Interaction and activation of luteinizing hormone receptor*

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The luteinizing hormone receptor is a G protein coupled receptor with unique structural and functional features, consisting of two halves. The N-terminal extracellular half (exodomain) binds the hormones, whereas the C-terminal membrane associated half (endodomain) is responsible for receptor activation. This article describes the sequence of the interactions among human chorionic gonadotropin, the exodomain and endodomain. For example, the hormone binds initially to the exodomain, the resulting hormone/exodomain complex modulates the interaction between the exodomain and endodomain, and this ternary complex is involved in signal generation. This sequence suggests that the exodomain and endodomain are intimately associated prior to the hormone binding, contrary to the view that the exodomain and endodomain are independent at least functionally.

Hormone, Receptor and Clinical Implication

Human chorionic gonadotropin (hCG) and luteinizing hormone (LH) are glycoprotein hormones. They belong to the glycoprotein hormone family, which also includes follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH). These glycoprotein hormones share the common α-subunit whereas the β-subunits are hormone specific. Only the αβ dimer is capable of endocrinologically binding to receptors to induce biological responses, whereas the dissociated subunits lose high affinity binding activity. The human α-subunit has 92 amino acids, the LHβ subunit has 121 amino acids, and the hCG β subunit has 145 amino acids long subunit. The sequences of LHβ and hCGβ are similar except 16 residues and the extra 24 amino acids at the hCG C-terminus. The two hormones have different oligosaccharides. Despite the differences, both hormones bind to the same receptor (LHR) and elicit similar physiological responses. hCG has been more frequently used than LH because it is more readily available, pure and stable. The glycoprotein hormones share similar receptors and hormonal responses.

LH is secreted from the pituitary gonadotropes and binds to the LHR expressed in thecal and granulosa cells in the ovary and Leydig cells in the testis. They control development of the gonads of both sexes and production of gametes. In the ovary, LH induces ovulation from a mature (Graffian) follicle and therefore, is essential for the ovulation (menstruation) cycle. On the other hand, hCG is produced in multinuclear syncytiotrophoblasts in the placenta. It is secreted seven days after fertilization and stimulates progesterone production in the ovarian corpus luteum, which in turn prevents the onset of the ovulation cycle and helps prepare and sustain implantation and thus, pregnancy. Therefore, the lack, insufficient quantity or blocking of hCG during early pregnancy will result in the termination of the pregnancy and miscarriage. The essential roles of the hormones and LH/CGR are underscored by the fact that numerous mutations cause infertility by rendering the receptor recessively inactive, the receptor dominantly active, or the hormones inactive. Approximately 16% of couples in the United States and also in the world suffer from infertility and/or other pathologies. Therefore, the receptor and hormones could be used as fertility drugs as well as contraceptives, including the morning after pill. The contraceptive drugs on the market are based on the steroid hormones, which have been established a half century ago. They impact gene expression in most body cells and cause serious side effects. Novel drugs based on gonadotropins and their receptors will be more specific, targeting primarily the gonads and thus reducing or eliminating the side effects. In fact, some of our mutant receptors and mutant hormones that were generated during our earlier studies could serve as the starting materials for such novel contraceptives and fertility drugs.

LHR and hCG play a central role in some cancers. Intact hCG and hCG fragments have been detected from trophoblastic and nontrophoblastic tumors including some ovarian and testicular tumors. On the
other hand, LHR is expressed in ovarian surface epithelium of heterogenous carcinoma and in approximately half of the ovarian carcinomas. LHR and hCG stimulate cell proliferation and inhibit apoptosis in ovarian surface epithelium and ovarian cancer cells, thus playing an important role in the development, progression, and chemoresistance of ovarian carcinomas. Most ovarian cancers are terminal because the current methods are only capable of detecting ovarian cancers at the terminal stage. Incidence of ovarian cancer in the US is ~20,700 patients per year with an annual death rate of 12,500. LHR and the hormones have also been implicated in prostate cancer, one of the most common male malignancies. Each year prostate cancer is diagnosed in 200,000 men in the United States, taking the lives of 38,000 American males. LHR has been also implicated in the attempt to restore some function, including induction of estradiol, of residual follicles in postmenopausal ovaries, thus improving the health of elderly women. Since more than 30 million elderly women are subjected to postmenopausal symptoms, this is one of the major health, social and political issues in this 21st century.

