The complex of human Gs protein with the beta3 adrenergic receptor: A computer-aided molecular modeling study

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Three-dimensional (3D) models of the human Gs protein, the human beta3 adrenergic receptor and their complex are constructed using computer-aided molecular modeling techniques. The structures of bacterial EF-tu 200 and bacteriorhodopsin were used as starting points for modeling the Gs protein and beta3 receptor, respectively. Experimental data are used as constraints to guide the modeling. The resulting 3D structures of the Gs protein, the beta3 receptor and their complex are in accord with the experimental data. It is found that the third interacellular loop of the beta3 receptor as well as its C-terminus are involved in the binding. Various residues of N-terminus and C-terminus of the Gs protein also participate in the binding. The model of the complex suggests that the Gs protein binds to the beta3 receptor in such a way that it will be placed in the interface of membrane and intracellular space. This orientation is supported by experimental data. It is concluded that the modeled structure of the complex of the alpha subunit of the human Gs protein and the beta3 adrenergic receptor is in agreement with the experimental data and it can provide a basis for understanding the way these proteins interact.

Keywords: adrenergic receptor, G protein, computer modeling

INTRODUCTION

The coupling of membrane-bound receptors such as the beta3 adrenergic receptor to enzyme effectors requires the activation of G proteins.[1,2] This involves GDP-GTP exchange on the alpha subunit of these proteins in the presence of Mg.[2,3] A common structural design and shared molecular mechanism distinguishes proteins in the GTPase superfamily. Of this superfamily, the only known crystal structures are of ras-p21[4–6] and the guanine nucleotide binding domain of bacterial EF-tu,[7–9] which show a remarkable similarity. The overall alpha/beta topologies of the two domains are the same and their polypeptide backbone appears, for the most part, superimposable. While the crystal structures of G proteins alpha subunits are not available, mutations and biochemical analysis as well as sequence similarity imply that these proteins have parallel molecular mechanisms and structural elements.[2,3] Considering the key role of G proteins in a variety of major cellular processes, it is of great interest to learn as much as possible about the structure-function relationships of these proteins and their interactions with membrane-bound receptors. Unfortunately, the structure of those G proteins that interact with membrane-bound receptors and also of the receptors themselves has not yet been elucidated. It has been suggested that in the absence of a detailed crystal structure of a membrane-bound protein, computer-aided molecular modeling could provide valuable information and insight regarding their structure-function relationships.[10,11] We have applied computer-aided molecular modeling techniques to generate reasonable three-dimensional (3D) model structures for the human Gs protein, the human beta3 adrenergic receptor and their complex to shed light on the structure-function relationships of these proteins and their coupling.

METHODS

Modeling of the human Gs protein

The sequence of human Gs protein was aligned with that of the *E. coli* EF-tu nucleotide binding domain and ras-p21 as well as human rod transducin[12] using the MULTALIN program.[13] The sequence of human transducin was added to the alignment to produce a balance between the human Gs protein and the sequences of ras-p21 and EF-tu. The results are shown in Figure 1. The secondary structure assignments were based on the consensus assignments from a battery of secondary structure prediction methods,[14–18] which agree with the results of the alignments. The crystal structure of

Color Plates for this article are on page 34.

