Research Article

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

ABRAHAM M. DE VOS,* MARK ULTSCH, ANTHONY A. KOSSIAKOFF

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 neutrophic factor, which is involved in neuropoiesis (*4*).

Like the receptor tyrosine kinases (*5*), members of the hematopoietic 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 activition 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

The authors are in the Department of Protein Engineering, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080.

*To whom correspondence should be addressed.

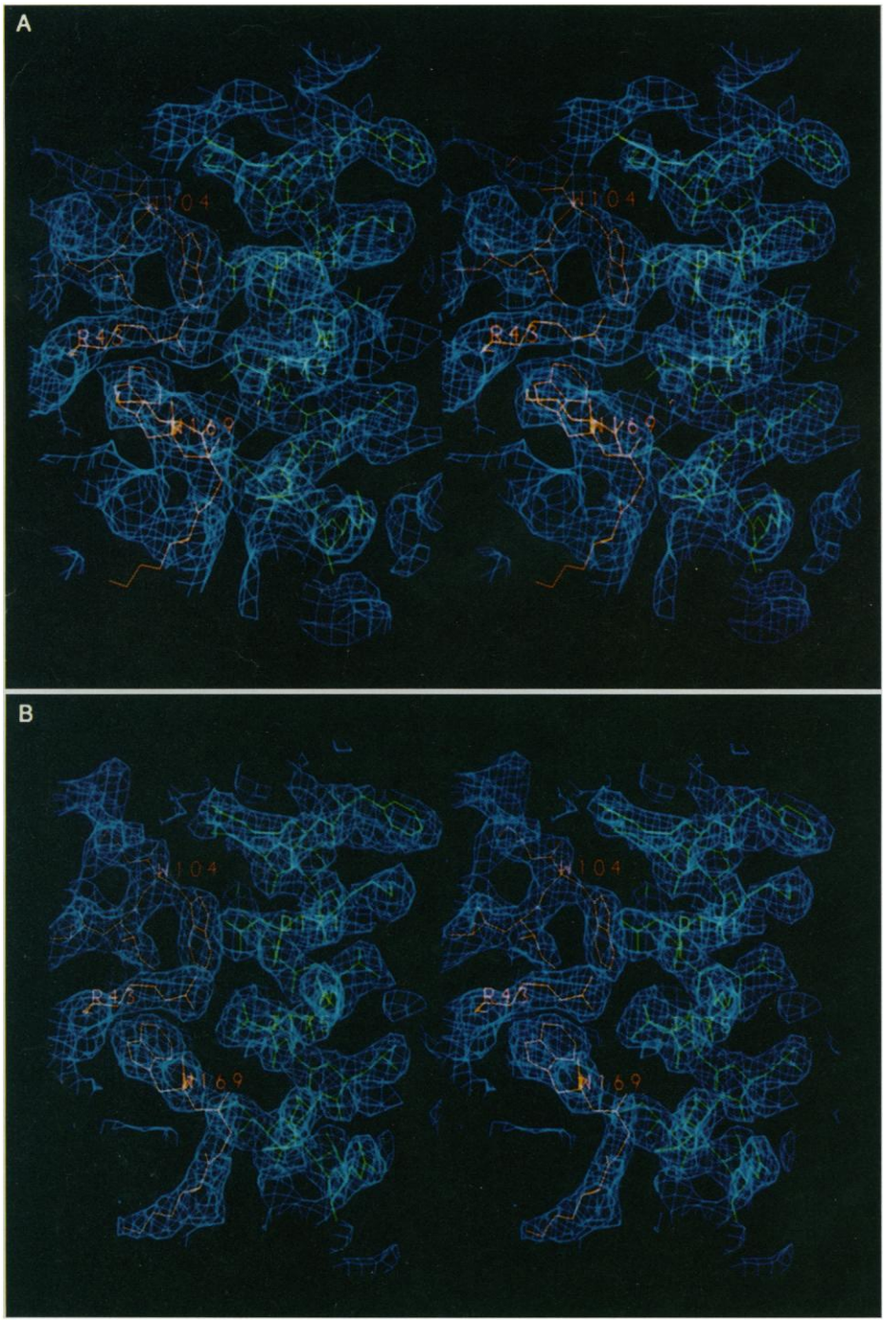

Fig. 1. Electron density for part of the hGH-hGHbp I interface. The current refined model in interface region I is superimposed on (**A**) the solvent flattened MIR map, and (**B**) the $2F_o$-F_c map, with phases calculated from the final model. The hGH atoms are green and receptor atoms are orange.

