

Human Growth Hormone and Extracellular Domain of Its Receptor: Crystal Structure of the Complex

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Binding of human growth hormone (hGH) to its receptor is required for regulation of normal human growth and development. Examination of the 2.8 angstrom crystal structure of the complex between the hormone and the extracellular domain of its receptor (hGHbp) showed that the complex consists of one molecule of growth hormone per two molecules of receptor. The hormone is a four-helix bundle with an unusual topology. The binding protein contains two distinct domains, similar in some respects to immunoglobulin domains. The relative orientation of these domains differs from that found between constant and variable domains in immunoglobulin Fab fragments. Both hGHbp domains contribute residues that participate in hGH binding. In the complex both receptors donate essentially the same residues to interact with the hormone, even though the two binding sites on hGH have no structural similarity. Generally, the hormone-receptor interfaces match those identified by previous mutational analyses. In addition to the hormone-receptor interfaces, there is also a substantial contact surface between the carboxyl-terminal domains of the receptors. The relative extents of the contact areas support a sequential mechanism for dimerization that may be crucial for signal transduction.

THE GROWTH HORMONE RECEPTOR IS ACTIVATED ON BINDING of growth hormone to stimulate the growth and metabolism of muscle, bone, and cartilage cells (1). This receptor is a member of a group of receptors that are found on various cell types and are generally involved in cell growth and differentiation. It has been recognized that a structural relationship exists between the extracellular domain of the endocrine hormone receptors and the extracellular domains of a group of cytokine receptors, including those for interleukins 2, 3, 4, 6, and 7, granulocyte and granulocyte-macrophage colony-stimulating factors, and erythropoietin (2, 3). Also, there is a more distant relationship with the extracellular domain of the receptors for tissue factor and the interferons (3). All these receptors are grouped together in the hematopoietic superfamily (2, 3). A recent addition to this superfamily is the receptor for ciliary neutrophilic factor, which is involved in neurogenesis (4).

Like the receptor tyrosine kinases (5), members of the hemato-

poietic receptor superfamily have a three-domain organization comprising an extracellular ligand binding domain, a single transmembrane segment, and an intracellular domain of unknown function, which within the family is not homologous. Beyond this, there is virtually no direct structural information bearing on possible mechanisms of activation or on details of molecular contacts. In analogy to receptor tyrosine kinases, the mechanism through which information from the ligand binding event is transmitted through the membrane by the activated receptor is assumed to involve some type of aggregation. However, the molecular details of aggregation of the ligand-bound receptors are not understood; most proposed models for receptor aggregation postulate complexes of ligand-receptor pairs, that is, a stoichiometry of two ligands and two receptors.

The extracellular domain of the human growth hormone (hGH) receptor (residues 1 to 246) occurs naturally in serum in the form of a hormone binding protein, which binds hGH with approximately the same affinity as the intact receptor (6) and which may play a physiological role in the regulation of hormone clearance. The complex between hGH and a slightly truncated form of this binding protein (hGHbp, residues 1 to 238) consists of one molecule of hGH and two molecules of hGHbp hGH·(hGHbp)₂ (7, 8). This was surprising because it was known from the structure of the porcine growth hormone (9) that there was no evidence for even pseudo-symmetrical binding surfaces that would support binding for two receptors simultaneously. This raised the possibility that either the two hormone binding sites interfaced with different regions of the receptor, or that the receptor binding surface could reconfigure to bind tightly a second set of hormone binding determinants.

Here, we report the structure of the hGH·(hGHbp)₂ complex which shows the novel manner in which a single monomeric protein molecule binds and brings together two receptor molecules. No other structures of protein-receptor complexes are known, although crystals of other such complexes have been reported (10). Interactions between receptors and ligands and between antibodies and antigens are examples of molecular recognition. However, unlike the antibody binding diversity that is expressed by changes in sequence of a limited number of residues on a relatively constant structural scaffold, the hormone-binding determinants of the hGH receptor as seen in the structure that we describe depend on conformational diversity in the presence of conserved sequence. Although the growth hormone system differs in detail from other hormone-receptor complexes in the hematopoietic superfamily, the general theme as to how receptors aggregate is likely to be a relatively common feature of the family as a whole.