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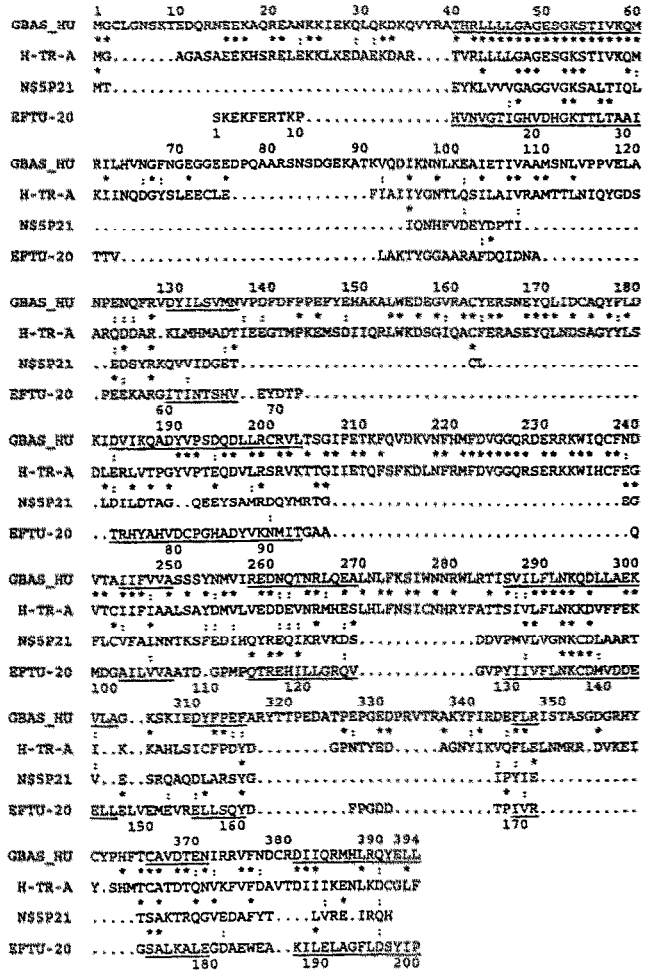

Figure 1. Alignment of the sequences of the human Gs protein (GBAS_HU) with that of human rod transducin (H-TR-A), ras-p21 (N$5P21) and the nucleotide binding domain of bacterial EF-tu (EFTU-20). The underlined residues of EF-tu 200 are mutated to the corresponding residues of human Gs protein to construct the core of the latter structure.

the EF-tu nucleotide binding domain[9] was used to generate a backbone framework with the program COMPOSER,[19,20] running as a part of the SYBYL package.[21] From this framework, a model of the human Gs protein was constructed by mutating the side chains of those residues whose predicted secondary structures were similar to that of corresponding residues of EF-tu as determined by sequence alignment (Figure 1).

It has been found that this procedure will provide the core of protein to which various surface loops can be added using the loop option of the COMPOSER program.[19,20] This option compares a database of 281 proteins to find the required loops. The best-fitted loops with no or minimum positive phi angles were chosen and added to the core of the modeled human Gs protein. For the N-terminus and those parts of the molecule where a reasonable loop could not be fitted, modeling was carried out on the basis of the predicted secondary structure of the Gs protein. The resulting structure was further refined using the CHARMm program.[22] First a dynamics run for 5 ps at 400 K with harmonic constraints on the backbone was carried out to reduce poor interactions between buried residues. Then another dynamics run for an additional 5 ps at 400 K with no constraints was performed to obtain a more realistic structure. The resulting structure was energy minimized and is illustrated in Color Plate 1.

Modeling of human beta3 adrenergic receptor

The seven helices of the human beta3 adrenergic receptor[23] were constructed by mutating corresponding residues of bacteriorhodopsin,[24] as suggested by Trumpp-Kallmeyer et al.[25] The extracellular and intracellular loops were added to these helices as described earlier using the SYBYL package. The N-terminus and C-terminus were built up following the predicted secondary structure of the human beta3 receptor. The resulting structure was further refined using the CHARMm program as previously described for the Gs protein and is shown in Color Plate 2.

Modeling the complex of the beta3 receptor and the Gs protein

It is believed that the C-terminus of the G proteins is involved in the binding of these proteins to the receptor.[26] Also, the third intercellular loop, as well as the C-terminus of G protein-coupled receptors bind to the G proteins.[27] Hence, docking was carried out manually to accommodate these facts using the QUANTA molecular modeling package.[28] The resulting structure was further refined in two stages as stated earlier and is shown in Color Plate 3.

The quality of the modeled structures was evaluated by aid of the Procheck package.[29] Plots of phi and psi angles showed that less than 1.5% of residues are located in disallowed regions.

