Serine and Threonine Residues Bend α-Helices in the $\chi_1 = g^-$ Conformation

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ABSTRACT   The relationship between the Ser, Thr, and Cys side-chain conformation ($\chi_1 = g^-$, t, g^+) and the main-chain conformation (ϕ and ψ angles) has been studied in a selection of protein structures that contain α-helices. The statistical results show that the g^- conformation of both Ser and Thr residues decreases their ϕ angles and increases their ψ angles relative to Ala, used as a control. The additional hydrogen bond formed between the O_γ atom of Ser and Thr and the i-3 or i-4 peptide carbonyl oxygen induces or stabilizes a bending angle in the helix 3–4° larger than for Ala. This is of particular significance for membrane proteins. Incorporation of this small bending angle in the transmembrane α-helix at one side of the cell membrane results in a significant displacement of the residues located at the other side of the membrane. We hypothesize that local alterations of the rotamer configurations of these Ser and Thr residues may result in significant conformational changes across transmembrane helices, and thus participate in the molecular mechanisms underlying transmembrane signaling. This finding has provided the structural basis to understand the experimentally observed influence of Ser residues on the conformational equilibrium between inactive and active states of the receptor, in the neurotransmitter subfamily of G protein-coupled receptors.

INTRODUCTION

Wide ranges of biologically active substances, such as neurotransmitters, elicit their action through signal transduction pathways that involve membrane proteins like G protein coupled receptors (GPCRs). The membrane-bound domain of GPCRs adopts the conformation of a bundle of seven transmembrane helices (TMH) (Baldwin et al., 1997; Unger et al., 1997). Pharmacological and mutagenesis studies (van Rhee and Jacobson, 1996) have shown that neurotransmitters bind, at the extracellular side of the membrane, with their protonated amine to the conserved Asp[3.32] (nomenclature of Ballesteros and Weinstein, 1995), in TMH 3. Similarly identified (van Rhee and Jacobson, 1996) are a series of conserved Ser residues (5.43 and 5.46), in TMH 5, which act as hydrogen bonding sites for the hydroxyl groups present in the chemical structure of many neurotransmitters. The molecular function of constitutively active receptors (Lefkowitz et al., 1993; Samama et al., 1993) and transgenic mice with receptor overexpression (Bond et al., 1995) provides direct evidence that GPCRs exist in equilibrium between inactive and active states. Spectroscopic studies (Gether and Kobilka, 1998) have suggested the movement of TMH 3 and TMH 6 during the formation of the active form of the receptor. Moreover, it has recently been shown that the Ser residues in TMH 5 do not only provide a

docking site for the agonist, but also control the equilibrium of the receptor between both conformational states (Ambrosio et al., 2000). Deletion of these —OH groups from the β_2-adrenergic receptor (Ala replacement of Ser[5.43] and Ser[5.46]) decreases the constitutive activity of the receptor (Ambrosio et al., 2000). Therefore, the side chain of Ser has a significant effect on the conformation of the helix and on consequence of the receptor.

A pioneer survey of protein α-helices in "hydrophilic" and "hydrophobic" environments revealed that additional hydrogen bonds between the peptide carbonyl oxygen to a solvent molecule produce a significant change in the main-chain torsion ϕ and ψ angles and in the curvature of the helix (Blundell et al., 1983). It has also been shown that Ser, Thr, and Cys residues might form an intrahelical hydrogen bond between the O_γ (or S_γ) atom and the i-3 or i-4 carbonyl oxygen (Gray and Matthews, 1984). This hydrogen bond interaction between side-chain and main-chain atoms is feasible in the $\chi_1 = gauche^-$ (g^-) or $\chi_1 = gauche^+$ (g^+) conformation (McGregor et al., 1987). It does not occur in the $\chi_1 = trans$ (t) conformation. We aim to explore the possibility that this intrahelical hydrogen bond of the polar side chain of Ser, Thr, and Cys could change the conformation of the α-helix. This would provide the structural basis to understand the experimentally observed influence of Ser on the conformational equilibrium between inactive and active states of the receptor (Ambrosio et al., 2000). We have analyzed the relationship between the Ser, Thr, and Cys side chain conformation (the torsion χ_1 angle) and the main chain conformation (the torsion ϕ and ψ angles and bend angle) in two independent samples of protein structures. First, in the four available helix bundle membrane protein structures: bacteriorhodopsin (Grigorieff

et al., 1996), cytochrome c oxidase (Tsukihara et al., 1996), the photosynthetic reaction center (Stowell et al., 1997), and the potassium channel (Doyle et al., 1998). Second, in a selection of soluble proteins that contains α-helices.

