Crystal Structure of an Antagonist Mutant of Human Growth Hormone, G120R, in Complex with Its Receptor at 2.9 Å Resolution*

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Human growth hormone binds two receptor molecules and thereby induces signal transduction through receptor dimerization. At high concentrations, growth hormone acts as an antagonist because of a large difference in affinities at the respective binding sites. This antagonist action can be enhanced further by reducing binding in the low affinity binding site. A growth hormone antagonist mutant Gly-120 → Arg, has been crystallized with its receptor as a 1:1 complex and the crystal structure determined at 2.9 Å resolution. The 1:1 complex is remarkably similar to the native growth hormone-receptor 1:2 complex. A comparison between the two structures reveals only minimal differences in the conformations of the hormone or its receptor in the two complexes, including the angle between the two immunoglobulin-like domains of the receptor. Further, two symmetry-related 1:1 complexes in the crystal form a 2:2 complex with a large solvent inaccessible area between two receptor molecules. In addition, we present here a native human growth hormone–human growth hormone-binding protein 1:2 complex structure at 2.5 Å resolution. One important difference between our structure and the previously published crystal structure at 2.8 Å is revealed. Trp-104 in the receptor, a key residue in the hormone-receptor interaction, has an altered conformation in the low affinity site enabling a favorable hydrogen bond to be formed with Asp-116 of the hormone.

Human growth hormone (hGH) is the structurally best characterized member of a family of closely related hormones. hGH and its relatives, prolactin (PRL) and placental lactogens, regulate a wide variety of physiological processes, including growth and differentiation of muscle, bone, and cartilage cells. hGH binds to the human growth hormone receptor (hGHR) and its soluble binding protein (hGHbp), consisting of the extracellular and transmembrane domain of the receptor. hGH also binds to the prolactin receptor (PRLR). In contrast, PRL binds only PRLR (for a recent review, see Ref. 1). In plasma, hGH and hGHbp exist predominantly as a 1:1 complex, and it has been speculated that hGHbp can serve as a dynamic buffer for bound and free hGH (2). Thus, variants of the hGHbp expressed in Escherichia coli can be used to study the extracellular events in the receptor dimerization (3).

To provide a framework for structural and functional studies de Vos and co-workers (4) crystallized and solved the structure of the hGH-hGHbp 1:2 complex in 1992 (hereafter referred to as the 1:2 complex). The crystal structure showed that two receptor molecules bind one hormone molecule (Fig. 1) by using virtually the same residues of the receptor but that hGH uses different residues in the two sites. The hGH site 1:hGHbp interaction is of considerably higher affinity than the hGH site 2:hGHbp interaction (4). hGHR dimerization is thus the likely event that activates intracellular signal transduction pathways where receptor-associated JAK kinases and phosphorylation-dependent STAT factors play critical roles (5, 6).

A mutant of hGH, where Gly-120 is replaced with an arginine residue (hereafter referred to as G120R), is an antagonist that binds to the hGHbp in an 1:1 stoichiometry in solution rather than the 1:2 stoichiometry observed in the native complex (7). A 1:1 complex between hGH and hGHbp mimics the first step in the formation of the ternary complex which is a prerequisite for binding of the second hGHbp (8). A crystal structure of the G120R-hGHbp complex (hereafter referred to as the 1:1 complex) has been discussed in the literature (9, 10) even though a detailed description of this structure has not yet been published.

To understand the structural basis for the formation of the G120R-receptor 1:1 complex we have expressed its constituents and determined its three-dimensional structure at 2.9 Å resolution using protein crystallographic methods. We compared our 2.5 Å structure of the 1:2 complex with that of the 1:1 complex, as well as our structure of the 1:2 complex with that reported previously by de Vos et al. (4).

MATERIALS AND METHODS

Chemicals and Equipment—If not stated otherwise, all chemicals were purchased from Sigma or Fluka. All protein purification equipment and material were from Pharmacia Biotech Inc.