Structure of the hormone and the binding proteins. The hGH binding protein (hGHbp, residues 1 to 238) was produced as a soluble protein from *Escherichia coli* (6). Purification of the binding

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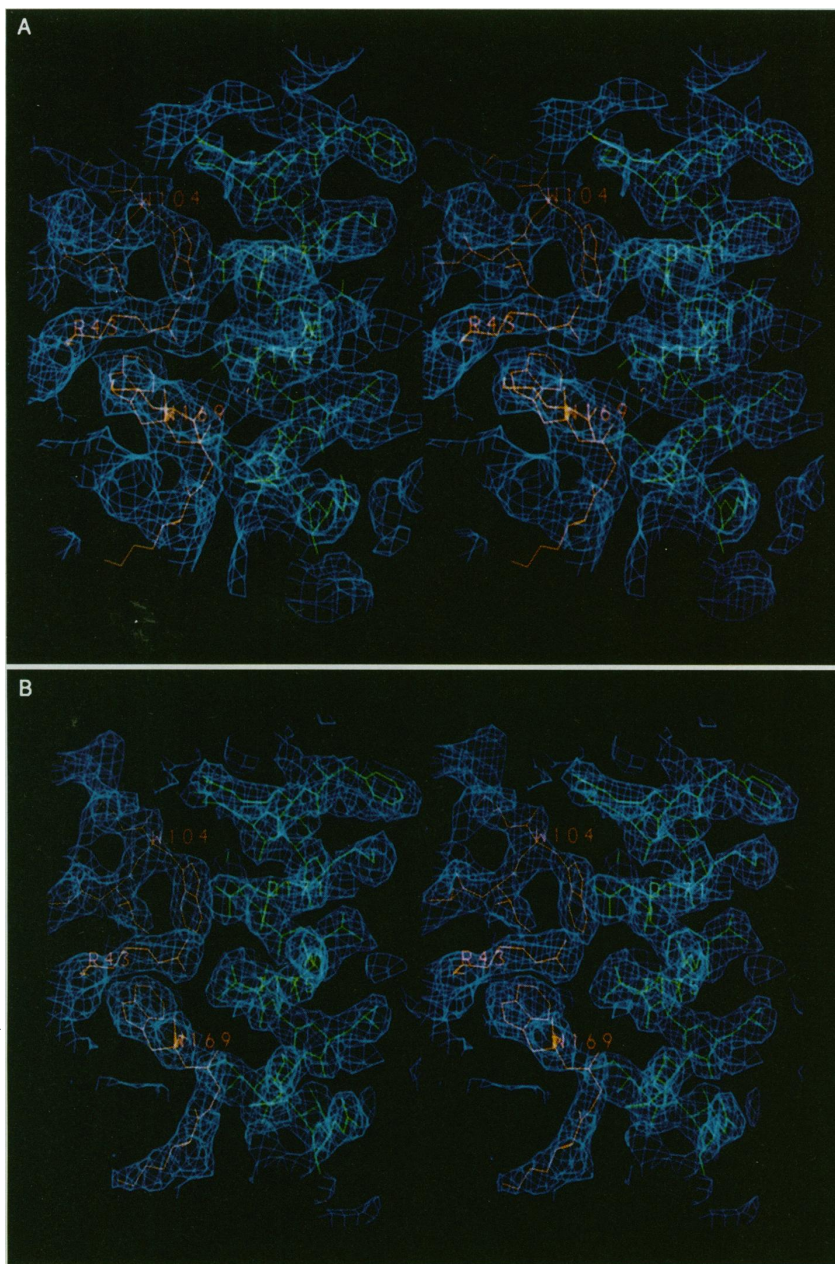


Fig. 1. Electron density for part of the hGH-hGHbp I interface. The current refined model in interface region I is superimposed on (A) the solvent flattened MIR map, and (B) the $2F_o - F_c$ map, with phases calculated from the final model. The hGH atoms are green and receptor atoms are orange.

helix. Alignment of the sequences to the density was straightforward, as there was good density for all the expected disulfide bonds and for almost all large side chains. Electron density was weak or absent for all termini, for part of one loop in hGH and for two loops in each receptor, both in the MIR map and in the solvent flattened map. The structure was refined to an *R* factor of 0.204 (10 to 2.8 Å) (Table 1).