Consequently, LHR and the hormones have been or can be used for tumor detection and cancer therapeutics in addition to the pregnancy test, fertility, contraception and postmenopausal health care. For this purpose, it is crucial to understand the function and mechanism of LHR and the hormones in reproduction, menopause and tumorigenesis.

**hCG**

In 1994, the research groups of Drs. Neil Isaacs and Joyce Lustbader determined the crystal structure of deglycosylated hCG. It shows five disulfides in hCGα and six in hCGβ. Among them, only three disulfides in each subunit are essential for the structural integrity. They are Cys10-Cys60, Cys26-Cys82 and Cys32-Cys48 in hCGα and Cys8-Cys57, Cys34-Cys88 and Cys38-Cys40 in hCGβ. They form the cystine knot motif in each subunit and form the frame work of the α and β subunits. The three disulfides tie up four peptide chains in each subunit, thus forming three peptide loops, αL1, αL2, αL3, βL1, βL2 and βL3 (Fig. 1). The two subunits are intimately associated, fitting like clasped hands, and a segment of the β-subunit wrapping around the α-subunit like a seat belt. This structure and other studies using chemical modification, proteolytic digestion, photoaffinity labeling, affinity crosslinking, antibody recognition, peptide mimics and mutational analyses (substitution, deletion, truncation and hybrid formation) over the past 25 years have produced a large body of information. Most of the studies suggest that the αC-terminal tail and the β-strap loop play an important role in receptor binding and hormone action. Since these peptide segments are present in the concave face of hCG, it has been generally thought to be the receptor binding face. However, there is little evidence of their direct interaction with the receptor except our report on the photoaffinity labeling of the receptor by the αC-terminal tail. In addition, αL1 and αL3 are also implicated in receptor binding as are βL1 and βL2,20,21

During the 70’s and 80’s we synthesized a number of novel, photosensitive, radioiodinatable and heterobifunctional reagents (for reviews22,23), introduced for the first time the macromolecular photoaffinity labeling technique24, and used them to study the receptor binding of hCG and other protein ligands. The hCG studies showed that Lys residues of the α subunit are involved in covalent labeling of three distinct regions of LHR. Two of the labeled regions of the receptors are in the extracellular domain and one is in the membrane associated domain.

**Both of α and β subunits in hCG αβ dimer interact with LHR exodomain**

In the early 80’s we demonstrated that both subunits of hCG interact with the receptor. The question was raised whether the hormone specific β subunit might be responsible for receptor binding, whereas the common α subunit might be responsible for inducing the common hormone action. It had become a more acute issue, when we showed in the early 90’s, that the endodomain is the site for signal generation...
and the exodomain is the hormone specific, high affinity binding site. A tempting question was whether hCGβ binds the exodomain with specificity and high affinity, in comparison to hCGα binding the endodomain and generating the hormone signals. After overcoming technical difficulties, we have showed in 1999 that both α and β subunits in the hCG αβ dimer make direct contact with the Glu'Leu'295 region in the LHR exodomain27,28. For the study and elaborate controls we employed a wide spectrum of independent methods and reagents, including truncated exodomains with and without the flag epitope (DYKDDDDK), photoaffinity labeling, affinity cross-linking, monoclonal anti-flag antibody, hCG, hCG derivatives containing either the α or β subunit that is derivatized with 125I and a photoactivatable group (azidobenzoyl group, AB), denatured hCG, FSH and TSH. For example, we prepared an hCG derivative consisting of untreated α and a 175I-AB-β (α/175I-AB-β) which binds with high affinity to the LHR expressed on intact cell, holo-LHR solubilized in Triton X-100 and solubilized LHR exodomain. AB was coupled to amino groups of the β subunit. To narrow down the binding region in the exodomain, two different sizes of the exodomain were produced and tested, one covering Glu'-Met'300 and the other Glu'-Leu'295. a/175I-AB-β bound to both of them and further, photoaffinity labeled them. The binding and labeling was saturable, required UV irradiation and the exodomains, and inhibited by unlabeled hCG but not by denatured hCG, natural FSH and natural TSH. Since the solubilized exodomain preparation contained other membrane proteins, it was necessary to purify the photoaffinity labeled exodomains. For this purpose, the exodomains containing the flag epitope were created, which were capable of binding 125I-hCG and a/175I-AB-β with the similar Kd value. They were photoaffinity labeled and immunoprecipitated using monoclonal anti-flag antibody. The immunoprecipitation required UV irradiation, the flag epitope, anti-flag antibody and protein G for precipitation. The immunoprecipitated and SDS-solubilized complexes appeared as discrete bands on SDS-PAGE, corresponding, in molecular weights, to the estimated 175I-AB-β/exodomain complex and α/175I-AB-β/exodomain complex. Normal mouse serum could not immunoprecipitate the labeled exodomain complexes. Based on the crystal hCG structure, Lys'20 in βL1 and Lys'104 in the seat belt are likely involved in the photoaffinity labeling. In addition to the hCGβ study, hCGα was examined as reported27. Because of the close similarity and page limitation, we will not describe the results.