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 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 NH_2- 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 Pro^{89}. 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 Cys^{53} and Cys^{165}. 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 NH_2-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 Cys^{182} and Cys^{189}.

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 Ser^{79} and Asp^{169}. The Oγ of Ser^{79} in helix 2 hydrogen-bonds back to the carbonyl

oxygen of Leu[75] (2.9 Å). The Oδ1 of Asp[169] in helix 4 hydrogen bonds to the Oγ of Ser[55] (3.0 Å) as well as to the Nε1 of Trp[86] (2.9 Å), as proposed on the basis of absorption spectroscopy (*12*) combined with mutagenesis (*13*). Oδ2 of Asp[169] is pointed outward from the core and appears to interact with Nζ of Lys[172] (4.1 Å). Other hydrophobic clusters can be found between the four-helix core and the connecting segments. Thus, Ile[36], Phe[44], Cys[53], Phe[54], and Ile[58] in the connection between helices 1 and 2 interact with Leu[75] and Ile[78] in helix 2, and with Leu[157], Tyr[160], Tyr[164], Cys[165], and Phe[176] in helix 4; but Leu[93], Val[96], and Phe[97] in the short segment between helices 2 and 3 interact with Phe[31] of helix 1 and with Leu[162] and Leu[163] 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 Tyr[222] and Ser[226], with a bulge at Gly[223] to Glu[224]. As a result, the side chains of Tyr[222] and Phe[225] both point into the solvent, whereas Oγ of Ser[226] forms a hydrogen bond to the main chain amine of Val[212] in the neighboring strand.

The NH_2-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 NH_2-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 [$R_{merge}(I)$ = 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 K_2PtCl_4 and K_2AuCl_4 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_2PtCl_4	3.0	25316	14794	94	0.077
K_2AuCl_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_2PtCl_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_2AuCl_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				
	R ($I > 0$)	R ($I > 2\sigma_I$)	Δ(bond) (Å)	Δ(angle) (°)	Δ(B) (Å²)
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.

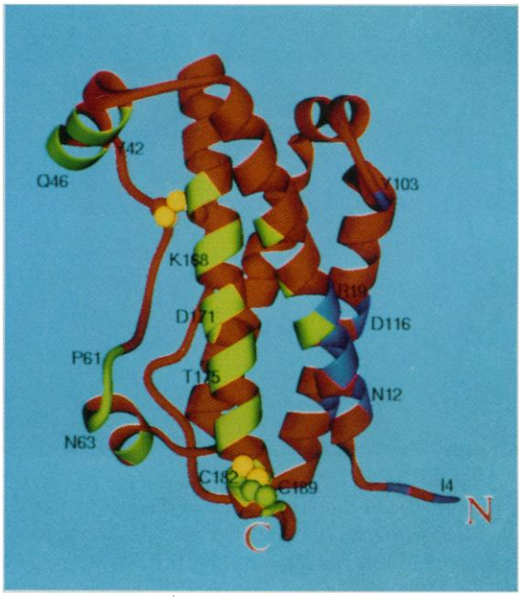

Fig. 2. Ribbon representation of the structure of hGH, viewed as perpendicular to the four-helix bundle. The NH_2-terminus is marked N, the COOH-terminus, C. Residues in the interfaces between the hormone and the two receptors are colored green (interface I) and blue (interface II), respectively, and 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 Phe[10], Ala[13], Ala[17], Leu[20], and Ala[24] of helix 1; Leu[76], Ser[79], Ile[83], Trp[86], and Val[90] of helix 2; Val[110], Leu[114], Leu[117], Ile[121], and Leu[124] of helix 3; and Phe[166], Asp[169], Met[170], Val[173], Leu[177], and Val[180] 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 Cα atoms of 1.1 Å (41 Cα positions were examined).

