). With this approach, it has been reported that both subunits of hCG interact with receptor (4–14). However, information regarding the spatial relationship of the subunits of hCG in the hormone–receptor complex is still obscure. In this communication, we report the results obtained by using a chemical cross-linking approach to probe the spatial relationships of the subunits of

hCG in its complex with receptor. Using this probe, we have compared the effectiveness of the cross-linking of the α subunit of hCG to the luteinizing hormone (LH)/hCG receptor with that of the β subunit. On the basis of these studies, a model for the assembly of the hCG–receptor complex is proposed.

MATERIALS AND METHODS

Purified α subunit (CR-119-3), β subunit (CR-119-3), and hCG (CR-121, 13,450 international units/mg) were generously supplied by Robert E. Canfield of Columbia University. Bovine LH (NIH-LHB9) was a gift from the Hormone Distribution Program, National Institutes of Health (Bethesda, MD). Gonadotropin-releasing hormone (GnRH) was a gift from Parke–Davis. Cholera toxin was a product of Schwarz/Mann. Collagenase (CLS, type I) was obtained from Worthington. Insulin, hCG, pregnant mare's serum gonadotropin, dithiothreitol, ϵ -amino-*n*-caproic acid, phenylmethylsulfonyl fluoride, and *N*- α -*p*-tosyl-L-lysine chloromethyl ketone were purchased from Sigma. Sephadex G-100 was obtained from Pharmacia. Eagle's minimal essential medium with Earle's salts (medium 109) was obtained from GIBCO. IODO-GEN, disuccinimidyl suberate (DSS), and 3,3'-dithiobis(succinimidyl propionate) (DSP) were purchased from Pierce. ^{125}I -labeled β subunit of hCG was prepared by the procedure of Salacinski *et al.* (specific radioactivity 15–20 mCi/ μg ; 1 Ci = 37 GBq) (15, 16). Crystallized bovine serum albumin was obtained from Miles. Reagents for electrophoresis were purchased from Bio-Rad. Kodak XR-5 film, Lightning-Plus intensifying screen, and X-Omatic cassette (8 $\times$ 10 in.) were obtained from Eastman Kodak. All other reagents used were of analytical reagent grade.

The subunits were recombined by using a modification of the method reported by other laboratories (17, 18). The purified ^{125}I - β subunit was recombined with unlabeled α subunit in a ratio of approximately 1:2 (β : α , wt/wt) in a 0.02 M sodium phosphate buffer, pH 7.5. Incubation was for 24 hr at 37°C. The resulting material was chromatographed on a Sephadex G-100 column (100 $\times$ 1.6 cm) equilibrated with 0.02 M sodium phosphate buffer, pH 7.5. The column was eluted with the same buffer and 3.0-ml fractions were collected. The elution profile of recombined ^{125}I -hCG(β) and free ^{125}I - β subunit was monitored with an automatic gamma counter (Searle Analytic model 1195).

The procedures for obtaining highly luteinized ovaries, preparation of collagenase-dispersed luteal cells, ^{125}I -hCG

Abbreviations: hCG, human chorionic gonadotropin; ^{125}I -hCG(α), ^{125}I -hCG with label in the α subunit; ^{125}I -hCG(β), ^{125}I -hCG with label in the β subunit; LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone; DSS, disuccinimidyl suberate (a noncleavable cross-linker); DSP, 3,3'-dithiobis(succinimidyl propionate) (a thiol-cleavable cross-linker).

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binding, cross-linking conditions, electrophoresis, and autoradiography have been described previously (19). Briefly, aliquots of cells (2×10^6 cells) were incubated with either recombinant $0.5 \text{ nM } ^{125}\text{I-hCG}(\beta)$ or $0.5 \text{ nM } ^{125}\text{I-hCG}(\alpha)$ for 2 hr at 37°C in the presence or absence of $1.5 \text{ }\mu\text{M}$ unlabeled hCG. After removal of the unbound $^{125}\text{I-hCG}(\beta)$ or $^{125}\text{I-hCG}(\alpha)$ from the cells by centrifugation and decantation, the cells were washed once and the $^{125}\text{I-hCG}(\beta)$ - or $^{125}\text{I-hCG}(\alpha)$ -equilibrated cells were suspended in $180 \text{ }\mu\text{l}$ of medium 109 and allowed to react with 1 mM DSS at 4°C for 15 min. The resulting $^{125}\text{I-hCG}(\beta)$ -linked luteal cells and $^{125}\text{I-hCG}(\alpha)$ -linked luteal cells were solubilized with 2% NaDodSO₄ solution and subjected to NaDodSO₄/PAGE followed by autoradiography.