**hCGα C-terminal region interacts with the receptor and partially activates the receptor**

The existing evidence from various studies using truncation, substitution, synthetic hormone peptides, and hCG crystals suggested that the C-terminal region of the α subunit contacts the LH/CG receptor and is involved in receptor activation. Despite a deluge of speculation and the importance of the αC-terminal region, direct evidence for its interaction with the receptor was elusive. Because of the significant biological activity, it was imperative to demonstrate the interaction. For this purpose, the decamer peptide mimic of the α subunit sequence of His'83-Ser'82 (α'83-92) were derivatized with the N-hydroxysuccinimide ester (NHS) of 4-azidobenzoylglycine (ABG) and radioiodinated. The resulting ABG-125I-α'83-92 was capable of binding and partially activating LH/CG receptor19. Furthermore, UV-sensitive ABG-125I-α'83-92 exclusively photoaffinity-labeled an ~86 kD molecule. This labeled molecule was shown to be LH/CG receptor by various methods including immunoprecipitation by anti-LH/CG receptor antiserum. In addition, evidence is presented that the amino group of αLys'91 of α'83-92 is in such close proximity to a carboxyl group of the receptor that this pair is cross-linked to form an amide, a zero length cross-link. This low affinity contact of α'83-92 and the receptor is sufficient for partial receptor activation and is crucial for the full understanding of the mechanism of the receptor activation steps. It is unclear whether α'83-92 interacts with the endodomain directly or indirectly via the exodomain for activation of the receptor.

**Structure and activation of LH receptor**

The glycoprotein hormone receptors comprise two equal halves, an extracellular N-terminal half (exodomain) and a membrane associated C-terminal half (endodomain) as shown in Fig. 2A. The exodomain is ~350 amino acids long which alone is capable of high affinity hormone binding26,31 with hormone selectivity22-24 but without hormone action11,13. The endodomain consists of seven transmembrane domains (TMs), three exoloops, three cytoloops, a C-terminal tail and a short extracellular extension connected to TM1. Receptor activation occurs in the endodomain8, which is structurally equivalent to the entire molecule.
of many other G protein-coupled receptors. Existing data suggest that glycoprotein hormones initially bind to the exodomain, and the resulting hormone/exodomain complex undergoes conformational adjustments and interacts with the endodomain. This secondary interaction is thought to be responsible for signal generation. Glycoprotein hormones, hCG, LH, FSH and TSH, share the same effectors, adenyl cyclase (AC) and phospholipase Cβ (PLC) in addition to the common α subunit. Therefore, it was speculated that the hormone specific β subunit would bind the receptor’s exodomain while the common α subunit would activate the endodomain to invoke the common hormonal signaling. During the past grant period, we showed that the exodomain interacts with both of the hCGα and β subunits. In addition, we showed that AC and PLC are activated distinctly. This raises a question whether both of the subunits interact and activate the endodomain. We plan to address this issue in the coming years.

The model described above for hormone binding and signal generation leaves the impression that the exodomain and endodomain are independent of each other until they contact each other in the secondary interaction. Our recent observations indicate otherwise. Hormone binding to the exodomain is dependent on the endodomain and in other words, the endodomain modulates hormone binding to the exodomain. These results suggest that the hormone binds to the exodomain in the binary complex of the exodomain and endodomain. Subsequently, the hormone, exodomain, and endodomain ternary complex undergoes conformational adjustments, leading to the final structure of the ternary complex. An outstanding question concerning this new paradigm is whether or not the hormone makes direct contact with the endodomain. In addition, little is known about the endodomain’s potential contact points with the exodomain and hCG. This becomes a crucial issue because the contact points are the primary candidates for the sites where distinct signals for cAMP and various IPs are generated by separate mechanisms. We plan to investigate the dynamic relationship among the three components of the ternary complex.