Cloning and Expression of hGH and hGHbp—A structural gene encoding the extracellular domain, transmembrane domain, and part of the cytoplasmic domain of the hGHR was amplified from commercially available adult liver cDNA (Clontech) using the polymerase chain reaction. The sequence amplified starts at nucleotide position 22 in the 5’ untranslated region and ends at position 1076 within the cytoplasmic domain according to the numbering of the sequence by Leung et al. (11). The polymerase chain reaction product was cloned into the Smal site of pUC19, and the nucleotide sequence was determined. The sequence was identical to that published by Leung et al. (11). A second round of
polymerase chain reaction amplifications was performed using the pUC19-hGHR clone as template. The sequence amplified encodes the first 237 residues of the hGHbp (nucleotide positions 98–808). After digestion with \textit{Mlu} I and \textit{Xba} I the polymerase chain reaction product was subcloned into standard \textit{E. coli} expression vectors. To simplify expression of wildtype and mutant forms of hGH, a synthetichGHgene was constructed. An \textit{Mlu} I site overlapping the codon for \textit{Phe} I and an \textit{Xho} I site at the 3\textsuperscript{9} end of the gene were incorporated into the sequence to allow \textit{E. coli} expression of the gene. The gene was constructed from a set of overlapping oligonucleotides derived from the cDNA sequence published by Goeddel \etal (12), which were allowed to hybridize and were subsequently extended using Sequenase (U.S. Biochemical Corp.). The gene was constructed in two segments that were then combined by standard cloning techniques and transferred into the expression vector.

\textbf{Purification of hGH and hGHbp—}Wild type and mutant forms of hGH were expressed in \textit{E. coli} using standard methods. Periplasmic proteins were released from the cells by a freeze-thaw procedure and thereafter extracted in hypotonicbuffer (10 mM Tris, pH 8.5, containing 2 mM EDTA and 1 mM phenylmethylsulfonyl fluoride). The hGH was purified in a two-step process. First the periplasmic extract was sepa-

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Parameter & 1:1 complex & 1:2 complex \\
\hline
No. of crystals & 2 & 2 \\
Resolution & 2.9 Å (20–2.9 Å) & 2.5 Å (20–2.5 Å) \\
Completeness & 91.8% (20–2.9 Å) & 94.3% (20–2.5 Å) \\
Multiplicity & 4.1 & 4.6 \\
\textit{R} merge & 9.9% (20–2.9 Å) & 9.7% (20–2.5 Å) \\
\hline
Cell & 67.78 67.78 229.24 Å & 145.61 69.02 76.04 Å \\
Space group & \textit{P}4\textsubscript{3}2\textsubscript{1}2 & \textit{P}2\textsubscript{1}2\textsubscript{1}2 \\
No. of solvent molecules & 0 & 283 \\
Unique reflections & 11596 & 25222 \\
\text{r.m.s.} bond deviations (Å) & 0.013 & 0.012 \\
\text{r.m.s.} angle deviations (°) & 1.98 & 1.77 \\
Model \textit{R} factor/ free \textit{R} & 21.3/31.2% & 19.1/28.3% \\
\hline
\end{tabular}
\caption{Crystallographic data for the 1:1 and the 1:2 complexes}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Parameter & 1:2 complex & 3hr \\
\hline
Total solvent accessibility & & \\
\hline
hGH & 10,295 & 10,426 \\
rec1 & 10,948 & 11,286 \\
rec2 & 11,146 & 11,151 \\
Total buried area & 5,221 & 4,817 \\
\hline
\end{tabular}
\caption{Solvent accessibility for the free components and for the 1:2 complex in Å\textsuperscript{2}, calculated using the program DSSP (22).}
\end{table}

\textbf{Purification of the 1:2 and 1:1 Complexes—}Wild type and mutant forms of hGH were expressed in \textit{E. coli} using standard methods. Periplasmic proteins were released from the cells by a freeze-thaw procedure and thereafter extracted in hypotonicbuffer (10 mM Tris, pH 8.5, containing 2 mM EDTA and 1 mM phenylmethylsulfonyl fluoride). The hGH was purified in a two-step process. First the periplasmic extract was sepa-

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Figure1.png}
\caption{Schematic representation of the general architecture of the 1:2 complex. The two receptor molecules are in red (high affinity) and yellow (low affinity). The picture was generated using MOLSCRIPT (36) and raster3D (37).}
\end{figure}
molar amounts of the constituents of the GI20R-hGHbp 1:1 complex and the hGH-hGHbp 1:2 complex were preformed by a short incubation at pH 7.5 in 20 mM Tris with 100 mM NaCl. The complex was concentrated to approximately 2 mg/ml before separation from noncomplexed proteins on a prepacked Hiloak XK 26/60 Superdex 75 column. The relevant peaks were collected and concentrated to 10 mg/ml in 10 mM ammonium acetate, pH 6.8, and thereafter shock-frozen in 100-µl aliquots in liquid nitrogen. The protein complexes were thereafter stored at -85°C prior to use in the crystallization experiments.