METHODS

Membrane protein structures

The atomic coordinates of *Halobacterium halobium* bacteriorhodopsin (PDB access number 2brd, 3.5 Å resolution), bovine cytochrome c oxidase (1occ, 2.8 Å), *Rhodobacter sphaeroides* photosynthetic reaction center (1aij, 2.2 Å), and *Streptomyces lividans* potassium channel (1bl8, 3.2 Å) were obtained from the Brookhaven Protein DataBank (Bernstein et al., 1977). The coordinates of the residues corresponding to transmembrane helices 1–7 of 2brd; 2–3, 7, 9, 12, 14–15, 19–20, 23, 28–30, 32–35, 41, 54, 59–60, and 63–66 of 1occ; 2, 5–11, 13, 17, 22–23, 28, 31–32, and 34 of 1aij; and 1 and 3 of 1bl8, in the HELIX annotation of the PDB files, were extracted for analysis. This results in a total of 45 TMHs. These TMHs were split into amino acid stretches of 12 residues long with either Ala (standard α-helix used as control), Cys, Ser, or Thr at the 8th position. Stretches with Pro residues in the sequence were removed from the database. The side chain conformation of Ser, Thr, and Cys was categorized into g^- ($0° < \chi_1 < 120°$), t ($120° < \chi_1 < 240°$), or g^+ ($240° < \chi_1 < 360°$) depending on the value of the torsional χ_1 angle. The following distribution of residues and conformations were observed: Ala (48), Cys (4; g^+: 4, t: 0, g^-: 0), Ser (34; g^+: 16, t: 5, g^-: 13), and Thr (41; g^+: 32, t: 0, g^-: 9).

Soluble protein structures

Iditis 3.1 (Oxford Molecular) was used for the selection of protein structures in the Brookhaven Protein DataBank (Bernstein et al., 1977). The chosen α-helices possess a resolution of 2.0 Å or better; a 12-residue length with Ala, Cys, Ser, or Thr at the 8th position; and no Pro residue in the sequence. If two α-helical segments have more than 80% sequence identity (if 10 or more than 10 residues of 12 are identical) only the structure with best resolution was considered. This systematic search provided the fol-

lowing distribution of residues and conformations: Ala (730), Cys (66; g^+: 46, t: 20, g^-: 0), Ser (245; g^+: 129, t: 74, g^-: 42), and Thr (247; g^+: 211, t: 2, g^-: 34).

Statistical analysis

The torsion angles of the backbone of the residues at positions 8, populated by Ala, Cys, Ser, or Thr (ϕ_i and ψ_i); 7 (ϕ_{i-1} and ψ_{i-1}); 6 (ϕ_{i-2} and ψ_{i-2}); 5 (ϕ_{i-3} and ψ_{i-3}); and 4 (ϕ_{i-4} and ψ_{i-4}) were calculated for statistical analysis with SAS 6.11 (SAS Institute, Cary, NC). One-way analysis of variance plus a posteriori two-sided Dunnett's T tests was employed for contrasting the calculated torsion angles in Ser, Thr, and Cys residues in the g^-, t, and g^+ rotamer conformations with the control Ala in both membrane and soluble proteins. No statistical difference was observed in the torsion ϕ_{i-1}, ϕ_{i-2}, ϕ_{i-3}, ϕ_{i-4}, ψ_{i-1}, ψ_{i-2}, ψ_{i-3}, and ψ_{i-4} angles in both membrane and soluble proteins. The only exceptions (2 of 112 comparisons) were found in ϕ_{i-3} in Cys/g^+ and ψ_{i-4} in Thr/g^+ for soluble proteins (results not shown). These two exceptions were not further considered because of the lack of consistency among residues, conformational classes, or protein type.