RESULTS

Characterization of the Recombined $^{125}\text{I-hCG}(\beta)$. The binding specificity of free $^{125}\text{I-}\beta$ subunit and $^{125}\text{I-hCG}(\beta)$ to rat luteal cells is shown in Table 1. Under the present experimental conditions, there is no specific binding of the free $^{125}\text{I-}\beta$ subunit to luteal cells. However, $^{125}\text{I-hCG}(\beta)$ prepared by recombining the unlabeled α subunit with $^{125}\text{I-}\beta$ subunit binds to luteal cells as effectively as $^{125}\text{I-hCG}$ prepared by radiolabeling the intact hCG. It has been demonstrated that radioiodination of intact hCG yields $^{125}\text{I-hCG}$ with label predominantly in the α subunit (20). The binding of $^{125}\text{I-hCG}(\beta)$ to luteal cells was partially inhibited by $1.5 \text{ }\mu\text{M}$ unlabeled α subunit of hCG and completely inhibited by $1.5 \text{ }\mu\text{M}$ unlabeled β subunit of hCG. This inhibition may be due to a minor contamination with intact hCG. The identity of recombinant $^{125}\text{I-hCG}(\beta)$ was also examined by NaDodSO₄/PAGE under reducing conditions after $^{125}\text{I-hCG}(\beta)$ was cross-linked with various concentrations of DSS from 0 to 2 mM (Fig. 1). A radioactive band with electrophoretic mobility slower than that of the $^{125}\text{I-}\beta$ subunit band became gradually more intense, and the intensity of $^{125}\text{I-}\beta$ subunit became gradually less prominent with increasing concentrations of DSS up to 2 mM. These results indicate that the slow-moving radioactive band is derived from the $^{125}\text{I-}\beta$ subunit after cross-linking with the α subunit. The identity of the slow-moving band was established as the $\alpha\beta$ dimer rather than an aggregate of β subunit since the electrophoretic mobility of this slow-moving band was similar to the product obtained from DSS treatment of $^{125}\text{I-hCG}$ in which the α subunit was labeled (19). This experiment also demonstrated that, except

Table 1. Binding specificity of $^{125}\text{I-}\beta$ subunit and $^{125}\text{I-hCG}(\beta)$ to rat luteal cells

Unlabeled protein added	Label bound, cpm	
	$^{125}\text{I-}\beta$ subunit	$^{125}\text{I-hCG}(\beta)$
—	784 ± 102	$13,962 \pm 724$
hCG	824 ± 35	786 ± 64
LH	—	$1,009 \pm 121$
α subunit of hCG	813 ± 132	$5,933 \pm 473$
β subunit of hCG	831 ± 50	$1,135 \pm 79$
Cholera toxin	—	$13,699 \pm 743$
GnRH	—	$13,545 \pm 899$
Insulin	—	$13,267 \pm 800$
Bovine serum albumin	—	$13,257 \pm 1067$

Luteal cells (2×10^6 cells) in medium 109 were incubated with either $^{125}\text{I-}\beta$ subunit of hCG ($150,000 \text{ cpm}$) or $^{125}\text{I-hCG}(\beta)$ ($150,000 \text{ cpm}$) in the presence of various unlabeled proteins ($1.5 \text{ }\mu\text{M}$) at a final volume of 0.4 ml . After incubation at 37°C for 2 hr, the cell-bound labeled ligand was separated from free labeled ligand by the addition of 1 ml of ice-cold 25 mM Tris-HCl buffer, pH 7.4, followed by centrifugation at $3000 \times g$ for 10 min. The supernatants were removed by suction and the radioactivities in the cell pellets were determined in a gamma counter (Searle Analytic model 1195). Results are mean $\pm$ SD.