Protein Crystallization—The 1:1 complex was crystallized by vapor diffusion. Three µl of protein solution (10 mg/ml) was mixed with 3 µl of 1.6 M LiSO₄ and 50 mM Bis-Tris at pH 6.25–6.75 in a sealed tissue culture 24-well plate (Falcon). The crystallization droplets were equilibrated at 18 °C with 1 ml of the mother liquor for 2–3 weeks to obtain optimal diffraction quality crystals. Crystals were 0.5 × 0.25 × 0.25 mm in size and diffracted to at least 2.9 Å with a conventional x-ray source. The 1:2 complex was crystallized under conditions similar to those for the 1:1 complex. To grow large crystals (1.2 × 0.5 × 0.3 mm) diffracting to 2.5 Å resolution using a conventional x-ray source, an addition of polyethylene glycol 500:dimethyl ether was essential. The best diffracting crystals were obtained by adding 1 µl of mother liquor at pH 6.25 with 46% (v/v) of a saturated LiSO₄ solution and 2% polyethylene glycol 500:dimethyl ether (v/v) to 5 µl of protein solution (10 mg/ml) and thereafter incubating the mixture at 18 °C for 1–2 weeks. To stabilize the crystals prior to x-ray diffraction data collection, the crystals were soaked in a 50% (v/v) saturated LiSO₄ solution, 0.1 M MES, pH 5.25, and 0.5 mM MgCl₂.

Data Collection and Processing—All data were collected using a MAR image plate system. The data were processed with MOSFLM (13) using the refix algorithm (14) for indexing and point group determination and were then further reduced and scaled using the CCP4 program package (13). For crystallographic data see Table I.

Structure Determination of the 1:2 Complex—At the time this work was initiated, only the Co coordinates of the 1:2 complex solved by de Vos and co-workers (4) were available from the Brookhaven PDB data bank (PDB entry 2hhr). Because of initial concern about the space group, we used a molecular replacement search procedure using the program AMORE (15). The highest scoring solution in the resolution interval 8–4 Å was found in space group P2₁2₁2₁ with one 1:2 complex molecule in the asymmetric unit. Since this confirmed that our LiSO₄:polyethylene glycol-grown crystals were virtually isomorphous to the crystals grown by Ultsch et al. (16), a polyalanine model of this backbone structure was generated, using the data base in O (17), and used as a starting model in the refinement of the structure. A rigid body refinement in X-plor (18) preceded a careful cyclic process of model building in O introducing only clearly visible side chains followed by POWELL minimizations in X-plor. This cyclic procedure was repeated until approximately 80% of the side chains were visible at 3.0 Å resolution. At this stage the first simulated annealing run (19) was performed using a slow cooling protocol from 2,500 to 300 K in 50-ns steps including data between 15 and 2.8 Å. An initial R value of 36.5% dropped to 22.1% (free R 31.5%) after 20 cycles of model building and X-plor refinement. Using this 2.8 Å model, all data between 10 and 2.5 Å were used in a process similar to the initial minimization and model building to create a refined model with a final R value of 21.3% (free R 30.1%) after B value refinement. Solvent molecules were introduced into F₂F₁ electron density maps calculated after a POWELL minimization followed by a simulated annealing run from 3,000 to 300 K in 50-ns steps including data between 10 and 2.5 Å excluding hGHbp residues Ile103, Trp-104, Ile-105, and hGH residue Asp-116 of the final 1:2 complex model from the calculation.
value (20) was used to validate the progress of the entire refinement. The final model consists of residues 1–147, 154–190 of hGH; 32–52, 63–235 of hGHbp 1; and 32–54, 62–72, 78–236 of hGHbp 2 as well as 283 solvent molecules. The R factor of this model is 19.1% (free R 28.3%), and the quality of the model has been assessed using PROCHECK (21). Solvent-accessible areas were calculated using the program DSSP (22).