The major structural feature of the hGH molecule is a four-helical bundle (Fig. 2) with unusual connectivity, which was described first for the structure of porcine growth hormone (9); the helices run up-up-down-down, in contrast to the more usual up-down-up-down case. The NH₂- and COOH-terminal helices (helices 1 and 4) are longer than the other two (26 and 30 residues compared to 21 and 23 residues), and helix 2 is kinked at Pro⁸⁹. A long crossover connection, consisting of residues 35 to 71, links helix 1 to helix 2, and a similar connection (residues 129 to 154) is found between helices 3 and 4. The first connection is disulfide-bonded to helix 4 through Cys⁵³ and Cys¹⁶⁵. In contrast, helix 2 is linked to helix 3 by a much shorter segment (residues 93 to 105). In addition to the four helices in the core, three much shorter segments of helix are found in the connecting loops: one each at the beginning and end of the connection between helices 1 and 2 (residues 38 to 47 and 64 to 70, respectively), and one in the short connection between helices 2 and 3 (residues 94 to 100). The NH₂-terminal eight residues extend away from the remainder of the molecule, whereas the COOH-terminus is linked to helix 4 with a disulfide bond between Cys¹⁸² and Cys¹⁸⁹.

The topography of the hormone appears to be similar to that described for porcine growth hormone (pGH) (9). Exceptions are the two short helices in the connecting segment between helix 1 and 2, which were not described for pGH; since they are involved in contacts between hormone and receptor (below), they may represent conformational changes in the hormone upon receptor binding. In addition, the connection between helices 2 and 3 has an omega-loop conformation in the porcine hormone (9). Since this connection does not participate in receptor binding (below), the difference in loop conformation represents a structural difference between hGH and pGH. The residues on the hormone that are color coded in Fig. 2 are directly involved in receptor binding.

The core of the four-helix bundle is made up of mostly hydrophobic residues (Fig. 2) with the exceptions of Ser⁷⁹ and Asp¹⁶⁹. The O γ of Ser⁷⁹ in helix 2 hydrogen-bonds back to the carbonyl

protein, formation and characterization of the complex, and crystallization procedures have been described (7). Crystals with cell parameters $a = 145.8$ Å, $b = 68.6$ Å, $c = 76.0$ Å were in space group $P2_12_12$. Before the data were collected, the crystals were stabilized in 40 percent saturated ammonium sulfate and 0.1 M sodium acetate, pH 5.5. The crystals contain a mixture of hGH and hGHbp (1:2) in the asymmetric unit (7, 8), and this is also the stoichiometry of the complex in solution (8). Phases for the observed intensities were determined by multiple isomorphous replacement with two heavy atom derivatives, combined with solvent flattening. The overall quality of the electron density maps was quite good (Fig. 1) (11), and the outline of the molecules and the individual domains was obvious. The electron density for the hormone was easily recognizable because of its four-helix bundle structure, whereas the density assigned to the binding protein did not contain any obvious

oxygen of Leu⁷⁵ (2.9 Å). The Oδ1 of Asp¹⁶⁹ in helix 4 hydrogen bonds to the Oγ of Ser⁵⁵ (3.0 Å) as well as to the Ne1 of Trp⁸⁶ (2.9 Å), as proposed on the basis of absorption spectroscopy (12) combined with mutagenesis (13). Oδ2 of Asp¹⁶⁹ is pointed outward from the core and appears to interact with Nζ of Lys¹⁷² (4.1 Å). Other hydrophobic clusters can be found between the four-helix core and the connecting segments. Thus, Ile³⁶, Phe⁴⁴, Cys⁵³, Phe⁵⁴, and Ile⁵⁸ in the connection between helices 1 and 2 interact with

Leu⁷⁵ and Ile⁷⁸ in helix 2, and with Leu¹⁵⁷, Tyr¹⁶⁰, Tyr¹⁶⁴, Cys¹⁶⁵, and Phe¹⁷⁶ in helix 4; but Leu⁹³, Val⁹⁶, and Phe⁹⁷ in the short segment between helices 2 and 3 interact with Phe³¹ of helix 1 and with Leu¹⁶² and Leu¹⁶³ of helix 4.