FIG. 1. Autoradiographic analysis of the effect of DSS at various concentrations on the cross-linking of α and β subunit in $^{125}\text{I-hCG}(\beta)$. Aliquots of recombinant $^{125}\text{I-hCG}(\beta)$ ($280,000 \text{ cpm}$ in $180 \text{ }\mu\text{l}$) were cross-linked at the indicated concentrations of DSS at 4°C for 15 min. The reaction was stopped by adding $50 \text{ }\mu\text{l}$ of 1 M ammonium acetate, followed 5 min later by the addition of 1 ml of 0.0625 M Tris-HCl buffer, pH 6.8, containing 10% (vol/vol) glycerol, 2% (wt/vol) NaDodSO₄, 0.001% bromophenol blue, 2% (vol/vol) 2-mercaptoethanol, and 8 M urea. Aliquots of each cross-linked sample ($25,000 \text{ cpm}$ in $100 \text{ }\mu\text{l}$) were boiled for 5 min in a water bath, electrophoresed in a 0.1% NaDodSO₄/10% acrylamide slab gel, and then stained, dried, and autoradiographed. The positions of the unlabeled α subunit, β subunit, and intact hCG are indicated on the left.

for the cross-linking of $\alpha\beta$ dimer, other nonspecific reactions produced by DSS were not detectable.

Effectiveness of Cross-Linking of the β Subunit of hCG to the LH/hCG Receptor. Attempts were then made to cross-link the β subunit of hCG to the LH/hCG receptor, using recombinant $^{125}\text{I-hCG}$ in which the β subunit was labeled. Experiments that examined the effect of the cross-linker concentration, reaction time, and temperature on $^{125}\text{I-hCG}$ -receptor cross-linking with $^{125}\text{I-hCG}$ containing the label in the α subunit have been reported previously (19). The conditions for the cross-linking reaction from our previous studies were applied to the $^{125}\text{I-hCG}(\beta)$ -receptor cross-linking experiments. The specificity of $^{125}\text{I-hCG}(\beta)$ cross-linking to the LH/hCG receptor was then examined (Fig. 2). Briefly, the cells (2×10^6 cells) were incubated with recombinant $^{125}\text{I-hCG}(\beta)$ for 2 hr at 37°C in the presence of various unlabeled proteins in the incubation medium. After the unbound $^{125}\text{I-hCG}(\beta)$ was removed by centrifugation followed by washing, the $^{125}\text{I-hCG}(\beta)$ -equilibrated cells were suspended in medium 109 and allowed to react with 1 mM DSS at 4°C for 15 min. The $^{125}\text{I-hCG}(\beta)$ -linked luteal cells were solubilized with 2% NaDodSO₄ and subjected to NaDodSO₄/PAGE followed by autoradiography. Cholera toxin, insulin, GnRH, or bovine serum albumin at a concentration of $1.5 \text{ }\mu\text{M}$ had no effect on the binding of $^{125}\text{I-hCG}(\beta)$ to luteal cells, but $1.5 \text{ }\mu\text{M}$ unlabeled hCG and LH abolished the binding. The autoradiograms prepared after NaDodSO₄/PAGE in the presence of reducing agents (50 mM dithiothreitol and 2% 2-mercaptoethanol) showed three bands: one band corresponding to the $^{125}\text{I-}\beta$ subunit, the second to the $^{125}\text{I-hCG}(\beta)$, and the third to $M_r 68,000$, which probably represents the complex of $^{125}\text{I-}\beta$ subunit and receptor subunit.

FIG. 2. Specificity of cross-linking of ^{125}I -hCG(β) to rat luteal cells in the presence of various unlabeled proteins or peptides. Recombinant ^{125}I -hCG(β) (0.5 nM) was incubated with rat luteal cells at 37°C for 2 hr in the presence of the indicated unlabeled proteins or peptides at $1.5\ \mu\text{M}$. ^{125}I -hCG(β)-equilibrated luteal cells were then cross-linked with 1 mM DSS at 4°C for 15 min. These cells were solubilized in the presence of reductant, and the solubilized membrane proteins were electrophoresed on a 0.1% NaDodSO₄/10% acrylamide slab gel. Migration distances of the molecular weight markers are shown to the left of the autoradiogram.