Structure Determination of the 1:1 Complex—For the 1:1 complex, our refined structure of the 1:2 complex, depleted of its low affinity receptor and solvent molecules, was used as a search model using the program AMORE. Data collected could be reduced in point group P422, and the highest resolution at which the electron density map was found was in the space group P4,2,2 with one 1:1 complex molecule in the asymmetric unit. Between data sets of 15 and 3.0 Å, the initial R value of 36.1% dropped to 29.1% (free R 35.1%) upon rigid body refinement and POWELL minimization in X-plor. Correction for changes in the external solvent density and side chains conformations in the NH2 terminus of hGH and the loop 75–79 in hGHbp as well as introduction of an arginine side chain at position 120 in G120R lowered the R value to 28.3% (free R 35.2%) after a POWELL minimization. At this stage the first simulated annealing run was performed using a slow cooling protocol from 2,500 to 300 K in 50-ps steps, followed by manual model building in O and POWELL minimizations in a cyclic procedure that produced a final model with an R value of 21.3% (free R 31.2%) using data between 8 and 2.9 Å. The final model consists of the residues 1–146, 155–191 of G120R and residues 31–53 and 63–237 of hGHbp. The quality of the model has been assessed using PROCHECK.

RESULTS

Structure of the 1:2 Complex—After we had refined the structure, de Vos and co-workers (4) released their 2.8 Å structure with side chains to the Brookhaven PDB data bank (PDB entry 3hhr). A comparison of this structure with ours revealed that the two models were essentially identical, although differences with functional importance are observed in the site 2 hGH-receptor interaction.

The overall structure of the 1:2 complex has been described thoroughly in several publications (4, 23, 24), thus we will discuss only briefly the general structural features of hGH and hGHbp. However, the major differences between our 1:2 structure and the previously published one will be discussed in detail.

The two extracellular domains (domain 1 consisting of residues 1–123 and domain 2 of residues 128–234 in hGHbp) of hGHbp have topologies similar to the immunoglobulin fold. Regions in hGHbp having poorly defined or absent electron densities are residues 1–14, 75–79, 103–119 and 224–237.

The hGH single domain four-helix bundle has the two first helices parallel to each other and antiparallel to the last two. This variant of a helix bundle was first seen in porcine growth hormone (25) but has later been observed in a variety of other cytokines (for review, see Ref. 24). hGH binds at the connecting region between the two domains of the receptor. The area buried between hGH site 1 and hGHbp (2,700 Å2) is considerably larger than that observed in the hGH site 2-hGHbp interaction (1,500 Å2). This difference is compensated for by the additional surface that is buried between the two COOH-terminal domains of the receptors (900 Å2) (Table II). Regions in hGH with poor degree of order are residues 39–45 and 128–147. Residues 148–153 and 191 completely lack electron density.

The overall similarity between the 1:2 complex presented here compared with the 1:2 complex structure deposited in the PDB data bank (3hhr) is very high with a root mean square difference for 566 Ca atoms positions of 0.51 Å, and a majority of the side chains is in similar or close to identical conformational. Differences between the two 1:2 complex structures involve mainly residues in the low affinity site between hGH and hGHbp. The most important difference in this area is that Trp-104 in hGHbp has adapted a conformation different from the rotation of Trp-104 of the receptor (Fig. 3). As seen in Table III, our Trp-104 orientation is very close to the most favorable rotamer of tryptophans according to the data base in O (17). As a test case, we used the 3hhr coordinates and refined this model with our data. Pronounced F, F, densities around Trp-104 clearly showed that our interpretation of the orientation of this key residue in the low affinity site was correct. In addition, our Trp-104 orientation is very close to the most favorable rotamer of tryptophans according to the data base in O (17).

The receptor surface area interacting with the hGH high affinity site 1 differs from the one seen in the lower affinity site 2 when structurally aligned mainly due to the different conformations of Trp-104 of the receptor (Fig. 3). As seen in Table III, the greater part of interacting residues from hGH in site 1 resides in the connecting loop between helix 1 and 2 (residues 41–68) and from the COOH-terminal part of helix 4 (residues 167–175). Site 2 interactions are mainly observed in the NH2 terminus (residues 1–16) and in helix 3 (residues 103–119) of hGH.

Members of the cytokine receptor family have a consensus WSXWS sequence (WS motif) in the extracellular domain. In interleukin-2, erythropoietin, and PRLRs, alteration of the WS sequence disrupts ligand binding and receptor signaling (26). In hGHbp, the WS sequence analogous motif is present in domain 2 involving the residues Lys-179, Trp-186, Arg-211, Phe-225, Arg-213, Tyr-222 and Lys-215 (Fig. 4). Since domain
2 lacks intramolecular disulfide bridges, this motif might serve to stabilize this domain.