The extracellular part of the receptor consists of two domains (residues 1 to 123 and 128 to 238, respectively), linked by a single four-residue segment of polypeptide chain (Fig. 3A). Each domain contains seven β strands (Fig. 3B) that together form a sandwich of two antiparallel β sheets, one with four strands and one with three, with the same topology in each domain. The two-domain structure and the presence in each domain of two β sheets were predicted by Bazan (3). He also proposed that the topology of the sandwich might be that of immunoglobulin constant domains. Instead, the topology of the hGHbp domains is identical to that of domain D2 of CD4 (14) and domain D2 of chaperone protein PapD (15), which differs from immunoglobulin constant domains in that "sheet switching" has taken place (14), with strand C' as part of the sheet formed by strands C, F, and G rather than of the other sheet. Strand G in the COOH-terminal domain is preceded by a stretch of irregular extended structure between Tyr²²² and Ser²²⁶, with a bulge at Gly²²³ to Glu²²⁴. As a result, the side chains of Tyr²²² and Phe²²⁵ both point into the solvent, whereas Oγ of Ser²²⁶ forms a hydrogen bond to the main chain amine of Val²¹² in the neighboring strand.

The NH₂-terminal 30 residues of both receptor molecules in the complex were not apparent in the electron density map and are not part of our model. Therefore, the ordered structure of the NH₂-terminal domain is smaller and more compact than that of the COOH-terminal domain. Superposition of the domains shows that

Table 1. Crystallographic statistics. Data were collected on an Enraf-Nonius FAST area detector, mounted on a Rigaku RU200 rotating anode generator operated at 45 kV, 110 mA. Crystals were mounted with the *b** axis parallel to the rotation axis, and two crystal settings were used to produce complete data sets. Processing was done with MADNES (25) and PROCOR (26). Two native data sets were collected to a resolution of 2.8 Å, and when combined gave 95 percent completeness [$R_{\text{merge}}(I) = 0.13$, all reflections between 15 and 2.8 Å with $F > 0$]. For derivatives, crystals were soaked in heavy atom compounds dissolved in stabilization solution. Both K₂PtCl₄ and K₂AuCl₄ gave a highly occupied single-site derivative. Anomalous differences were used during phase refinement with PROTEIN (27). The final figure of merit was 0.55 (15 to 3.0 Å, 14,787 reflections). Solvent flattening (28) increased the figure of merit to 0.76. The resulting solvent flattened map was used for chain tracing and model building with the original MIR map as a reference. The starting model for refinement consisted of hGH residues 3 to 134 and 154 to 189, residues 33 to 51, 65 to 70, and 79 to 231 for the first receptor, and residues 35 to 51, 65 to 69, and 80 to 235 for the second receptor. Of these 516 amino acids (out of 667), 52 side chains were trimmed back to alanine. Crystallographic refinement was done with XPLOR (29). The starting *R* factor was 0.47 (10 to 3.0 Å); conventional positional refinement decreased the *R* factor to 0.32, and one cycle of simulated annealing to 0.27. The resolution was extended to 2.8 Å, and combination of map fitting and refinement resulted in $R = 0.249$ (10 to 2.8 Å, 17,985 reflections, or 95 percent of the possible number). At this stage, tightly restrained individual temperature factors were refined. The final model consisted of residues 3 to 146 and 154 to 190 of hGH, residues 29 to 54, 59 to 72, and 79 to 234 of the first receptor, and residues 31 to 53, 61 to 72, and 76 to 238 of the second receptor. No water molecules were added to the model.

| Sample | Diffraction data | | | | |
|----------------------------------|------------------|--------------------|-------------------|-------------------|-------------------------|
| | Resolution (Å) | Measurements (No.) | Reflections (No.) | Data coverage (%) | R_{sym} (on I) |
| Native 1 | 2.8 | 48635 | 17302 | 89 | 0.063 |
| Native 2 | 2.8 | 47414 | 18368 | 95 | 0.061 |
| K ₂ PtCl ₄ | 3.0 | 25316 | 14794 | 94 | 0.077 |
| K ₂ AuCl ₄ | 3.0 | 42964 | 14482 | 92 | 0.067 |

| | Phase refinement at resolution (Å): | | | | | | | | |
|----------------------------------|-------------------------------------|------|------|------|------|------|------|------|---------|
| | 10.0 | 7.5 | 6.0 | 5.0 | 4.3 | 3.7 | 3.3 | 3.0 | Overall |
| Native | | | | | | | | | |
| Figure of merit | 0.79 | 0.79 | 0.74 | 0.65 | 0.58 | 0.47 | 0.43 | 0.39 | 0.51 |
| Reflections (No.) | 316 | 601 | 976 | 1414 | 1916 | 2484 | 3165 | 3915 | 14787 |
| K ₂ PtCl ₄ | | | | | | | | | |
| R_{Cullis}^* | 0.61 | 0.61 | 0.60 | 0.67 | 0.66 | 0.74 | 0.71 | 0.76 | 0.71 |
| Phasing power† | 0.93 | 1.22 | 1.46 | 1.30 | 1.21 | 1.19 | 1.16 | 1.10 | 1.20 |
| K ₂ AuCl ₄ | | | | | | | | | |
| R_{Cullis}^* | 0.51 | 0.56 | 0.51 | 0.62 | 0.68 | 0.78 | 0.77 | 0.72 | 0.66 |
| Phasing power† | 1.61 | 1.85 | 2.13 | 1.63 | 1.27 | 1.26 | 1.32 | 1.41 | 1.56 |