The NH_2-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, Cys^{38} in strand A is bridged to Cys^{48} 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 Cys^{108} and Cys^{122}, 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 Cys^{83} in strand C′ to Cys^{94} 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 NH_2-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 NH_2-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 (Val^{125} and Asp^{126} have $\varphi,\psi \approx -70°, -20°$; Glu^{127} and Ile^{128} have $\varphi,\psi \approx -115°, 10°$). 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 Arg^{39} in the NH_2-terminal domain and Asp^{132} 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, 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.

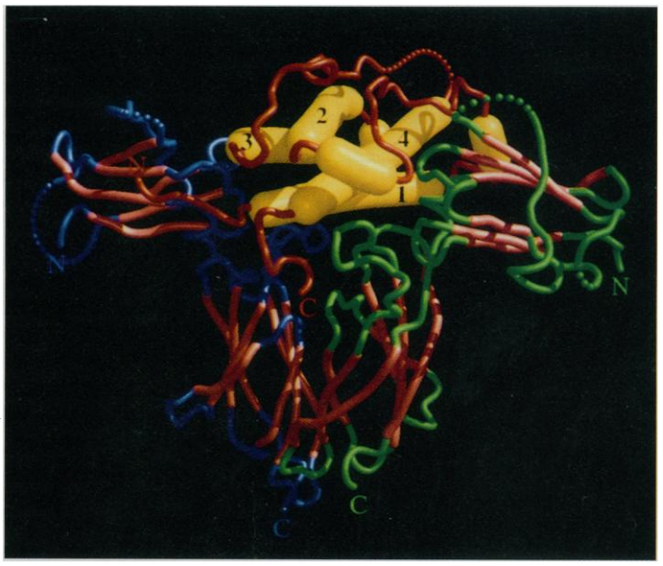

Fig. 4. Backbone structure of the hGH·$(hGHbp)_2$ complex. The hormone is shown as yellow cylinders representing the helices connected by red tubes. The β strands of the binding proteins are shown in brown, the loops are green (hGHbp I) and blue (hGHbp II). The viewing direction is approximately down 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 Cα of 1.0 Å (179 atoms). Superposition of the individual domains gives rms differences of 0.7 Å for the NH_2-terminal domain (74 atoms), and 0.9 Å for the COOH-terminal domain (93 atoms).

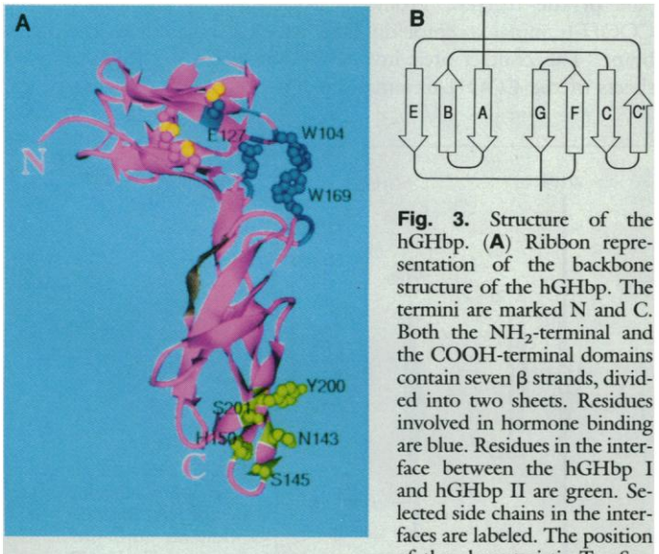

Fig. 3. Structure of the hGHbp. (**A**) Ribbon representation of the backbone structure of the hGHbp. The termini are marked N and C. Both the NH_2-terminal and the COOH-terminal domains contain seven β strands, divided into two sheets. Residues involved in hormone binding are blue. Residues in the interface between the hGHbp I and hGHbp II are green. Selected side chains in the interfaces are labeled. The position of the characteristic Trp-Ser-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.)