Bend angle of the amino acid stretches of 12 residues long was calculated from the two axes that minimize the distance to the main chain atoms of residues 1–4 and 9–12 (Chou et al., 1984). One-way analysis of variance plus a posteriori one-sided Dunnett's T tests was employed to contrast if the bend angle of Ser, Thr, and Cys residues in the g^-, t, and g^+ rotamer conformations is greater than the control Ala in the sample of soluble proteins.

The χ^2 distribution was employed to compare the frequencies of residues and conformations in membrane and soluble proteins.

RESULTS

Table 1 summarizes the means and standard deviations for the backbone ϕ_i and ψ_i dihedral angles of α-helices containing Ala (standard α-helix used as control) and Ser, Thr, and Cys residues in the three possible rotamer conformations: g^-, t, and g^+. The histograms in Fig. 1 depict the mean values and the lines extending from the bar represent

TABLE 1 Backbone ϕ_i and ψ_i dihedral angles of α-helices

| | | Membrane proteins | | | | | | | Soluble proteins | | | | | | |
| | | ϕ_i | | | ψ_i | | | | ϕ_i | | | ψ_i | | |
	n	$\bar{x}$	s	Δ	$\bar{x}$	s	Δ	n	$\bar{x}$	s	Δ	$\bar{x}$	s	Δ
Ala	48	−60.9	6.5		−44.4	8.6		730	−63.1	5.6		−41.2	5.6	
g^-	22	−65.2	8,6	−4.3*	−34.3	8,5	10.1***	76	−67.9	9.2	−4.8***	−34.1	9.4	7.0***
Thr	9	−66.3	7.8	−5.4	−35.7	8.9	8.7***	34	−69.6	9.2	−6.5***	−35.0	10.5	6.2***
Ser	13	−64.5	9.3	−3.6	−33.3	8.4	11.1***	42	−66.5	9.0	−3.4**	−33.5	8.4	7.7***
Cys	0	—	—	—	—	—	—	0	—	—	—	—	—	—
g^+	52	−61.8	6.6	−0.9	−45.3	9.3	−0.9	386	−63.4	5.6	−0.3	−43.2	6.3	−2.0***
Thr	32	−61.8	7.6	−0.9	−46.3	9.3	−1.9	211	−63.4	5.3	−0.3	−44.2	5.2	−3.0***
Ser	16	−62.5	4.9	−1.6	−43.9	9.9	0.5	129	−63.3	6.1	−0.2	−42.2	7.6	−1.0
Cys	4	−58.9	1.5	2.0	−42.9	6.4	1.5	46	−63.7	5.7	−0.6	−41.6	6.8	−0.4
t	5	−56.9	9.6	4.0	−44.4	8.0	0.0	96	−62.6	5.7	0.4	−44.4	5.3	−3.2***
Thr	0	—	—	—	—	—	—	2	−68.8	8.8	−5.7	−40.1	6.1	1.1
Ser	5	−56.9	9.6	4.0	−44.4	8.0	0.0	74	−62.7	5.3	0.4	−44.0	4.9	−2.8**
Cys	0	—	—	—	—	—	—	20	−62.0	6.7	1.1	−46.1	6.2	−5.0**

Means ($\bar{x}$), standard deviations (s), and the difference in degrees (Δ) relative to Ala (in bold) of the backbone ϕ_i and ψ_i dihedral angles of α-helices containing Ala and Ser, Thr, and Cys residues in the *gauche⁻* (g^-), *trans* (t), and *gauche⁺* (g^+) rotamer conformations. The results are presented for membrane and soluble proteins.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

FIGURE 1 Analysis of the torsion ϕ (*a* and *b*) and ψ (*c* and *d*) angles of α-helices, in membrane (*a* and *c*) and soluble (*b* and *d*) proteins, containing Ala (control in black) and Ser, Thr, and Cys residues in the *gauche⁻*, *trans*, and *gauche⁺* rotamer conformations. Histograms depict the mean values and the lines extending from the bar represent the standard deviation.

the standard deviation of ϕ_i (*a* and *b*) and ψ_i (*c* and *d*) dihedral angles. The results are presented for membrane (*a* and *c*) and soluble (*b* and *d*) proteins. The difference in degrees (Δ) relative to the control Ala (*black solid bar* in Fig. 1) is also shown in Table 1.