Structure of the 1:1 Complex—The initial electron density map of the 1:1 complex was of very good quality despite the relatively low resolution of the data. The overall similarity of the 1:1 complex to the 1:2 complex depleted of the low affinity receptor is very high with a root mean square difference of 0.81 Å comparing 368 Cα atoms. The majority of the side chains as well as the loop regions are remarkably similar to the 1:2 complex depleted of the receptor binding to hGH site 2. The angle between the two domains in hGHbp is virtually identical to that seen in the 1:2 complex, indicating that the low affinity site interaction between hGH and hGHbp does not induce any domain movements in the hGH site 1 binding receptor. One of the more striking differences compared with the 1:2 complex is that the NH₂ terminus of the hormone, residues 1–5, extends out from the hormone with the side chain of Phe-1 packing against a hydrophobic motif around the hGHbp residues Trp-76 and Trp-80 of a symmetry-related molecule. From residue Leu-6 in the hormone, the main chain is very similar to the 1:2 complex.

The exposed surfaces that are normally buried between the two receptors and the low affinity site in hGH form extensive interactions with symmetry-related molecules. In fact, the interactions are so extensive that one could argue that a 2:2 complex with two binding proteins and two hormone molecules has been formed across a crystallographic 2-fold axis (Fig. 5). The total area of the four molecules in the “2:2 complex” not accessible to solvents compared with the free components is approximately 7,300 Å². Approximately 2,000 Å² is buried in hormone-receptor or receptor-receptor interactions compared with approximately 2,400 Å² in the 1:2 complex, excluding the high affinity sites from the calculations.

Arg-8 in the NH₂-terminal part of helix 1 of the hormone has adapted a conformation similar to the one we see in our 1:2 complex, differing from the 3hhr structure. Another residue in site 2 of hGH, Asn-12, which in the 1:2 complex structure is within hydrogen bond distance to Asp-126 and Arg-43 of the low affinity receptor, now is within favorable hydrogen bond distance and angle to the main chain oxygen of Pro-234 of the 2:2 complex thus stabilizing the COOH-terminal residues in hGHbp. Most other changes of side and main chain conformations are due to minor crystal packing effects and are therefore not discussed in any detail because of the relatively low resolution of the data.

DISCUSSION

When the 2.8 Å crystal structure of the 1:2 complex structure was published in 1992, a new understanding of the events involved in protein-protein interactions was obtained. A protein complex that utilized virtually the same residues in both receptor sites was shown to interact with completely different residues in the hormone. The 1:2 complex structure as well as other studies indicated that a sequential dimerization would be sufficient for signal transduction.

The wild type hGH as well as the hGH single site mutant Gly-120 → Arg in complex with its soluble binding protein crystallize under similar conditions. Wild type hGH and the hGHbp crystallize as a 1:2 complex and the G120R and hGHbp as a 1:1 complex. The crystal structure of the 1:1 complex shows, at least at high protein concentrations, that a 2:2 complex can be formed with an altered arrangement of the interface of two binding proteins. It is interesting to speculate whether such a 2:2 complex also could be biologically active, if the geometry of the receptors with respect to the mobility in the membrane allows it to be formed. A recent report by Harding et al. (27) most likely show that G120R can form 2:2 complexes with the porcine GH receptor. However, the significance of this observation remains to be shown with similar experiments using the hGHR.

It has become generally accepted that GH receptors and PRL receptors are activated by dimerization. Monoclonal antibodies directed against the hGHR can induce signal transduction, whereas their monovalent fragments could not. This indicates that the dimerization itself, without structural constraints such as domain movements within the receptor molecule, is sufficient for signal transduction (7). However, in the absence of any structural information of the unliganded receptor one can only speculate on this matter.

A mutant erythropoietin receptor (Arg-129 → Cys) is constitutively active when disulfide-linked homodimers are formed in the extracellular domain (28), suggesting that the wild type erythropoietin receptor is activated by ligand-induced homodimerization without structural constraints induced by the hormone. The high affinity receptor for the granulocyte macro-
phage colony-stimulating factor (GM-CSF) is a heterodimer composed of an α-receptor and a β-receptor (GMR-αβ). The GM-CSF-GMR-αβ complex is biologically active. A mutant in GM-CSF, Glu-21 → Ala, inhibits high affinity binding, leaving low affinity binding unaffected. Despite near to wild type biological response in cell assays, no detectable binding interaction of this mutant with GMR-β in the context of GMR-αβ was observed. This clearly demonstrated that GM-CSF receptor activation can occur independently of high affinity binding (29).

