Conformational Changes at The Carboxyl Terminus of Ga Occur during G Protein Activation‡

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To understand the dynamics of conformational changes during G protein activation, surface exposed cysteine residues on Ga were fluorescently labeled. Limited tryptophanolysis and mutational analysis of recombinant Ga1/Ga41 determined that two cysteines are the major fluorescent labeling sites, Cys210, located in the switch II region, and Cys347, at the C terminus. Mutants with serines replacing Cys210 (Chi6a) and Cys347 (Chi6b) were single fluorescently labeled with lucifer yellow (LY), while a double mutant (Chi6ab) was no longer labeled. When Chi6b was labeled with LY on Cys210, AlF4− caused a 220% increase in LY fluorescence, indicating that the fluorescent group at Cys210 is a reporter of conformational change in the switch II region. Chi6a labeled at Cys347 also showed an AlF4−-dependent increase in LY fluorescence (91%), indicating that Ga activation leads to a conformational change at the COOH terminus. Preactivation of the protein with AlF4− before labeling led to a decreased incorporation of LY into Cys347 suggesting that Ga activation buries Cys347. This COOH-terminal conformational change may provide the structural basis for communication between the GDP-binding site on Ga and activated receptors, and may contribute to dissociation of activated Ga subunit from activated receptor.

Heterotrimeric G proteins are activated by seven-transmembrane-spanning receptors and relay signals to downstream effectors, including cellular enzymes and ion channels. Upon agonist binding, receptors become activated and in turn interact with G proteins and catalyze GDP release from G protein α subunits. After the release of GDP, the Ga subunit, together with Gβγ subunits, remains in a tight complex with the receptor, which dissociates when GTP binds to the empty Ga subunit. Both the GDP-bound Ga subunit and the Gβγ subunit complex are then capable of regulating a variety of effectors on the intracellular face of the plasma membrane. The binding of Ga and Gβγ subunits is restored when the intrinsic GTPase activity in the Ga subunit hydrolyzes the bound GTP to GDP.

In order to have a reliable method for studying the conformational changes in G proteins, we developed a fluorescent monitor because fluorescence is easily detectable and responsive to local environmental change. Cysteines are highly reactive and can be labeled with a variety of Cys-directed fluorescent groups. We determined the cysteines that are accessible to sulfhydryl-specific fluorescence labeling on Ga subunits and characterized the fluorescence changes on Ga when different sites are labeled. We replaced those accessible cysteines with serine to make a functionally cysteineless mutant in which to place additional cysteines at sites where we would like to monitor conformational changes or interaction with other proteins.

To study structural changes in Ga, upon its activation, we used the functional derivative of Ga1α, Ga1α/Ga41 chimera (Chi6) in which residues 216–294 of Ga1α were replaced with the corresponding residues 220–298 from Ga41. Chi6 can be conveniently expressed in Escherichia coli, and it was shown to have a similar rate of rhodopsin-catalyzed GDP/GTP exchange as Ga1α does, implying that its receptor and Gβγ binding properties are Ga1α-like (1). The crystal structure of Chi6 in complex with Gβγ1γ1 has been solved and revealed an identical geometry with wild type, native Ga1α (2). High expression levels of this protein in E. coli provided us milligram amounts of pure protein for biochemical and fluorescent studies as well as ease in constructing Ga1α mutants.

Lucifer yellow, an environmentally sensitive fluorescent probe, was selected because it is a good reporter of local changes. We previously used this probe as a reporter of the binding of the inhibitory subunit of cGMP phosphodiesterase to Ga1α (3).

Ho and Fung (4) previously reported that by using 5,5'-dithio-bis(2-nitrobenzoic acid) titration and N-ethylmaleimide modification, a total of five reactive sulfhydrys in native Ga1α and nine reactive sulfhydrys in the SDS-denatured Ga1α protein were found. Eight cysteines were found by DNA sequencing of Ga1α (5). In 1988, by using 32P-labeled GDP-Ga1α and the [32P]-ATCTP, a crosslinking reagent, Dhanasekar et al. (6) showed that Cys210 and Cys347 were the major reactive cysteines. Here, we show that the major labeling sites for LY are located on Cys210 and Cys347 in Ga1α/Ga41 chimeras. Also, we show that single labeling at either Cys210 or Cys347 can be used to report the local conformational changes around the labeled sites. As expected, Cys210 in the switch II region reports an AlF4−-dependent activating conformational change. Unexpectedly, there is also an AlF4−-dependent conformational change at Cys347, which may be...
Targeted Fluorescent Probe Reports Conformational Changes in Ga

EXPERIMENTAL PROCEDURES

Materials—GTP, GTP-S, GDP, deoxyribonucleotides, and imidazole were purchased from Boehringer-Mannheim. All restriction and DNA modification enzymes were obtained from Boehringer-Mannheim and Pharmacia Biotech Inc. Lucifer yellow vinyl sulfone was purchased from Sigma. All other reagents were from sources described previously (1).