The g^- conformation

The g^- conformation significantly decreases ϕ_i (Δ of $-4.3°$) and increases ψ_i (Δ of $10.1°$), relative to Ala, in membrane proteins (Table 1). Moreover, the effect caused by both Ser and Thr is similar in magnitude. Ser/g^- decreases ϕ_i $-3.6°$ and increases ψ_i $11.1°$, whereas Thr/g^- decreases ϕ_i $-5.4°$ and increases ψ_i $8.7°$ (Table 1 and Fig. 1, *a* and *c*). However, these differences relative to Ala, calculated independently for Ser/g^- and Thr/g^-, are significant from a statistical point of view only in ψ_i. The lack of statistical significance of ϕ_i is attributed to the smaller number of points in the split Ser/g^- (13 structures) and Thr/g^- (9 structures) categories than in the total g^- (22 structures) category (Table 1). Thus, in order to reinforce this finding of the influence of the g^- conformation in both ϕ_i and ψ_i angles, we have undertaken a similar analysis in soluble proteins for which larger number of high-resolution structures are available (see Methods). The g^- conformation of Ser and Thr residues in soluble proteins has a

statistically significant effect in both ϕ_i and ψ_i (Table 1). Notably, the magnitude and direction of the effect is the same as observed in membrane proteins. The g^- conformation of Ser and Thr decreases ϕ_i (Δ of $-3.4°$ and $-6.5°$, respectively) and increases ψ_i ($7.7°$ and $6.2°$) relative to Ala (Table 1 and Fig. 1, *b* and *d*). Similar behavior in ϕ_i and ψ_i cannot be observed in the Cys residue since no experimental data is available in either membrane or soluble proteins. The g^- conformation of Cys is totally forbidden because of the steric clash between the S_γ atom and the carbonyl oxygen of residue i-3 (McGregor et al., 1987).

The conformation of the α-helix, driven by the g^- conformation of Ser or Thr is illustrated in Fig. 2. Fig. 2 *a* shows the conformation of a polyAla α-helix (red) and a polyAla α-helix with a single Ser or Thr (blue) residue in between. The location of either Ser or Thr in the α-helix is shown throughout the C_α—C_β bond. The helices were constructed with the average ϕ_i and ψ_i angles reported in Table 1 for Ala ($-60.9°$ and $-44.4°$) and the g^- conformation ($-65.2°$ and $-34.3°$) in membrane proteins. Clearly, the g^- conformation induces a bending angle in the helix (see below). Incorporation of this bending angle at one side of the cell membrane results in a significant displacement of the residues located at the other side of the membrane. The magnitude of the relocation might be estimated from the models depicted in Fig. 2 *a*. Thus, the distance between the
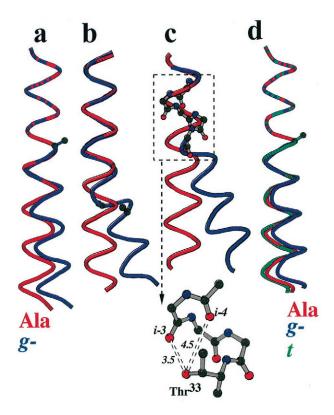
FIGURE 2 Comparison of helix bending between a polyAla α-helix (red) and (*a*) a polyAla α-helix with a single Ser or Thr residue (the C_α—C_β bond is shown) in the *gauche*[−] (g^-) conformation (blue); (*b*) helix 32, which contains Thr[277] and Thr[279] (the C_α—C_β bonds are shown) in g^-, from the photosynthetic reaction center; (*c*) helix 1, which contains Thr[33] also in g^-, from the potassium channel; and (*d*) a polyAla α-helix with a Ser residue (the C_α—C_β bond is shown) in *trans* (*t*) conformation (green) or g^- (blue) conformation. (*c*) A detailed view of the amino acids from i (the residue in g^-) to i-4 are shown as ball and stick. Figures were created using MOLSCRIPT (Kraulis, 1991).

α-carbon positions, in the straight helix (red) and the bent helix (blue), is 3.3 Å for an amino acid located 15 residues away from Ser or Thr.