| Resolution (Å) | Crystallographic refinement | | | | |
|----------------|-----------------------------|---|-------------|--------------|------------------------|
| | <i>R</i> (<i>I</i> > 0) | <i>R</i> (<i>I</i> > 2σ ₁) | Δ(bond) (Å) | Δ(angle) (°) | Δ(B) (Å ²) |
| 10–2.8 | 0.228 (17985) | 0.204 (15632) | 0.015 | 3.6 | 2.0 |

* R_{Cullis} : Cullis *R* factor for centric reflections. †Phasing power: mean value of heavy atom structure factor amplitude divided by residual lack of closure error.

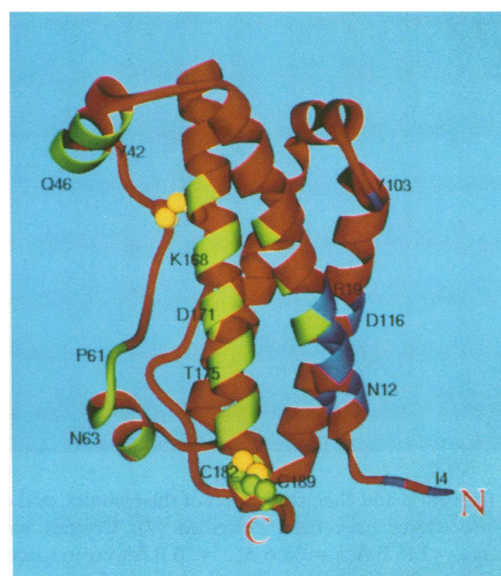


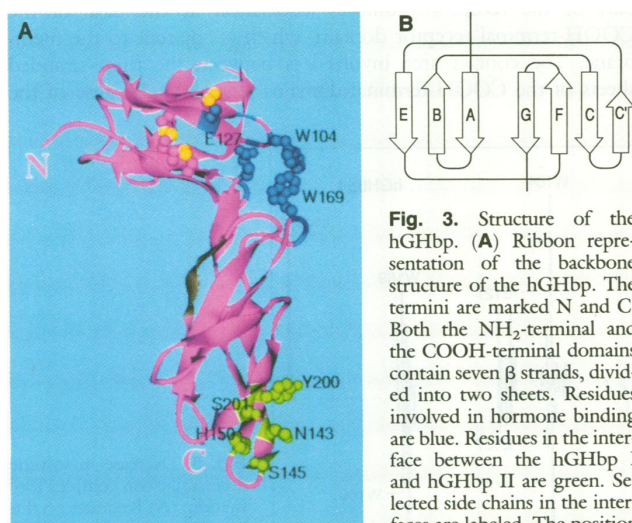
Fig. 2. Ribbon representation of the structure of hGH, viewed as perpendicular to the four-helix bundle. The NH₂-terminus is marked N, the COOH-terminus, C. Residues in the interfaces between the hormone and the two receptors are colored green (interface I) and blue (interface II), respectively, and selected interface residues are labeled; helix 1, 9 to 34; helix 2, 72 to 92; helix 3, 106 to 128; and helix 4, 155 to 184. Additional short helical segments are 38 to 47, 64 to 70, and 94 to 100. The core of the four-helix bundle is formed by the side chains of Phe¹⁰, Ala¹³, Ala¹⁷, Leu²⁰, and Ala²⁴ of helix 1; Leu⁷⁶, Ser⁷⁹, Ile⁸³, Trp⁸⁶, and Val⁹⁰ of helix 2; Val¹¹⁰, Leu¹¹⁴, Leu¹¹⁷, Ile¹²¹, and Leu¹²⁴ of helix 3; and Phe¹⁶⁶, Asp¹⁶⁹, Met¹⁷⁰, Val¹⁷³, Leu¹⁷⁷, and Val¹⁸⁰ of helix 4. (Residues 1 and 2, 147 to 153, and 191 are not visible in the electron density map and are not included in the model).

they are similar in their core, with a root-mean-square (rms) difference between corresponding C α atoms of 1.1 Å (41 C α positions were examined).