RESULTS AND DISCUSSION

Alpha subunit of the human Gs protein

The result of the alignment of the alpha subunit of the human Gs protein with that of ras-p21 and the nucleotide binding domain of *E. coli* EF-tu is shown in Figure 1. The human rod transducin sequence[12] has been added to the alignment calculation to produce a balance between the sequence of the human Gs protein and those of ras-p21 and EF-tu. It has been found that the human Gs protein correlates better with EF-tu than with ras-p21. The backbones of EF-tu and ras-p21 are almost superimposable.[9] But since the EF-tu nucleotide binding domain has a longer sequence, the backbone of EF-tu was chosen as a frame of reference for the modeling. There is good agreement between the resolved crystal structure of EF-tu 200 and that of the predicted structure of corresponding residues of the alpha subunit of the human Gs protein. Inspection of the EF-tu 200 structure shows that those residues that align with the human Gs protein could provide the core of protein to which various surface loops could be added to build up a model of the Gs protein. Thus, the backbone of EF-tu 200 was used as starting structure for the modeling as described in the methods section.

The final structure of the alpha subunit of the human Gs protein, after several runs of dynamics and energy minimization, is shown in Figure 2 and Color Plate 1. As can be seen from Figure 2, the core structure of the Gs protein is preserved with respect to that of EF-tu and extra residues form various surface loops. The GDP binding site is shown in Figure 3. The final secondary structure of the Gs protein is shown in Table 1 and Color Plate 1. This shows that the alpha subunit of the human Gs protein consists mainly of alpha helices. Some 127 of a total 394 residues have an alpha helix conformation. Altogether, there are 14 helices and five beta sheets. The core of protein is hydrophobic and the whole structure is stable. Plots of phi and psi angles show that less than 1.5% of the residues are in the disallowed region. Other statistics are comparable with those of EF-tu 200, which was used for the modeling.

It is interesting to note that the surface of protein around the GDP binding site is predominantly hydrophobic, which may indicate that this side of the molecule would be bound to the membrane. This will place the N-terminus and some part of the C-terminus of the Gs protein in the intracellular space. The resulting structure conforms with the experimental data of Neer et al.,[30] who showed that both the N-terminus and the C-terminus of the G proteins could be cleaved with proteolytic enzymes, showing that these parts of the G proteins are not buried inside the molecule and are accessible from the surface (Figure 2). It has been shown[31] that deletion of up to 59 residues from the N-terminus and up to 42 residues from the C-terminus of the rat Gs protein results in a G protein that still binds to the membrane. Juhnn et al.[31] have concluded that deletion of these regions is insufficient to cause cytozolic localization of the expressed protein. The modeled structure of the Gs protein supports this view (Color Plate 1).

It has been postulated that there are four conserved areas in G proteins that correspond to residues R 42–V 57 (G1), D 196–E 210 (G2), F 219–R 228 (G3) and I 288–D 295 (G4). It has been suggested that these regions might be involved in the GDP/GTP binding site.[3] In our model, only G1, G2 and G4 are located around the GDP binding site. The G3 region is further away and is a part of the loop that is located in the vicinity of the N-terminus of the enzyme. It is believed that the N-terminus may be involved with the coupling of G proteins with G protein-coupled receptors.[32] This region is connected via the helix H7 (D 194–L 198, Table 1 and Figure 2) to the binding site of GDP. However, some experimental data point to the validity of the modeled structure. It has been shown[33] that H21a mutant cells contain a mutant Gs protein in which G226 is replaced by alanine. This mutant protein, which does bind GTP analogs, is not capable of Gpp(NH)p or GTP + isoproterenol-induced stimulation of adenylyl cyclase activity. These results suggest that the H21a mutation is in a region of Gs that could interact either with the receptor or adenylyl cyclase—an idea that is supported by our present model. Similarly, another mutant of Gs, Q2271,[34,35] in the same region could not be ADP-ribosylated with the cholera toxin and its activity toward adenylyl cyclase did not increase in the presence of isoproterenol. Masters et al.[35] have interpreted this mutation as primarily affecting the switch mechanism that mediates the conformational transition between the GTP- and GDP-bound forms of these proteins. According to this view, GTP hydrolysis should be slowed because the Q → L substitution slows or prevents the transition of these proteins from the

Figure 2. Stereo view of the modeled structure of the human Gs protein (solid line) superimposed on the crystal structure of the bacterial EF-tu 200 (broken line). Only the N-terminus (1 MET) and C-terminus (394 LEU) of the human Gs protein are marked.