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 $Å^2$. 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 NH_2-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 $Å^2$, 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 $Å^2$ 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^{166}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 NH_2-terminal domain include Arg^{43} (on the loop between strands A and B), Trp^{104} (on the loop between strands E and F), and some residues on strand G immediately preceding the linker between the two domains. The Glu^{127} in the linker is part of the interface, as is the loop between strands B and C (notably Trp^{169}) in the COOH-terminal domain. The only receptor determinant that is different in both interfaces between hormone and receptors is Asn^{218} 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 Cα after superposition of 1.0 Å (179 atoms). Because, overall, the receptors superimpose so well, it is possible that the linker between the NH_2- 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 Arg^{43}, Glu^{127}, Trp^{169}, and Asn^{218}. Exceptions are the conformations of Trp^{104} and of the loop comprising residues 163 to 168. The difference in Cα position of Trp^{104} 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 Cα 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 Lys^{168} and Lys^{172} of hGH with the side chain of Trp^{104} of hGHbp I. In both interfaces, Trp^{104} of the receptors buries most surface area with a decrease in solvent accessibility of 170 $Å^2$ in site I and of more than 210 $Å^2$ 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 Arg^{43} 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 Trp^{104} of hGHbp I and Asp^{171} and Thr^{175} of hGH. In site II, the cluster consists of Arg^{43} and Asp^{126} of hGHbp II and Asn^{12} of hGH (Fig. 6B). Another residue with multiple interactions is Glu^{127} of hGHbp I, which forms salt bridges to Lys^{41} and Arg^{167} 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

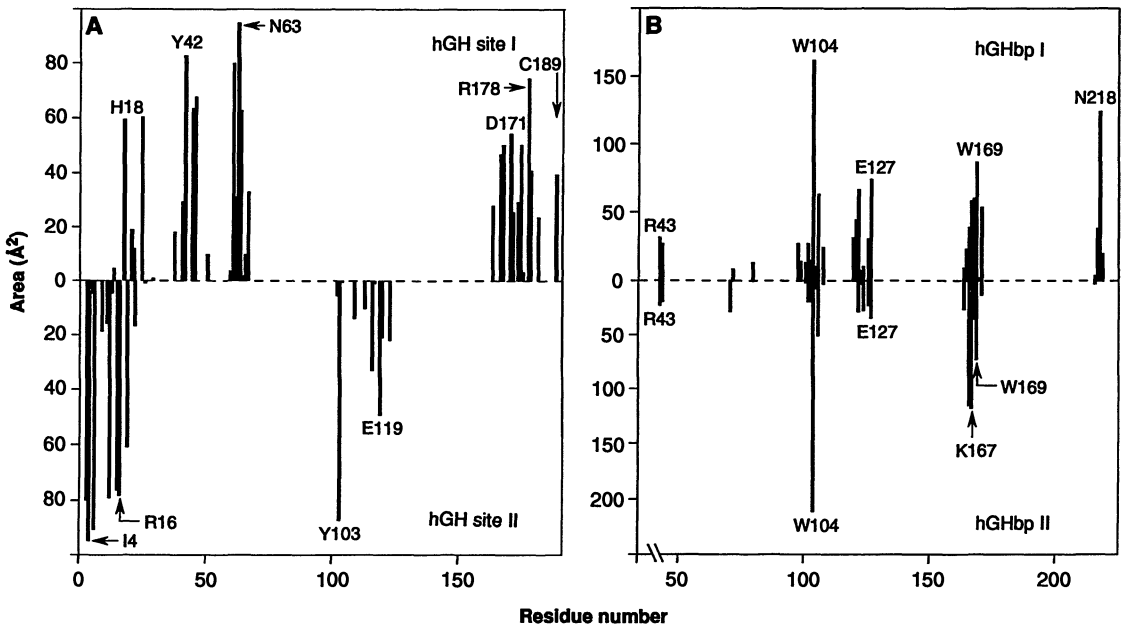

Fig. 5. Decrease in solvent accessibility on complex formation. (**A**) Residues on the hormone: top, site I; and bottom, site II. (**B**) Residues on the receptors: top, hGHbp I; bottom, hGHbp II. Solvent accessibility was calculated with the program written by Lee and Richards (*24*); a probe radius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