The NH₂-terminal domain of the receptor contains three disulfide bridges (Fig. 3A), and the disulfide connections observed in the structure confirm the previous assignments made on the basis of chemical methods (6). Two of the disulfide bonds link neighboring strands. Thus, Cys³⁸ in strand A is bridged to Cys⁴⁸ in strand B with the disulfide packed in the interior between the two sheets, while strands F and G of the other sheet are linked by Cys¹⁰⁸ and Cys¹²², the disulfide in this case being exposed on the solvent-accessible side of the barrel. The third disulfide cross-links the two sheets of the sandwich, thereby connecting Cys⁸³ in strand C' to Cys⁹⁴ of strand E (Fig. 3). The loops between the strands that are disulfide-linked are relatively short (only 3 to 6 residues), whereas the other connections are longer (9 to 14 residues). Although two of the disulfides are part of the hydrophobic core of the NH₂-terminal domain, their presence is apparently not required for the observed fold; the COOH-terminal domain, and domain D2 of PapD (15) do not have any disulfides, and domain D2 of CD4 has only one (14).

The two domains of the hGHbp are linked by a four-residue segment that immediately follows strand G of the NH₂-terminal domain. The main-chain torsion angles of these four residues are unusual for a linker between immunoglobulin-like domains in that they generate a helical turn (Val¹²⁵ and Asp¹²⁶ have $\phi, \psi \approx -70^\circ, -20^\circ$; Glu¹²⁷ and Ile¹²⁸ have $\phi, \psi \approx -115^\circ, 10^\circ$). The result of this is that the relative orientation of the two domains is completely different from that found between the constant and variable domains of immunoglobulins. A salt bridge (2.9 Å) between Arg³⁹ in the NH₂-terminal domain and Asp¹³² in the COOH-terminal domain may participate in stabilization of the relative orientation between the domains.

Structure of the complex. The two receptor molecules in the hGH·(hGHbp)₂ complex show apparent twofold symmetry about an axis approximately perpendicular to the helical axes of the hGH bundle (Fig. 4). The COOH-terminal domains are closely parallel,



X-Trp-Ser pattern occurring in other members of the superfamily is gray. (B) Topology diagram of the domains of the hGHbp. Strands are labeled as described (14). A, B, and E belong to one sheet; C, C', F, and G to the other sheet. C' is significantly shorter than the other strands. (Amino acids not visible in the electron density map and not included in the current model are residues 1 to 28, 55 to 58, 73 to 78, and 235 to 238 of hGHbp I; and residues 1 to 30, 54 to 60, and 73 to 75 of hGHbp II.)



Fig. 4. Backbone structure of the hGH·(hGHbp)₂ complex. The hormone is shown as yellow cylinders representing the helices connected by red tubes. The β strands of the binding proteins are shown in brown, the loops are green (hGHbp I) and blue (hGHbp II). The viewing direction is approximately down the four-helix bundle of hGH. In this orientation, the COOH-termini of the extracellular domains, and therefore the cell membrane, are at the bottom. A rotation of 159° , followed by a translation of 8 Å, superimposes the two receptor molecules with an rms difference in C α of 1.0 Å (179 atoms). Superposition of the individual domains gives rms differences of 0.7 Å for the NH₂-terminal domain (74 atoms), and 0.9 Å for the COOH-terminal domain (93 atoms).

each having its COOH-terminus pointing away from the hormone in the direction where the membrane surface would presumably be. Intact receptors would have an additional eight residues between the COOH-terminus at the end of strand G of the hGHbp and the putative membrane-spanning helix. The structure suggests a model in which this eight-residue segment provides the flexibility and freedom of orientation needed for the hormone to bring together efficiently the extracellular domains.