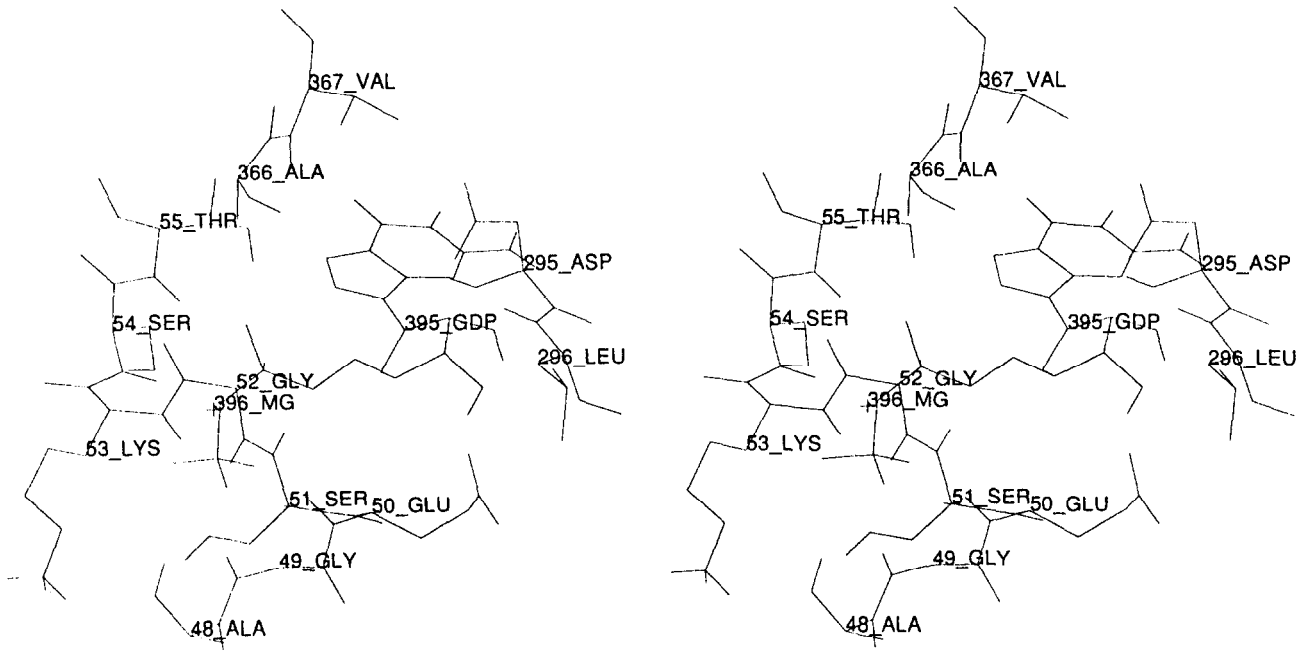

Figure 3. Stereo view of the GDP binding site of the human Gs protein. The residues within 5 Å of GDP are shown.

Table 1. Secondary structure of the modeled alpha subunit of the human Gs protein.

Residues	Secondary structure
Glu 12–Lys 32	alpha helix H1
His 41–Leu 44	beta sheet B1
Lys 53–Ile 62	alpha helix H2
Gln 78–Arg 81	alpha helix H3
Thr 105–Leu 113	alpha helix H4
Pro 122–Phe 126	alpha helix H5
Val 135–Asn 136	beta sheet B2
Ala 152–Glu 155	alpha helix H6
Lys 186–Tyr 190	beta sheet B3
Asp 194–Leu 198	alpha helix H7
Phe 212–Val 224	alpha helix H8
Thr 242–Phe 246	beta sheet B4
Asn 254–Ser 275	alpha helix H9
Val 287–Phe 290	beta sheet B5
Leu 302–Lys 305	alpha helix H10
Phe 312–Ala 316	alpha helix H11
Thr 319–Glu 374	alpha helix H12
Asp 368–Arg 374	alpha helix H13
Ile 383–Leu 393	alpha helix H14

GTP- to the GDP-bound conformation, but not because the mutation directly alters the rate of GTP hydrolysis. According to our modeled structure, the mutation in this region of Gs affects binding to the receptor and therefore prevents the cascade of conformational changes that will be necessary for activation and deactivation of the Gs protein. It is interesting to note that another mutant, G49V,[34,35] in which the mutation site is located in the vicinity of the GDP binding site (Figure 3), in spite of Q227L, does not respond to stimulation by AlF4 or GTP S, but will be ribosylated with the cholera toxin to the same extent as wild-typed Gs.