Exodomain, high affinity hormone binding. Leu Rich Repeats, disulfides and oligosaccharides

There is ample evidence that the exodomain of LHR alone is capable of binding hCG with high affinity. Dufau’s group and Seagaloff’s group are the first to demonstrate that the truncated exodomain lacking the endodomain binds hCG with a high affinity similar to or slightly better than the wild type affinity. When the LHR exodomain was fused to the single-transmembrane domain of CD8, the hybrid protein was capable of binding hCG with a high affinity. Puett’s group identified a number of ionic amino acids in the exodomain that are important for hormone binding. In addition, other mutations in the exodomain abolished hormone binding. Ryan’s group showed that peptides corresponding to three regions in the exodomain were capable of inhibiting hCG binding to the receptor. During this grant period, we reported the first evidence that the exodomain makes direct contacts with both subunits of hCG.

There are several notable features in the structure of LHR exodomain. The sequence alignment shows a Leu Rich Repeat (LRR) motif. This motif is present in a large number of proteins that belong to the LRR family. Each LRR consists of 20-28 amino acids and has a short β strand linked to an α helix approximately parallel to each other. LRR is stabilized by hydrophobic interactions among residues, particularly involving Leu and Ile. In the
crystal structures of some LRR proteins, up to 15 LRRs form a nonglobular, horseshoe-shaped structure with the inner lining of curved parallel β strands and the outer lining of helices. The β strand inner lining interacts with the protein ligands. Based on the established LRR crystal structures, the glycoprotein hormone receptors have been modeled. These computer models show an 1/3 donut structure consisting of 7-9 LRRs and the inner lining presumably interacts with the hormones, in particular the center of the LRRs interacting with the central groove of hCG.

Despite the general attention and deluge of models and reports on the LRRs, little is known about whether LRRs are functional in the exodomains of LHR and other glycoprotein hormone receptors and if so, whether they indeed assume the one third donut structure. If they do so, it will be necessary to determine whether all of the LRRs contact the hormones. If not, which LRRs interact with the hormones has to be determined. In addition, there are the N- and C-flanking regions of the LRRs in the exodomain and their size is substantial. While others were focusing on and modeling the LRR region, we decided to

Table 1 — Effect of substitutions for Lys583 in exoloop 3
Lys583 of exoloop 3 was substituted with a panel of amino acids and the resulting mutant LHRs were expressed on 293 cells. Cells were assayed for hCG binding and induction of cAMP, inositol phosphate 1 (IP1), inositol phosphate 2 (IP2) and inositol phosphate 3 (IP3).

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<th>Kd (pM)</th>
<th>Max xAMP (%)</th>
<th>Max IP1 (%)</th>
<th>Max IP2 (%)</th>
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Fig. 3 — hCG concentration dependent photolability labeling. Increasing concentrations of hCG was incubated with a constant concentration of AB-125I-LHR peptide or ABG-125I-LHR peptide. The mixture was irradiated with UV, solubilized and electrophoresed. Dried gels were analyzed on phosphoimager and radioactive band intensities were determined.
examine the flanking regions. Interestingly, our results show that the N-flanking region of the LRRs makes strong contact with both subunits of hCG.

The LHR exodomain has 12 Cys residues. Cys\(^8\), Cys\(^{12}\), Cys\(^{14}\) and Cys\(^{22}\) are located upstream of LRR1 and substitution for any one of them abolishes the receptor activity\(^1\), consistent with our observation that the region interacts with hCG. Cys\(^{106}\) and Cys\(^{131}\) are in LRR4 and LRR5, respectively, and are not found in FSH-R and TSH-R. They are important for hCG binding and thought to form a disulfide \(^5\) and link LRR4 and LRR5 as suggested in computer models. Therefore, they are considered important for hCG binding and receptor activation\(^7\). The LHR exodomain has six consensus N-glycosylation sites, which are actually glycosylated in transfected cells. After a considerable controversy on their role, it is now generally accepted that they are important for targeting but not for hormone binding and receptor activation\(^7\).