As a result of complex formation, some of the surface area is buried in the interfaces between hormone and receptor (Fig. 5). The receptor-binding sites on hGH (Figs. 2, 5, A and B, 6) are located on the faces of opposite sides of the four-helical bundle. The first binding site on hGH for the hGHbp (site I; color coded green in Fig. 2) has a concave character. It is formed by residues on exposed faces of mainly helix 4 but also of helix 1, of the four-helix bundle, together with residues in the connecting region between helices 1 and 2. The total surface buried by the hormone on the receptor in this interface is about 1230 Å². The second binding site on hGH (site II) (Fig. 2) is made up of the exposed sides of helices 1 and 3 and, in contrast to the concave character of site I, it is relatively flat. The NH₂-terminal tail of hGH is extended, pointing away from the helical bundle, and contributes to site II (Fig. 2). The total surface buried in this interface is approximately 900 Å², and thus smaller by about 25 percent compared to interface I. A third region contributing to the stabilization of the complex is the contact surface between the membrane-proximal halves of the COOH-terminal domains of the receptors, which buries about 500 Å² on each receptor (see below). The ratio of the polar to the nonpolar atoms buried in the interfaces between hormone and receptors shows a small excess of polar surface, whereas the interface between the two receptors is more apolar (16).

Although the overall shapes of the two binding sites on the

Table 2. Salt bridges and hydrogen bonds in intermolecular contact areas

| hGH-hGHbp I interface | | | hGH-hGHbp II interface | | | hGHbp I-hGHbp II interface | | |
|------------------------|------------------------|--------------|------------------------|------------------------|--------------|----------------------------|------------------------|--------------|
| hGH atom | hGHbp atom | Distance (Å) | hGH atom | hGHbp atom | Distance (Å) | hGHbp I atom | hGHbp II atom | Distance (Å) |
| Lys ⁴¹ Nζ | Glu ¹²⁷ Oε2 | 2.9 | Asn ¹² Oδ1 | Arg ⁴³ Nη2 | 2.9 | Ser ¹⁴⁵ Oγ | Asp ¹⁵² Oδ2 | 3.0 |
| Gln ⁴⁶ Nε2 | Glu ¹²⁰ Oε2 | 3.3 | Asn ¹² Nδ2 | Asp ¹²⁶ Oδ2 | 3.0 | Leu ¹⁴⁶ N | Ser ²⁰¹ Oγ | 3.1 |
| Pro ⁶¹ O | Ile ¹⁰³ N | 2.9 | Arg ¹⁶ Nη1 | Glu ⁴⁴ Oε2 | 3.1 | Thr ¹⁴⁷ Oγ | Asp ¹⁵² Oδ1 | 2.7 |
| Arg ¹⁶⁷ Nη1 | Glu ¹²⁷ Oε1 | 3.2 | Arg ¹⁹ Nη2 | Gln ¹⁶⁰ Oε1 | 3.0 | His ¹⁵⁰ Nε2 | Asn ¹⁴³ Oδ1 | 2.9 |
| Arg ¹⁶⁷ Nη2 | Glu ¹²⁷ Oε1 | 2.9 | | | | Asp ¹⁵² Oδ2 | Tyr ²⁰⁰ Oη | 2.7 |
| Lys ¹⁶⁸ Nζ | Trp ¹⁰⁴ O | 3.1 | | | | Ser ²⁰¹ Oγ | Tyr ²⁰⁰ Oη | 3.3 |
| Asp ¹⁷¹ Oδ2 | Arg ⁴³ Nη2 | 3.1 | | | | | | |
| Thr ¹⁷⁵ Oγ1 | Arg ⁴³ Nη1 | 3.2 | | | | | | |
| Arg ¹⁷⁸ Nη2 | Ile ¹⁶⁵ O | 2.9 | | | | | | |

hormone are quite different, the residues on both receptors that interact with these sites are largely the same (Fig. 5, C and D). On both receptors, binding determinants in the NH₂-terminal domain include Arg⁴³ (on the loop between strands A and B), Trp¹⁰⁴ (on the loop between strands E and F), and some residues on strand G immediately preceding the linker between the two domains. The Glu¹²⁷ in the linker is part of the interface, as is the loop between strands B and C (notably Trp¹⁶⁹) in the COOH-terminal domain. The only receptor determinant that is different in both interfaces between hormone and receptors is Asn²¹⁸ in interface I on the loop between strands F and G of the COOH-terminal domain of the hGHbp (Fig. 5B).