Human beta3 adrenergic receptor

The modeled structure of the human beta3 adrenergic receptor is shown in Color Plate 2 and its secondary structure is shown in Table 2. The resulting structure is stable and plots of phi and psi angles show that less than 1.5% of the residues are in the disallowed region. We have chosen the beta3 receptor because little modeling of this receptor has been carried out. Human beta3 adrenergic receptors, like other beta adrenergic receptors, activate adenylyl cyclase via the Gs protein.[23] Therefore, we should be able to study its complex with the Gs protein.

The final structure of the human beta3 adrenergic receptor shows an overall similarity to that of the modeled beta2 receptor.[11] However, our model shows the second and third intracellular loops to be more helical, and the first and third transmembrane helices are somewhat extended into the intracellular space. The helical structure of the second, fourth and seventh transmembrane helices is distorted to some extent. This is expected, because there are several glycine and proline residues in these helices. The hydrophobicity of the structure is in accord with its location in the membrane; hydrophobic side chains of residues of helices are pointed outward (Color Plate 2).

Complex of the Gs protein and the beta3 receptor

It has been shown that the C-terminus of the Gs protein, as well as its N-terminus, is involved in binding to the G protein-coupled receptors (GPCRs).[26,32] From the receptor point of view, it is believed that the third intracellular loop

Table 2. The secondary structure of the modeled human beta3 adrenergic receptor.

Residues	Secondary structure	Segment	Location
Met 1–Pro 59	coil	N-terminus	extracellular
Trp 35–Val 60	alpha helix	TMH-1	transmembrane
Ala 61–Leu 68	alpha helix	i–1	intracellular
Gln 69–Met 71	turn	i–1	intracellular
Thr 72–Val 76	coil	i–1	intracellular
Thr 77–Leu 79	coil	TMH-2	transmembrane
Ala 80–Leu 88	alpha helix	TMH-2	transmembrane
Leu 89–Val 91	coil	TMH-2	transmembrane
Pro 92–Leu 99	alpha helix	TMH-2	transmembrane
Thr 100–His 102	turn	o–1	extracellular
Trp 103–Gly 106	coil	o–1	extracellular
Ala 107–Thr 127	alpha helix	TMH-3	transmembrane
Leu 128–Tyr 136	alpha helix	i–2	intracellular
Leu 137–Leu 143	coil	i–2	intracellular
Arg 144–Gly 146	turn	i–2	intracellular
Ala 147–Arg 155	alpha helix	i–2	intracellular
Thr 156–Val 159	coil	i–2	intracellular
Leu 160–Val 164	alpha helix	TMH-4	transmembrane
Ser 165–Ser 175	turn	TMH-4	transmembrane
Gln 176–Gly 181	turn	o–2	extracellular
Ala 182–Gln 187	alpha helix	o–2	extracellular
Arg 188–Ser 191	coil	o–2	extracellular
Asn 192–Ala 199	alpha helix	o–2	extracellular
Ser 200–Tyr 204	coil	o–2	extracellular
Val 205–Ala 225	alpha helix	TMH-5	transmembrane
Arg 226–Ala 231	alpha helix	i–3	intracellular
Thr 232–Arg 233	turn	i–3	intracellular
Gln 234–Glu 249	alpha helix	i–3	intracellular
Ser 250–Ala 253	coil	i–3	intracellular
Pro 254–Pro 262	turn	i–3	intracellular
Val 263–Glu 270	coil	i–3	intracellular
Gly 271–Arg 277	alpha helix	i–3	intracellular
Arg 278–Leu 283	coil	i–3	intracellular
Pro 284–Cys 292	alpha helix	i–3	intracellular
Thr 293–Leu 314	alpha helix	TMH-6	transmembrane
Arg 315–Ser 321	coil	o–3	extracellular
Leu 322–Gly 335	alpha helix	TMH-7	transmembrane
Tyr 336–Ile 345	turn	TMH-7	transmembrane
Tyr 346–Leu 367	coil	C-terminus	intracellular
Pro 368–Ala 375	turn	C-terminus	intracellular
Arg 376–Leu 379	alpha helix	C-terminus	intracellular
Phe 380–Gly 402	coil	C-terminus	intracellular