Surprisingly, it was shown recently that the PRLR becomes constitutively active when the membrane proximal domain is deleted from the extracellular part of the receptor, when expressed in Chinese hamster ovary cells (30). Neither of these properties discussed above has so far been shown for the hGHR. However, the extracellular part of the hGHR contains one unpaired cysteine at position 241, as expected in close proximity to the membrane, which possibly could be involved in covalent linkage of hGHR dimers (31). The formation of such a disulfide bridge might be an important prerequisite for efficient signal transduction via extracellular receptor dimerization.

The single substitution Gly-120 → Arg in hGH creates a powerful hGH antagonist at moderate levels (20 nM) whereas wild type hGH exhibits antagonist action at much higher concentrations (2 μM). Further, G120R has been shown to act as an antagonist on human PRLR (32) and also to cause rodent PRLR-mediated proliferation of Nb-2 cells in the presence of Zn²⁺ (10 μM) (33). Zn²⁺ as a prerequisite for proliferation could possibly be explained with metal ion restoration of the ternary complex of hGH-PRLR. Crystals of the complex of wild type hGH-PRLR were initially expected to contain a 1:2 complex, but high performance liquid chromatography purification of the complex showed it to consist of stoichiometrically equivalent amounts of hormone and receptor (34). Because of better crystal growth-promoting properties the G120R mutant was used in the structural analysis. However, a crystal structure of a 1:2 complex of hGH-PRLR would be of extreme interest for validation of the biological significance of the 1:2 complex of hGH-hGHRP.

The 1:1 complex is remarkably similar to a 1:2 complex.
depleted of its low affinity receptor with no large domain shifts.

The majority of side chain conformations and the main chain loop regions are close to identical to the 1:2 complex depleted of its site 2 binding receptor. The only main chain change clearly visible in the hGHbp is that the loop region 71–79 of the hGHbp is ordered because of symmetry molecule interactions with the hGH NH₂ terminus. Because of this interaction the five NH₂-terminal residues in hGH which normally are bound in site 2 interactions have an altered conformation and now extend out from the hormone. One could argue that this region in the absence of low affinity site interactions is readily available to bind to target receptors and thus might play a key role in hGH-mediated signal transduction. Thirty-one residues in the NH₂ terminus of hGHbp are completely lacking electron density in both the 2:1 as well as in the 1:1 complex. The function of the NH₂ terminus of the receptor is not understood, but a potential N-linked glycosylation site in this region (Asn-26) could be important for both function and stability.

The Gly-120 → Arg substitution of hGH resides in a sequence motif consisting of residues of 111–129. The substitution of these residues to the corresponding residues in hPRL decreases activity more than 100-fold (EC₅₀mut/EC₅₀wt). A similar response is seen with a truncated variant of hGH lacking residues 1–8. Single site alanine mutations within these motifs revealed residues Phe-1, Ile-4, Leu-6, Arg-8, and Asp-116 as critical residues in the dimerization event upon site 2 binding with the Ile-4 → Ala as the single most critical mutation (8). As shown in this study, the most striking difference between the two independently refined 1:2 complex structures is the orientation of the indol ring of Trp-104 in the low affinity receptor, enabling a favorable hydrogen bond to be formed with Asp-116 in the hormone, in addition to its hydrophobic interactions. Thus, the functional importance of the observed interaction between Asp-116 in hGH and Trp-104 of the receptor correlates well with mutational data. The dramatic effect of the Gly-120 → Arg substitution in hGH can, at least partly, be explained by steric hindrance of the hGH Asp-116 → hGHbp Trp-104 interaction in addition to disruption of other site and main chain conformations in this area (Fig. 6).

In another study, the effects of mutants of hGH were studied with respect to tyrosine kinase-dependent phosphorylation of intracellular proteins and promotion of growth in mice. G120R was shown to be a potent hGH antagonist, but surprisingly it was also observed that the hGH mutant Ile-4 → Ala increased the amount of intracellular phosphorylation as well as exhibited full growth promoting activity (35) clearly in contrast to the predicted properties of this mutant hGH.

The 1:1 complex of G120R-hGHbp mimics the first step in a dimerization process required for intracellular signal transduction. The small differences between the 1:1 complex and the 1:2 complex depleted of its site 2 binding hGHbp would suggest that conformational changes in the high affinity site upon binding of the second receptor are not required for efficient signal transduction. However, high resolution structures of the free and solvated forms of hGH and hGHbp will contribute significantly to our understanding of the events involved in binding of the hormone to its receptor as well as to the understanding of the requirements for efficient signal transduction in cells.

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