Not only are the binding determinants on both receptors largely the same, but their structures are similar, as shown by an rms difference in C α after superposition of 1.0 Å (179 atoms). Because, overall, the receptors superimpose so well, it is possible that the linker between the NH₂- and COOH-terminal domains is fairly rigid and confers a special orientation between them. The similarity in structure extends to the backbone of most of the binding determinants, and is even observed for the side chain conformations of many of the residues involved in interactions with the hormone, such as Arg⁴³, Glu¹²⁷, Trp¹⁶⁹, and Asn²¹⁸. Exceptions are the conformations of Trp¹⁰⁴ and of the loop comprising residues 163 to 168. The difference in C α position of Trp¹⁰⁴ is 2.8 Å, and the side chain orientation differs in the two receptors. Loop 163 to 168 also takes on a different conformation, resulting in

differences in C α positions after superposition of 2 to 4 Å.

Many of the interactions in the binding sites are apolar; most of the hGH side chains that have binding functionality interact primarily through hydrophobic contacts. Examples are the van der Waals contacts between the methylene groups of Lys¹⁶⁸ and Lys¹⁷² of hGH with the side chain of Trp¹⁰⁴ of hGHbp I. In both interfaces, Trp¹⁰⁴ of the receptors buries most surface area with a decrease in solvent accessibility of 170 Å² in site I and of more than 210 Å² in site II.

The hydrogen bonds and salt bridges in the three intermolecular interfaces in the complex are shown in Table 2. The side chain of Arg⁴³ of the hGHbp is involved in specific hydrogen-bonding interactions in both hormone-receptor interfaces (Table 2). It participates in a network of H bonds in site I (Figs. 1 and 6A) that includes Trp¹⁰⁴ of hGHbp I and Asp¹⁷¹ and Thr¹⁷⁵ of hGH. In site II, the cluster consists of Arg⁴³ and Asp¹²⁶ of hGHbp II and Asn¹² of hGH (Fig. 6B). Another residue with multiple interactions is Glu¹²⁷ of hGHbp I, which forms salt bridges to Lys⁴¹ and Arg¹⁶⁷ of hGH (Table 2). The total number of possible intermolecular salt bridges and hydrogen bonds in binding site I is 9, compared to only 4 in binding site II (Table 2).

The structure shows that hormone binding to the extracellular part of the receptor promotes association at the base of the COOH-terminal receptor domain, which is adjacent to the membrane. The contact area involved is between the three-stranded sheets of the COOH-terminal domains (Fig. 3A). Because of the

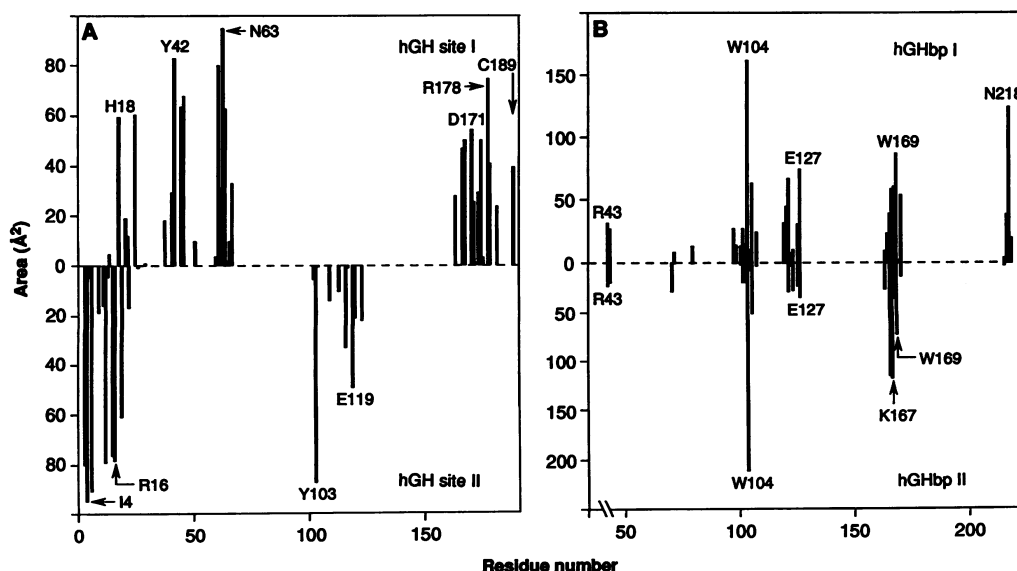


Fig. 5. Decrease in solvent accessibility on complex formation. (A) Residues on the hormone: top, site I; and bottom, site II. (B) Residues on the receptors: top, hGHbp I; bottom, hGHbp II. Solvent accessibility was calculated with the program written by Lee and Richards (24); a probe radius of 1.4 Å was used.

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