and the receptor C-terminus are involved in the binding to G proteins.[27] Inspection of the modeled structures of the Gs protein and the beta3 receptor shows that there is only one way that these proteins could form a complex if these criteria have to be satisfied. The modeled complex, after several runs of dynamics calculation and energy minimization, is shown in Color Plate 3.

The calculations suggest that this complex is energy stable and the resulting structure is supported by experimental data. According to this model, the Gs protein is bound to the beta3 receptor in the interface of the membrane with the intracellular space, which is in agreement with experimental data.[2] In this orientation, the binding site of GDP will be placed in the membrane and its N-terminus in the cytoplasmic space, which is expected from the overall hydrophobicity pattern of the Gs protein. In addition, the result of the mutation studies concerning the effect of N-terminus and C-terminus deletion on the binding of the Gs protein to the membrane[31] lend credence to this view.

It has been shown that deletion of the residues 222–229 and 258–270 of the beta2 receptor greatly reduces the ability of this receptor to activate adenylyl cyclase via stimulation of the Gs protein.[36] Our model (Color Plate 3) shows that corresponding residues of the beta3 adrenergic receptor,[23] residues 227–234 and residues 276–289 are located in the vicinity of the C-terminus of the Gs protein. It is believed

the C-terminus of the G protein plays an important role in the formation of the complex.[26] In this region, the His-288 residue of the beta3 receptor is bound to the Gln-390 of the Gs protein. The Gln-390 residue is highly conserved among the G proteins (Figure 1). Another important segment of the Gs protein, its N-terminus, is in close contact with the previously mentioned residues of the beta3 receptor as well as the second intracellular loop. As mentioned before, the Gln-227 residue of the Gs protein is believed to be important in the activity of Gs proteins.[34,35] It has been found that the NH2 of this amino acid forms a hydrogen bond to Glu-248 of the beta3 receptor, which is located in its third intracellular loop.

It has been demonstrated that the mutation of residues Cys-327 and Cys-341 of the beta2 receptor to arginine and glycine respectively, greatly reduces the ability of this receptor to activate adenylyl cyclase in response to isoprenaline.[37] Our model shows that Cys-347 of the beta3 receptor, which corresponds to Cys-327 of beta2, is located in the binding site of the Gs protein and its carboxyl group forms a hydrogen bond to the side chain amine of Arg-289 of the beta3 receptor. Therefore, this mutation will produce a repulsion between two arginine residues that could result in the conformational change of this part of the molecule and an inhibition of binding. It is interesting to note that Arg-362 of the beta3 receptor (which corresponds to residue 342 of the beta2 receptor) forms an ionic bond to Asp-323 of the Gs protein. These data are in agreement with the mutation experiments of O'Dowd et al.[37] It has also been shown that the mutation of Asp-130 of the beta2 receptor is critical for proper G protein coupling.[38] The corresponding residue in the beta3 receptor (Asp-134) does not bind directly to the Gs protein. However, it forms an ionic bond to Arg-348 of the C-terminus of the beta3 receptor. It is possible that this binding is responsible for proper orientation of the C-terminus of the beta3 receptor and formation of the complex.

It will be of great interest to add to this complex the structures of other subunits of the Gs protein, namely the beta and gamma subunits, which are necessary to see the whole picture. However, the modeled structure of the complex of the alpha subunit of human Gs protein and the beta3 adrenergic receptor is in very good agreement with experimental data and can provide a basis for understanding the way in which these proteins interact.

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