Several hCG contact sites, including upstream region of LRRs

Our earlier studies indicate multiple contact sites between hCG and LHR\(^5\). To approximate the hCG contact sites in the exodomain, LHR was sequentially truncated one exon at a time out of the C-terminus. Interestingly, exons 2-9 correspond to LRRs 1-8, respectively. The study revealed several discrete regions which impact hormone binding\(^5\). They are around the boundaries of exons 1-2 (upstream of LRR1), exons 4-5 (=LRRs 3-4), exons 6-7 (LRRs 5-6), and exons 9 and 10 (downstream of LRRs 8-9). In 1992, Ryan's group reported the inhibition of hCG binding by LHR peptides covering exons 1-2, 4-5 and 9-10\(^1\). Ala scan of the Asp\(^{17}_-\)Arg\(^{36}\) region upstream of LRR1 uncovered three alternating residues (Leu\(^{20}\), Cys\(^{22}\) and Gly\(^{24}\)) crucial for hormone binding. Ala substitution for any one of these residues abolished hormone binding albeit the successful surface expression of the mutants. In contrast, Ala substitution for their flanking and intervening residues did not impair hormone binding. The surface expression was proven by three methods, antibody staining of intact cells expressing the mutant receptors and visualization using confocal laser immunofluorescent microscopy. \(^{125}\)I-antibody binding to mutants on intact cells, and \(^{125}\)I-hCG binding to intact cells and cells solubilized in nonionic detergent solution.

**Direct interaction of LHR Gly\(^{18}_-\)Tyr\(^{36}\) sequence with the α and β subunits of hCG**

To test if the Asp\(^{17}_-\)Arg\(^{36}\) region upstream of LRR1 interacts with hCG, the hormone was photoaffinity labeled with a peptide mimic corresponding to Gly\(^{18}_-\)Tyr\(^{36}\) of the receptor (LHR\(^{18}_-\)\(^{36}\)). This peptide was derivatized with a UV activable agent 4-azidobenzoyl (AB) or 4-azidobenzoyl-glycyl (ABG) at the amino terminus and radiiodinated to produce \(^{125}\)I-AB-LHR\(^{18}_-\)\(^{36}\) or \(^{125}\)I-ABG-LHR\(^{18}_-\)\(^{36}\). AB and ABG have the same structure except that ABG is ~0.3 nm longer due to the presence of a Gly. The maximum labeling distance is 0.7 nm for AB and 1.0 nm for ABG, respectively. \(^{125}\)I-AB-LHR\(^{18}_-\)\(^{36}\) and \(^{125}\)I-ABG-LHR\(^{18}_-\)\(^{36}\) specifically photoaffinity labeled both α and β subunits of hCG with a Kd value of 25 μM (Fig. 3). This

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**Table 2—Effects of Ala substitution for exoloop 3 amino acids**

| Exoloop 3 residues individually substituted with Ala and the resulting mutant LHRs were individually expressed on 293 cells. HCG binding and cAMP induction were assayed. The Kd value and EC\(_{50}\) value of the wild type was divided with the corresponding values of the mutants |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Kd\(^{wild}\) | WT | K573 | V574 | P575 | L576 | I577 | T578 | V579 | TSS0 | N581 | S582 | K583 |
| 1.00 | 1.43 | 0.70 | 0.92 | 0.79 | 1.28 | 1.33 | 1.31 | 0.93 | 1.01 | 3.35 | 1.06 | 1.68 |
| EC\(_{50}\)\(^{wild}\) | 1.00 | 0.56 | 0.34 | 0.12 | 0.51 | 0.25 | 0.80 | 0.37 | 0.61 | 0.43 | 0.83 | None |
| Max cAMPr\(^{wild}\) | 1.00 | 0.88 | 0.95 | 0.28 | 0.77 | 0.74 | 0.83 | 0.53 | 0.83 | 0.80 | 0.95 | 0 |

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**Table 3—Effects of Ala substitution for exoloop 3 amino acids**

| Exoloop 2 residues were individually substituted with Ala and the resulting mutant LHRs were individually expressed on 293 cells. HCG binding and cAMP induction were assayed. The Kd value and EC\(_{50}\) value of the wild type was divided with the corresponding values of the mutants |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kd\(^{wild}\) | WT | S484 | N485 | Y486 | M487 | K488 | V489 | S490 | H491 | C492 | L493 | P494 |
| 1.00 | 0.24 | 1.28 | 0.68 | 0.79 | 1.48 | 0.44 | 1.37 | 0.82 | 0.74 | 1.00 | 0.51 | 0.44 |
| EC\(_{50}\)\(^{wild}\) | 1.00 | 0.25 | 0.34 | 0.23 | 1.03 | 0.30 | 0.19 | 0.24 | 0.46 | 0.56 | 0.34 | 0.47 | 0.32 |
| Max cAMPr\(^{wild}\) | 1.00 | 0.68 | 0.82 | 1.29 | 1.29 | 0.88 | 0.48 | 1.12 | 0.78 | 1.22 | 0.85 | 1.08 | 1.12 |