Fig. 2, *b* and *c* show the crystal structure of helix 32, which contains Thr[277] and Thr[279] in g^-, from the photosynthetic reaction center and helix 1, which contains Thr[33] also in g^-, from the potassium channel, respectively. To emphasize the structural consequences of the g^- conformation, the transmembrane α-helices were superimposed to an ideal α-helix (red). The backbone atoms of the amino acids from i (the residue in g^-) to i-4 are shown as ball and stick, whereas tube ribbons represent the rest of the backbone atoms (Fig. 2 *c*). Remarkably, the presence of these polar residues in the g^- conformation modifies the direction of the α-helix. The additional intrahelical hydrogen bond formed between the side chain OH_γ of Ser or Thr and the i-3 or i-4 peptide carbonyl oxygen of the preceding turn seems to produce this effect (Blundell et al., 1983). Fig. 2 *c* also shows a detailed view of this hydrogen bond network in the

potassium channel. The average hydrogen bond $O_\gamma \cdots O_{i-3}$ and $O_\gamma \cdots O_{i-4}$ distances (broken lines in Fig. 2 *c*) are 3.4 and 3.5 Å in membrane proteins and 3.1 and 3.5 Å in soluble proteins, respectively. Thus, the O_γ atom is located between O_{i-3} and O_{i-4}, closer in average to O_{i-3}. However, the small difference between the $O_\gamma \cdots O_{i-3}$ and $O_\gamma \cdots O_{i-4}$ distances and the absence of the H_γ atom in the crystal structures does not allow identifying to which carbonyl oxygen the OH_γ side chain preferentially hydrogen bonds.

The g^+ conformation

The g^+ conformation is the most abundant rotamer conformation in both membrane and soluble proteins (Table 1). Thus, the statistical contrasts between Ala and g^+ possess higher statistical power than between Ala and g^- or *t*. Despite this fact, the g^+ conformation produces a statistically significant change only in ψ_i of Thr in soluble proteins (Δ of $-3.0°$, Table 1 and Fig. 2). The lack of consistency of this variation among protein type and the other residues (Ser and Cys) does not led us to conclude that an α-helix with Ser, Thr, or Cys in the g^+ conformation leads to a different conformation than an α-helix with Ala.

The *t* conformation

The hydrogen bonding capacity of either Ser, Thr, or Cys must be satisfied, in a hydrophobic environment like the cell membrane, by the hydrogen bond interaction, in either the g^+ or g^- conformation, with the carbonyl oxygen in the preceding turn of the helix (Gray and Matthews, 1984). Thus, only 5 residues in the *t* rotamer conformation are found in membrane proteins. This lack of structures prevents the statistical analysis on membrane proteins. The *t* conformation produces in soluble proteins a statistically significant change in ψ_i, without modifying ϕ_i (Table 1 and Fig. 2, *b* and *d*). Thus, both Cys and Ser residues in the *t* conformation decrease, relative to Ala, ψ_i by $-5.0°$ and $-2.8°$. No statistical differences are obtained for Thr because only 2 cases are found in the analysis. The steric clash between the methyl group and the carbonyl oxygen of residue i-3 (Gray and Matthews, 1984) explains the lack of Thr residues in this conformation. The conformation of the α-helix caused by Ser in *t* conformation (green, ϕ_i of $-62.7°$ and ψ_i of $-44.0°$), compared with the g^- conformation (blue, $-66.5°$ and $-33.5°$) and the ideal polyAla (red, $-63.1°$ and $-41.2°$) are illustrated in Fig. 2 *d*. The fact that ϕ_i does not change and the smaller change in ψ_i produced by the *t* conformation, relative to the g^- conformation, is reflected in the reported structures. The α-helix with Ser in *t* (green) is comparable to Ala α-helix (red). However, it is important to note that the obtained changes in ψ_i, in g^- and *t* conformations, occur in opposite directions (increases in g^- and decreases in *t*, relative to Ala) which

results in a bend of the helices pointing toward different positions in space (Fig. 2 *d*).