Experiments were then designed to characterize the M_r 68,000 species. To accomplish this, the thiol-cleavable cross-linking agent DSP, which has properties analogous to DSS with respect to reactivity toward nucleophilic residues of protein, was used (21–23). This reagent serves as a cross-linker only when the electrophoresis is performed in the absence of reducing agents. The ^{125}I -hCG(β)-equilibrated luteal cells were covalently cross-linked with DSP, and the solubilized samples were then subjected to electrophoresis in the

presence of reductants (Fig. 3A, lane 3). As expected, samples cross-linked with DSP and then subjected to electrophoresis under reducing conditions lost the ^{125}I -hCG(β) and the M_r 68,000 species. Use of noncleavable cross-linker (DSS), on the other hand, produced the ^{125}I -hCG(β) and the M_r 68,000 species (Fig. 3A, lane 1). To examine whether the M_r 68,000 radioactive species was the product of ^{125}I - β subunit–receptor subunit complex or ^{125}I - β dimer, NaDodSO₄/PAGE was performed in the absence of reducing agent (Fig. 3B). If the M_r 68,000 radioactive species was the product of ^{125}I - β dimer, its mobility would not be expected to change in NaDodSO₄/PAGE under nonreducing conditions. However, if this radioactive species was the product of ^{125}I - β subunit–receptor subunit complex, it would disappear from the M_r 68,000 region concomitantly with the accumulation of radioactivity in the high molecular weight region, since we have previously shown that the LH/hCG receptor is an oligomeric molecule linked by disulfide bonds with M_r about 230,000–280,000 (19). The autoradiograms developed from the NaDodSO₄/PAGE performed in the absence of reducing agent showed that the M_r 68,000 band disappeared concomitantly with the accumulation of the radioactivity in the high molecular weight region (Fig. 3B). This observation clearly indicated that the M_r 68,000 radioactive species represents ^{125}I - β subunit-linked receptor subunit complex rather than the dimer of ^{125}I - β subunit.

Effectiveness of Cross-Linking of the α Subunit of hCG to the LH/hCG Receptor. To examine the effectiveness of cross-linking of the α subunit of hCG to the LH/hCG receptor, attempts were made to cross-link ^{125}I -hCG with the LH/hCG receptor containing label in the α subunit. The ^{125}I -hCG(α)-equilibrated luteal cells were covalently cross-linked with either DSS or DSP at 1 mM, and the solubilized samples were then subjected to NaDodSO₄/PAGE in the presence or absence of reductants (Fig. 4). As expected, samples cross-linked with cleavable cross-linker (DSP) and then subjected to NaDodSO₄/PAGE under reducing conditions (50 mM dithiothreitol and 2% 2-mercaptoethanol) lost the ^{125}I -hCG(α) and the ^{125}I - α subunit-linked protein species (Fig. 4A, lane 3). Use of noncleavable cross-linker (DSS), on the other hand, produced ^{125}I -hCG(α) and four ^{125}I - α subunit–receptor subunit complexes (M_r = 62,000, 74,000, 96,000, and 105,000) (Fig. 4A, lane 1). When NaDodSO₄/PAGE was per-

FIG. 3. Effectiveness of cross-linking of the β subunit of hCG to the LH/hCG receptor and effect of reductant on the electrophoretic migration of the ^{125}I - β subunit–receptor complex on NaDodSO₄/PAGE. Recombinant ^{125}I -hCG(β) (0.5 nM) was incubated with rat luteal cells at 37°C for 2 hr in the absence or presence of unlabeled hCG at $1.5\ \mu\text{M}$ as indicated. ^{125}I -hCG(β)-equilibrated luteal cells were then cross-linked with either 1 mM DSS (lanes 1, 2, 5, and 6) or 1 mM DSP (lanes 3, 4, 7, and 8). The ^{125}I -hCG(β)-linked luteal cells were solubilized with 50 mM dithiothreitol and 2% 2-mercaptoethanol (A) or without reductants (B) and subjected to electrophoresis on a 0.1% NaDodSO₄/10% acrylamide slab gel followed by staining, drying, and autoradiography. The positions of the molecular weight markers are indicated on the left side of the autoradiogram.