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Endodomain and signal generation

Distinct mechanisms for generating the signal AC/cAMP signal and the individual PLC/IP signals

When some of the exoloop 3 mutants were assayed for hCG dependent IP production (Table 1), the results were interesting. The Lys

A1a substitution and deletion of Lys

abolished cAMP induction but did not impair hCG binding and IP induction. In contrast, the Ile

A1a substitution abolished IP induction without impairing hormone binding and cAMP induction. These results clearly indicate that the AC/cAMP signal and PLC/IP signal are generated by distinct mechanisms. The Lys

Glu mutant induced IP

but not cAMP and IP, On the other hand, the Lys

Arg mutant induced IP but not induce cAMP, IP, and IP. These results indicate that the three IP species are different mechanisms, an indication of separate signals.

Exoloops 3 constrains hormone binding to the exodomain and plays an important role in cAMP induction

Exoloops are logical candidates for the interaction with the exodomain and therefore, we examined exoloop 3, connecting TMs 6 and 7, which have been implicated in signaling of LHR. The 11 residues of exoloop 3 were Ala scanned and the resulting mutants were assayed for hCG binding and cAMP induction. The Kd values, EC50 for cAMP induction and Max cAMP level were determined. To readily compare these values, the wild type Kd value was divided with each mutant Kd value (Table 2). Likewise, the wild type EC50 for cAMP induction was divided with each individual mutant's value. In addition, the Max cAMP levels of individual mutants were divided with the wild type Max cAMP level. The resulting Kd and EC50 ratios over 1 reflect the affinities better than the wild type. Similarly, a Max cAMP level over 1 means that it is higher than the wild type level. The Kd and EC50 ratios show that some Ala substitutions significantly improved the binding affinity, in particular the 3-fold increase in the binding affinity of the Asn

A1a mutant. The result suggests the potential interaction of exoloop 3 with the exodomain. On the other hand, all of the EC50/

ratios for cAMP production decreased, indicating that Ala substitutions impaired the affinity for cAMP induction. Particularly, some were severely impaired and interestingly, those residues position at one side of exoloop 3 as shown in the black
Exoloop 2 constrain hormone binding to the exodomain and is not essential for cAMP induction.

The 20 residues in exoloop 2, Ser484-Gln503, were individually substituted with Ala. The mutants were expressed on 293 cells and assayed for 125I-binding and cAMP induction. All of those expressed on the cell surface induced cAMP in response to hCG (Table 3). This is in contrast to exoloop 3 mutants. Ala substitution for several residues, Ser484, Tyr485, Cys492 and Pro494, prevented the surface expression. Next, the affinity of hormone binding to intact cells or after solubilization was noticeably improved over the wild type affinity for Ser484, Asn485, Lys488, Ser489 and Ser499. Computer modeling suggests that these residues may be positioned on one side of exoloop 2 and constrain the hormone binding at the exodomain. To test this hypothesis we carefully examined the binding affinity of the exodomain after truncating the endodomain. The truncation consistently improved the affinity by 30-100%. These results suggest the potential interaction of exoloop 2 with the exodomain.

The disulfide bridge between Cys462 and Cys463 in exoloop 2 and Cys428 in exoloop 1 has been suggested but not been proven. The Cys428/Ala substitution did not significantly impact hCG binding but blocked the surface expression. Apparently, it is related to targeting but not important for hCG binding. It will be interesting to see the effect of the Cys428/Ala substitution.

Concluding remarks

In conclusion, glycoprotein hormones initially bind to the exodomain and that the resulting hormone/exodomain complex undergoes a conformational change and interacts with the endodomain. This secondary interaction separately generate distinct signals to activate adenyl cyclase and phospholipase Cβ in the endodomain. Our studies show that the exodomain and endodomain intimately associated before and after hCG binding.

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