approximate twofold symmetry in the complex, this interface is formed by the same residues of each receptor (Table 2). The segments buried in the interface are the very end of strand A, most of the loop between A and B, some residues on strand B, part of the loop between D and E, and three or four residues on strand E. In both cases, on the basis of surface area buried, Tyr^{200} contributes most. Only about half of the side chains buried in the interface are hydrophobic; examples are Leu^{146} and Ile^{149} of hGHbp I, and Leu^{142} and Pro^{198} of hGHbp II. Most of the hydrophilic side chains are involved in specific interactions; for example, Asp^{152} of hGHbp II interacts with Ser^{145} (3.0 Å) and Thr^{147} (2.7 Å) of hGHbp I; Asp^{152} of hGHbp I is close to Tyr^{200} of hGHbp II (3.0 Å).

Comparison with mutational studies. The receptor binding determinants on hGH for site I have been mapped by means of homolog- and alanine-scanning mutagenesis (*17*). Binding site I was identified as a patch consisting of three discontinuous segments of hGH, the loop between residues 54 and 74, the COOH-terminal half of helix 4 and, to a lesser extent, the NH_2-terminal region of helix 1. Subsequent to that work, analysis of our crystals of the complex revealed the presence of the second hGHbp (*7, 8*). Mutational analysis was again used to identify this second binding site, showing it to consist of residues near the NH_2-terminus and on the hydrophilic faces of helices 1 and 3 (*8*). The three-dimensional structure of the complex confirms this interface region (Fig. 2). From the structure, there is one additional segment of polypeptide chain that is part of the interface in binding site I, namely, the small piece of helix (residues 38 to 47) at the beginning of loop 1 (Fig. 5). Since mutation of these residues did not have significant effects on binding of hGHbp I, the interface in this region may not contribute significantly to the binding energy, or may be able to adjust to different side chains. On a residue by residue basis, the correspondence between the structure and the mutagenesis mapping is also good. Most of the residues identified by alanine scanning can be classified as direct binding determinants in that they are found in the hormone-receptor interface; the structure also shows that some mutations resulting in decreased binding probably interfere with the proper folding of the hormone (Phe^{10}, Phe^{54}, Ile^{58}, and Phe^{176} in binding site I). Changing Phe^{1} in binding site II to alanine reduced the binding affinity by a factor of 5 (*8*). From the structure, however, it is unclear what the role of this amino acid side chain is since the NH_2-terminal two residues cannot be seen in the electron density map.

A similar mutational analysis involving changes of charged residues or selected tryptophans to alanine was applied to the hGHbp (*18*). By far, the largest decrease (2500 times) in hGH binding was observed for the change of Trp^{104} to alanine, while even the more conserved substitution to phenylalanine resulted in a large reduction (110 times) in binding. The next largest effect (84 times) was on substitution of Pro^{106}, whereas other variants were affected much less (less than eight times). Overall, the results are again in good agreement with the interactions seen in the crystal structure of the complex. A notable exception is Arg^{43}, mutation of which to alanine had little effect on binding of hGHbp I. Considering the network of interactions in which this residue pointing out (Figs. 1 and 6), it is hard to reconcile the differences in this instance, pointing out the difficulties in cross-referencing hormone and receptor binding determinants on the basis of mutational analysis.

Signal transduction by the growth hormone receptor. Analysis of the composition of our crystals (*7*), biophysical measurements on the complex in solution (*8*), and mutational alterations for mapping the second receptor binding site on hGH (*8*) show that the growth