FIG. 4. Effectiveness of cross-linking of the α subunit of hCG to the LH/hCG receptor and effect of reductant on the electrophoretic migration of the ^{125}I - α subunit-receptor complex on NaDodSO₄/PAGE. ^{125}I -hCG(α) (0.5 nM) was incubated with rat luteal cells at 37°C for 2 hr in the absence or presence of 1.5 μM unlabeled hCG as indicated. ^{125}I -hCG(α)-equilibrated luteal cells were then cross-linked with either 1 mM DSS (lanes 1, 2, 5, and 6) or 1 mM DSP (lanes 3, 4, 7, and 8). The ^{125}I -hCG(α)-linked luteal cells were solubilized with 50 mM dithiothreitol and 2% 2-mercaptoethanol (A) or without reductants (B) and subjected to electrophoresis on a 0.1% NaDodSO₄/10% acrylamide slab gel followed by staining, drying, and autoradiography. The positions of the molecular weight markers are indicated on the left side of the autoradiogram.

formed under nonreducing conditions, a high molecular weight species was seen concomitant with the disappearance of the ^{125}I - α subunit-receptor subunit complexes (Fig. 4B). These observations suggest that the α subunit of hCG, unlike the β subunit, can effectively cross-link with all four subunits of the LH/hCG receptor.

DISCUSSION

In this communication, we have used a chemical cross-linking approach to probe the spatial relationship of the subunits of hCG in the hormone-receptor complex by comparing the efficacy of the α subunit of hCG in cross-linking to the LH/hCG receptor with that of the β subunit of hCG. The autoradiographic profile of ^{125}I -hCG-receptor complex, in which the α subunit of hCG was labeled, showed that the α subunit of hCG can cross-link with all four subunits of the LH/hCG receptor (19). However, when the radioactivity of ^{125}I -hCG was present on the β subunit, only one faint labeled band of M_r 68,000 was detected, corresponding to ^{125}I - β subunit-receptor subunit complex. The molecular weight difference between α subunit (16,000) and β subunit (22,000) is about 6000, which is the approximate difference between the ^{125}I - β subunit-receptor subunit complex (M_r , 68,000) and the smallest ^{125}I - α subunit-receptor subunit complex (M_r , 62,000), suggesting that these two species represent the same receptor subunit species interacting with the labeled α or β subunit. Given the constraint that the cross-linker is only 11 Å in length and on the basis of the differential effectiveness of the cross-linking of ^{125}I - α subunit and ^{125}I - β subunit of hCG to the LH/hCG receptor, we are proposing a model in which both α and β subunits of hCG are intimately associated with the receptor but the bulk of the β subunit is buried between the receptor and the α subunit (Fig. 5). Thus, the α subunit is accessible to be cross-linked to all four subunits of the receptor, since the α subunit is exposed. On the other hand, the β subunit can only weakly cross-link with the smallest subunit of the receptor, since the α subunit in the exterior masks the cross-linking sites between the β subunit and the other three receptor subunits. While proposing this model, we fully realize that hormone-receptor interaction is a dynamic complex process involving internalization and intracellular processing. Our model accounts for the spatial arrangement of the hormone-receptor complex only under equilibrium binding conditions since the cross-linking was

performed after the hormone-receptor complex had been formed.