Bend angle

Bend angles of the helices are calculated from the two axes that minimize the distance to the main chain atoms of the residues at the beginning and the end of the helix (Chou et al., 1984). Thus, only 4 residues (12 atoms) at the beginning and the end of the helix are employed in the calculation of the axes. Therefore, a small variation in the undersized number of main chain atoms results in an intermediate variation in the helical axis and a large variation in the calculated bend angle. This effect is very noticeable in membrane proteins because of the low resolution structural information available and the limited number of them. Therefore the analysis of bend angle is presented only for soluble proteins. Fig. 3 and Table 2 shows the means and standard deviations for the bending angle calculated from high resolution crystallographic structures. Notably, the g^- conformation significantly increases the bend angle (Δ of 3.8°), relative to Ala. No statistical differences are observed for the g^+ (Δ of 0.5°) or t (Δ −0.4°) conformations. The observed statistical significance for the g^- conformation is not preserved when the analysis is independently done for Ser/g^- and Thr/g^- despite the magnitude of the differences continues similar to the g^- category: Ser/g^- increases the bend angle 4.3° and Thr/g^- 3.2° relative to Ala. The smaller number of points in the Ser/g^- and Thr/g^- categories seems responsible for this lack of significance.

DISCUSSION

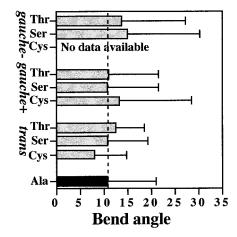The ability of all naturally occurring amino acids to form a turn when placed in the middle of a transmembrane helix has recently been measured (Monne et al., 1999). The observed rank order for turn-stabilizing tendencies are Asn = Arg = Pro (1.7) > Asp = Glu = His = Lys = Gln = (1.6) > Gly (1.3) > Ser = Trp (0.7) > Cys = Ile = Tyr (0.6) > Ala = Met = Val (0.5) > Leu = Phe = Thr (0.4). Clearly, there are two sets of residues with either high ($\geq$1.3) or low ($\leq$0.7) turn propensity. Charged or polar residues induce a turn ($\geq$1.3), whereas hydrophobic residues plus Ser, Thr, and Cys remain α-helical ($\leq$0.7). Moreover, statistical analysis of transmembrane sequences has shown that the most frequent amino acids are Leu > Ile > Val > Ala > Phe > Gly > Ser > Thr (Senes et al., 2000). These amino acids comprise more than two-thirds of the total. Thus, Ser and Thr are regularly found in transmembrane segments. Consistent with these findings, the ratio of Ala:Ser:Thr:Cys residues found in the present survey of protein α-helices is 12:8.5:10.2:1 in membrane proteins and 11.1:3.7:3.7:1 in soluble proteins. Ser and Thr residues occur almost as often as Ala in membrane proteins and three times less in soluble proteins. In addition, the ratio of g^+:g^- for Ser and Thr residues are 1.2:1 and 3.5:1 in membrane proteins and 3.1:1 and 6.2:1 in soluble proteins, respectively. There is a noticeable increase of the population of g^- conformation if the α-helix is embedded in a hydrophobic environment like the cell membrane. Notably, Ser possesses as many side chains in g^- as in g^+ in membrane proteins. These findings suggest a structural role of Ser and Thr residues in transmembrane segments. We have shown that the presence of Ser and Thr residues adopting the g^- conformation correlates with a significant bending of the α-helix at this locus. Therefore, we hypothesize that local alterations of the rotamer configurations of these Ser and Thr residues may result in significant conformational changes across transmembrane α-helices, and thus participate in the molecular mechanisms underlying transmembrane signaling.

FIGURE 3 Analysis of the bend angle of α-helices containing Ala (control in black) and Ser, Thr, and Cys residues in the *gauche*$^-$, *trans*, and *gauche*$^+$ rotamer conformations in soluble proteins. Histograms depict the mean values and the lines extending from the bar represent the standard deviation.

TABLE 2 Bend angle of α-helices

	n	$\bar{x}$	s	Δ
Ala	**730**	**10.7**	**10.2**	
g^-	76	14.5	14.4	3.8*
Thr	34	13.9	13.4	3.2
Ser	42	15.0	15.3	4.3
Cys	0	—	—	—
g^+	386	11.2	11.3	0.5
Thr	211	10.9	10.7	0.2
Ser	129	10.8	10.7	0.1
Cys	46	13.2	15.4	2.5
t	96	10.3	8.2	−0.4
Thr	2	12.5	6.1	1.8
Ser	74	10.8	8.5	0.1
Cys	20	8.1	6.7	−2.6

Means ($\bar{x}$), standard deviations (s), and the difference in degrees (Δ) relative to Ala (in bold) of the bend angle of α-helices in soluble proteins containing Ala and Ser, Thr, and Cys residues in the *gauche*$^-$ (g^-), *trans* (t), and *gauche*$^+$ (g^+) conformations.
*$p < 0.05$.