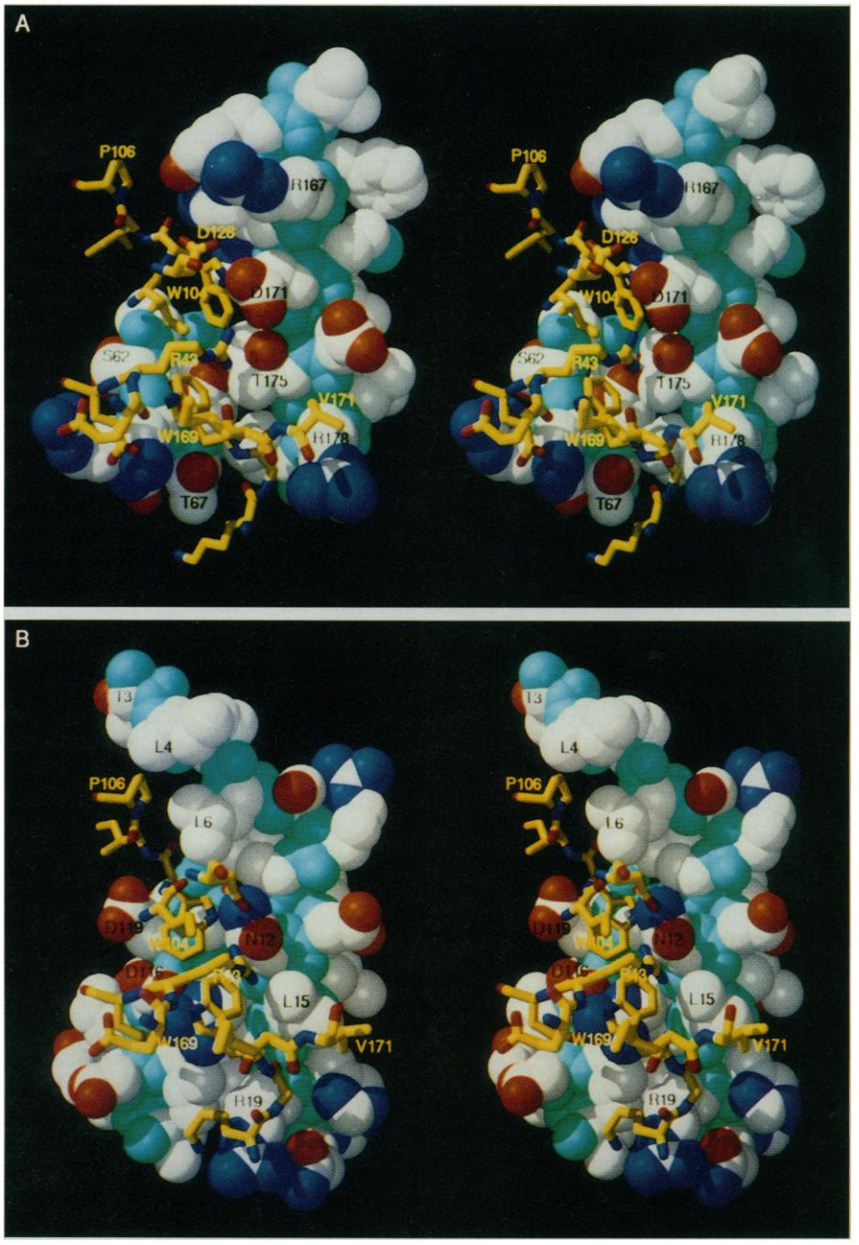

Fig. 6. Close-up of interfaces between hormone and receptors. (**A**) Binding site I; (**B**) binding site II. The hGH is represented by a space filling model, the receptors by a stick model. The hGH backbone atoms are cyan, side chain carbons are white, and side chain oxygens and nitrogens are red and blue, respectively. The receptor carbon atoms are in yellow, with red oxygens and blue nitrogens. Selected residues are labeled.

hormone-receptor complex has the form hGH·(hGHbp)$_2$. The crystal structure of the complex reveals how the hormone, a nonsymmetrical molecule, binds two copies of the receptor that use essentially the same binding determinants. The difference in surface area between interfaces I and II supports the sequential mechanism for receptor dimerization proposed by Cunningham *et al.* (*8*), who showed that the second receptor can only bind to hGH if the first receptor is already bound. This is consistent with the observation that the contact surface between receptor I and the hormone (1230 Å^2) is significantly larger than that between the hGHbp II and hGH (900 Å^2). We propose that binding of the second receptor is supported by the extra stabilization acquired by the interaction between the two extracellular domains near the COOH-terminus (500 Å^2).