It may be argued that the differential effectiveness of the cross-linking of ^{125}I - α subunit and ^{125}I - β subunit of hCG to the LH/hCG receptor may be due to the fact that the ^{125}I - β subunit of hCG has no available nucleophilic residues to cross-link with the receptor or that the ^{125}I - β subunit of hCG is situated distal to the receptor such that interaction is not feasible. However, the former possibility can be ruled out because the β subunit of hCG has four lysine residues. In addition, the ^{125}I - β subunit can cross-link with the α subunit of hCG. Nonetheless, we realize that not all the nucleophilic

FIG. 5. Model of the spatial relationships of subunits of hCG in its complex with receptor. The α subunit of hCG is exposed at the surface of the hormone-receptor complex and intimately bound to the LH/hCG receptor. The β subunit of hCG is also intimately bound to the LH/hCG receptor, and the bulk of this subunit is buried in between the receptor and the α subunit of hCG.

groups may be equivalent with respect to their reactivity. The extent to which those on the β subunit are less reactive may have a bearing on the proposed model. In light of other investigators' findings that the β subunit of hCG directly interacts with the LH/hCG receptor (24, 25), the second possibility is also considered unlikely.

It may be noticed that in Figs. 2 and 3A the intensity of the band corresponding to the ^{125}I - β subunit-receptor subunit complex is less than that of the band corresponding to the ^{125}I -hCG(β). Since DSS consists of two functional groups, one of which reacts with ^{125}I - β subunit of hCG while the other functional group is free to react with either the α subunit of hCG or the receptor subunit, the ^{125}I - β subunit of hCG should have the same probability of cross-linking to the receptor subunit as to the α subunit. However, the intensity of the band corresponding to ^{125}I -hCG(β) is stronger than that of the band corresponding to ^{125}I - β subunit-receptor subunit complex, suggesting that this is not the case. The uneven distribution of ^{125}I -hCG(β) and ^{125}I - β subunit-receptor subunit complex, as shown in Figs. 2 and 3A, may be due to the exposure of the α subunit of hCG in the exterior of hCG-receptor complex. Thus, there appeared to be more sites on the α subunit that reacted with DSS than on the ^{125}I - β subunit. After the first functional group of DSS has reacted with the α subunit, the second free functional group of DSS may cross-link with ^{125}I - β subunit to form ^{125}I -hCG(β). This leads to a greater distribution of radioactivity in ^{125}I -hCG(β) rather than in ^{125}I - β subunit-receptor subunit complex since most of the ^{125}I -hCG(β) has resulted from DSS first reacting with the α subunit and then cross-linking the ^{125}I - β subunit.

The effectiveness of ^{125}I - β subunit in covalently cross-linking with the LH/hCG receptor in rat luteal cells by the affinity cross-linking method is quite different from that observed in porcine granulosa cells with the photoaffinity labeling method (25). The difference in the autoradiographic profile between our findings and those of Ji and Ji (25) is caused by the differences in method. For example, the photoactive agent can remain in the interface between hormone and the receptor. Since the photoactive agent is already coupled to the ^{125}I - β subunit, it can be buried between the hormone and receptor during the binding reaction. However, DSS, in the present experiment, was added after the hormone-receptor complex had been formed and could not be inserted into the interface between the hormone and receptor. Therefore, the cross-linker reacted only on the surface of hormone-receptor complex. Thus, the results of Ji and Ji (25) are not contradictory to our finding.

The observation that a portion of the β subunit remains exposed after the hormone-receptor complex formation is consistent with the findings of Louvet *et al.* (26), who showed that antibodies to the COOH-terminal 30 amino acid region of the β subunit of hCG failed to block the ^{125}I -hCG binding activity. On the other hand, Moyle *et al.* (24) have used monoclonal antibodies to hCG to examine the effect of the immunological determinants of hCG on hormone-receptor binding. In their studies the addition of a monoclonal antibody, designated as B 101, in the ^{125}I -hCG binding assay medium abolished the formation of the hormone-receptor complex, suggesting that a portion of the β subunit is buried in between the hormone and the receptor. They also found

that an antibody to the α subunit, designated as A 103, had no effect upon ^{125}I -hCG binding to its receptor, suggesting that a portion of the α subunit is exposed. These observations are consistent with our model of hCG-receptor interaction as proposed in Fig. 5. Moyle *et al.* (24) also observed that an antibody specific to the α subunit, designated as A 102, blocked the binding and biological action of hCG, suggesting that this A 102 antigenic site on the α subunit is adjacent to the receptor. This observation is not contradictory to our proposed model since the α subunit of hCG, in our model, is intimately associated with the receptor.

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