It should be noted that the statistical correlation found between Ser and Thr adopting the g^- conformation and helix bending does not clarify whether the Ser/Thr side chain induces or stabilizes the observed helix bending. However, we would favor the causal relationship between side chain to main chain H-bonding and helix bending, following the argument put forward by Blundell et al. (1983). The authors compared the 180° angle of a linear NH⋯O = C α-helical backbone H bond that occurs in a straight helix, with the 120° of the same angle in a bifurcated (NH, HOH)⋯O = C H bonding when a water molecule also H bonds the backbone carbonyl. This difference in the H bonding angle would explain the characteristic bending observed in high resolution α-helical structures, where the water-exposed face is bent (120°) relative to the more straight (180°) buried face of the helix (Blundell et al., 1983). For the case of the Ser and Thr side chains, the side chain hydroxyl moiety may play a similar role as the water hydroxyl, inducing a similar bifurcated (NH, OH)⋯O = C H bond with an angle of 120° that would, by itself, induce a local bend in the α-helix.

We suggest that Ser 5.43 and 5.46 in the β_2-adrenergic receptor, which provide the docking site for the agonist (see above), adopt the g^- conformation, in the absence of the extracellular ligand. Possibly, Ala replacement of Ser 5.43 and Ser 5.46 by site-directed mutagenesis changes the conformation of helix 5, from the bent helix (Ser/g^- in blue, see Fig. 2 d) to the straight helix (Ala in red). This would explain the influence of these Ser residues in helix 5 on the conformational equilibrium between inactive and active states of the receptor (Ambrosio et al., 2000). Moreover, substitution of two Ser residues, located three residues apart and thus in the same face of the helix, augments the magnitude of the relocation of helix 5 by Ala substitution.

Finally, we would like to remark the structural consequences derived from the hydrogen bond formation between the neurotransmitters and the Ser residues in helix 5. Ser must adopt the t conformation, if it acts as hydrogen bond donor, in the process of hydrogen bonding to the hydroxyl moieties of the ligand. Thus, ligand binding might require the conformational transition of Ser from the g^- (see α-helix in blue in Fig. 2 d) to the t (green) conformation. This process of rotation around χ_1, from g^- to t, induces a change in the direction of the helix toward different positions in space (see above and Fig. 2 d).

It is important to note that Ala replacement of Ser 5.43 and 5.46 (conformational transition from Ser/g^- in blue to Ala in red, see Fig. 2 d) decreases the levels of intracellular cAMP (Ambrosio et al., 2000). In contrast, ligand binding to Ser 5.43 and 5.46 (conformational transition from Ser/g^- in blue to Ser/t in green, see Fig. 2 d) increases the levels of intracellular cAMP (Ambrosio et al., 2000). This opposite effect cannot merely be understood from these reported conformational changes of helix 5. Thus, ligand binding might trigger more complex processes that finally lead to the active form of the receptor. It has been suggested that agonists of the β_2-adrenergic receptor also induce conformational changes in transmembrane domains 3 and 6 (Gether et al., 1997b). Moreover, the ligand might produce unfavorable changes (Gether et al., 1997a) in the receptor binding site that triggers the significant change in the conformational properties of the receptors that are transmitted to the intracellular site (Pardo et al., 1997).

This statistical analysis on the influence of Ser and Thr residues to the curvature of α-helices has provided the structural basis to understand the mechanism by which the Ser residues in helix 5 in the neurotransmitter family of GPCR control the equilibrium between inactive and active states of the receptor. Because our findings are based on general principles of protein structure, it is conceivable that Ser and Thr residues on α-helices of other integral membrane proteins, such as gap junctions (Ri et al., 1999), may also participate in the conformational changes underlying transmembrane signaling.

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