This significant contact surface between the two extracellular domains demonstrates that dimerization of the extracellular domains does indeed take place. The fact that this association brings together that part of the COOH-terminal domain that is closest to the cell membrane suggests that similar association would also occur between the intracellular domains of the receptor. This structural picture, together with the fact that hGH mutants that cannot induce receptor dimerization (*8*) are biologically inactive (*19*), make a compelling argument that the mechanism of signal transduction on hormone binding is this dimerization event. Although it is at present unknown what the function of the intracellular domain is, association may generate a site of interaction with intracellular substrates or effector proteins.

Implications for the hematopoietic superfamily. The extracellular domain of the receptors belonging to the hematopoietic superfamily has a conserved set of four cysteine residues in the NH_2-terminal half of the sequence and some limited sequence homology, including a characteristic Trp-Ser-X-Trp-Ser sequence (X can be any amino acid) near the COOH-terminus (*2, 3*). In addition, Bazan (*3*) proposed that each of these receptors contains two 100-residue domains, each folded in an immunoglobulin-like barrel. Overall, the structure of the hGHbp confirms that hypothesis. At present, no other structures of the extracellular domains of hematopoietic receptors have been determined, but the structure of the hGHbp can probably serve as a good model for the related receptors of the superfamily. The structure shows that the conserved cysteines in the NH_2-terminal domain are linked to form disulfide bonds that are buried in the interior of the β barrel. Many of the other conserved residues are part of the core of the barrels, for example Trp^{50} in the NH_2-terminal domain and Trp^{157} in the second domain. A strictly conserved proline (Pro^{134} in hGHbp) is part of a sequence Pro^{131}-Asp^{132}-Pro^{133}-Pro^{134} in hGHbp, which follows the linker between the two domains and immediately precedes the first β strand of the COOH-terminal domain; these residues presumably contribute to the special disposition of the two receptor domains with respect to each other.

The structure of the hGH·(hGHbp)$_2$ complex provides no clear insight into the function of the characteristic Trp-Ser-X-Trp-Ser pattern. In hGHbp, the tryptophans and the first serine are not conserved, and the sequence is Tyr^{222}-Gly^{223}-Glu^{224}-Phe^{225}-Ser^{226}. This sequence of amino acids is part of the distorted segment of extended chain preceding strand G of the COOH-terminal domain (Fig. 3) and is located away from all binding interfaces. A pattern is generated by alternating series of charged and aromatic residues, the aromatic side chains packing between the methylene groups of the charged side chains (*20*). A similar pattern seems to be conserved in many of the other receptors of the superfamily.

The receptor residues in the hormone-receptor interfaces that appear to be most important are not conserved in the other members of the superfamily. This is not unexpected, because each particular receptor has to interact with its own ligand. Trp^{104} and Trp^{169} are conserved only between the growth hormone and prolactin receptors; conservative changes are found in the erythropoietin receptor with phenylalanine for Trp^{104}, and in the receptors for interleukin 4, interleukin 6, and granulocyte-macrophage colony-stimulating factor with tyrosine or phenylalanine for Trp^{169}. Asn^{218}, a binding determinant in interface I only, is His in the prolactin receptor, consistent with the proposal that the zinc dependence of the interaction between prolactin and its receptor is the result of a zinc binding site involving this His residue (*21*).

The interaction between the COOH-terminal domains of the extracellular part of the receptors is assumed to be weak, since receptor association should not take place in the absence of the ligand (*22*). Thus, there are no strong constraints against variation of residues in this interface, consistent with the observation that there is no apparent conservation among different members of the superfamily.

The dimerization observed for the growth hormone-receptor complex results from the interaction of two identical receptor molecules with a single hormone molecule. No other examples of this particular type of interaction are known, the closest analogy being the binding of interleukin 2 to two different subunits of its receptor, but more examples of a growth hormone–like mechanism may be found. As proposed for many receptor tyrosine kinases (*5*) and in agreement with indirect evidence available for the prolactin receptor (*23*), our structure shows that for the hematopoietic superfamily, too, ligand-induced receptor dimerization is likely to be the common mechanism of signal transduction.

REFERENCES AND NOTES

1. C. S. Nicol, G. L. Mayer, S. M. Russel, *Endocrine Rev.* 7, 169 (1986).
2. D. Cosman *et al.*, *Trends Biochem. Sci.* **15**, 265 (1990); K.-I. Arai *et al.*, *Annu. Rev. Biochem.* **59**, 783 (1990).
3. J. F. Bazan, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6934 (1990).
4. S. Davis *et al.*, *Science* **253**, 59 (1991).
5. Y. Yarden and A. Ullrich, *Annu. Rev. Biochem.* **57**, 443 (1988); A. Ullrich and J. Schlessinger, *Cell* **61**, 203 (1990).
6. G. Fuh *et al.*, *J. Biol. Chem.* **265**, 3111 (1990).
7. M. Ultsch, A. M. de Vos, A. A. Kossiakoff, *J. Mol. Biol.*, in press.
8. B. C. Cunningham *et al.*, *Science* **254**, 821 (1991).
9. S. S. Abdel-Meguid *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6434 (1987).
10. G. Lambert, E. A. Stura, I. A. Wilson, *J. Biol. Chem.* **264**, 12730 (1989); N. Gunther, C. Betzel, W. Weber, *ibid.* **265**, 22082 (1990).
11. Figures 2, 3A, 4, and 6 were produced with the MIDAS molecular graphics package [T. E. Ferrin *et al.*, *J. Mol. Graphics* 6, 13 (1988)], with software written by T. Hynes for Figs. 4 and 6.
12. T. A. Bewley and C. H. Li, *Arch. Biochem. Biophys.* **233**, 219 (1984).
13. M. G. Mulkerrin, B. C. Cunningham, J. A. Wells, T. A. Bewley, unpublished results.
14. J. Wang *et al.*, *Nature* **348**, 411 (1990); S.-E. Ryu *et al.*, *ibid.*, p. 419.
15. A. Holmgren and C.-I. Bränden, *Nature* **342**, 248 (1989).
16. In interface I, 53 percent of the surface of the hormone and 59 percent of the surface of the hGHbp is polar. The corresponding numbers for interface II are 60 and 56 percent. In the dimer interface between the two receptors, 36 percent of the surface of hGHbp I and 44 percent of the surface of hGHbp II are polar.
17. B. C. Cunningham *et al.*, *Science* **243**, 1330 (1989); B. C. Cunningham and J. A. Wells, *ibid.* **244**, 1081 (1989).
18. S. H. Bass, M. G. Mulkerrin, J. A. Wells, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 4498 (1991).
19. R. Clark *et al.*, unpublished results.
20. Residues contributing to this motif are Glu^{173} and Lys^{215}; Tyr^{222}; Glu^{175} and Arg^{213}; Phe^{225}; Gln^{177} and Arg^{211}; Trp^{186}.
21. B. C. Cunningham *et al.*, *Science* **250**, 1709 (1990).
22. The binding of the two extracellular domains is weaker than micromolar (B. C. Cunningham and J. A. Wells, personal communication).
23. G. Elberg *et al.*, *J. Biol. Chem.* **265**, 14770 (1990).
24. B. Lee and F. M. Richards, *J. Mol. Biol.* **55**, 379 (1971).
25. A. Messerschmidt and J. W. Pflugrath, *J. Appl. Cryst.* **20**, 306 (1987).
26. W. Kabsch, *ibid.* **21**, 916 (1988).
27. W. Steigemann, thesis, Technische Universitat Munchen (1974).
28. B. C. Wang, *Methods Enzymol.* **115**, 90 (1985).
29. A. T. Brünger, J. Kuryian, M. Karplus, *Science* **235**, 458 (1987).

30. We thank K. Olson for hGH, B. Snedecor and M. Covarrubias for fermentation, M. Mulkerrin for suggestions on purification, J. Wells for scientific discussions, B. Henzel for amino acid analyses, and T. Hynes for help with Figs. 4 and 6. R. Stroud made valuable comments on the manuscript. Cα coordinates will be deposited in the Protein Data Bank.

18 September 1991; accepted 18 November 1991