Construction of Mutants and Chimera Expression and Purification—The construction of Chi6 was described previously (1). The construction of all mutants were based on Chi6 (1), composed of Ga, cDNA except amino acids 216 to 294, which were from Ga, cDNA. Mutants were all constructed by using the QuikChange™ site-directed mutagenesis kit from Stratagene Ltd. Strategy of oligo design for polymerase chain reaction-based mutagenesis is suggested by the QuikChange™ kit instruction manual. The NH₂-terminal sequence of Chi6 is derived from Ga expression construct (1) and preceded by MA(His)₆A. All chimera proteins were expressed and purified by nickel affinity chromatography followed by anion exchange chromatography using Protein Pak Q15 HR resin (Millipore) as described previously (1). The concentrations of chimera in the elutes were determined spectrophotometrically and resolved by HPLC using protein Pak Q15 HR column (capacity of 2 ml).

To determine the solvent-accessible surface areas the method of Eisenberg et al. (10) was used. The solvent-accessible surface areas of all eight cysteines in Chi6 were summarized in Table I. Among these cysteines, Cys210 has greater surface exposure area in its GDP binding form than Cys62, Cys321, and Cys282. Three other cysteine residues (135, 216, and 250) are completely buried inside the protein according to this calculation. Cys210 becomes less accessible in the Ga-GTP,S binding form. Since the COOH terminus of Ga is disordered and not visible in the crystal structure, it is likely that Cys347 is highly accessible.

RESULTS

Determination of Solvent-accessible Surface Areas of Cysteines in Ga.—There are eight cysteines in Ga, and according to the crystal structure (11), only four of them are surface exposed: namely Cys62, Cys210, Cys321, and Cys347 (Fig. 1). To obtain a quantitative criterion of surface exposure, we calculated the solvent-accessible surface area by the method of Eisenberg and McLachlan (10) using the coordinates of Ga-GDP (11) and Ga-GTP,S (12). The solvent-accessible surface areas of all eight cysteines in Chi6 are summarized in Table I. Among these cysteines, Cys210 has greater solvent exposed area in its GDP binding form than Cys62, Cys321, and Cys282. Three other cysteine residues (135, 216, and 250) are completely buried inside the protein according to this calculation. Cys210 becomes less accessible in the Ga-GTP,S binding form. Since the COOH terminus of Ga is disordered and not visible in the crystal structure, it is likely that Cys347 is highly accessible.

Determination of LY Labeling Sites on Chi6.—To determine accessible cysteines in Chi6, we first labeled Chi6 with LY under mild conditions and identified the labeling sites. After the excess LY was removed, the labeled samples were purified by anion-exchange HPLC. The presence of LY adds negative charges to the protein and, thus, allows separation of variously labeled species by anion exchange chromatography (Fig. 2). Chi6 showed three major labeled peaks as well as one unlabeled peak which co-migrates with the original protein. Each peak in the HPLC elution was collected separately and the stoichiometry of labeling was determined. Proteins from labeled peaks 1 and 2 each contains a single fluorescent group per molecule, while protein in labeled peak 3 contains two fluorescent groups (Table II).

To identify the labeling site(s) for each peak, limited tryptic digestion and mutagenesis studies were performed. The potential tryptic cleavage sites in Chi6 protein are lysine 18, arginine 204, and arginine 310 (8, 9) as illustrated at the bottom of Fig. 3. Protein from labeled peak 3 of Chi6 was treated with trypsin for 0, 5, 15, and 30 min before the reactions were stopped by TLCK. The LY-labeled fragments on the gel were observed with UV illumination. As shown in Fig. 3, the double labeled peak 3 showed fluorescent fragments of 38, 19, 15, and 5 kDa, which suggested the labeled cysteines were within the region, composed of amino acids 205–350, which contain Cys210, Cys321, and Cys347. To identify the labeled site(s), a mutant (Chi6a) was constructed in which Cys210 was changed to serine. As shown in Table II, after being labeled with LY, Chi6a-LY (Cys347-LY) migrated on Mono-Q column as a single labeled
peak with a retention time of 13 min similar to labeled peak 2 in Chi6-LY (Table II). After this peak was treated with trypsin, the size of the LY-labeled fragments were 38, 19, and 5 kDa, which suggested the labeled site was either Cys347 or Cys321 (Fig. 3).

To further clarify the possible labeling sites suggested in tryptic digestion experiments, two more mutants were constructed: Chi6b, with Cys347 mutated to serine, and Chi6ab, which has both Cys210 and Cys347 mutated to serine. As shown in Table II, after labeling with LY, Chi6b showed a single labeled peak with a retention time of 12 min, which corresponded to peak 1 for Chi6, whereas in the double mutant (Chi6ab), all major labeled peaks disappeared. We therefore conclude that the protein eluted in labeled peak 1 was labeled at Cys210 while protein eluted in peak 2 was labeled at Cys347. Thus, cysteine 210 and cysteine 347 are the major accessible sites for LY labeling. A comparison of the areas of the peaks shows that the efficiency of labeling at Cys347 was 6.5 times higher than that of Cys210. However, for mutants with one cysteine available for labeling (Chi6a and Chi6b), the efficiency of LY labeling at Cys210 and Cys347 was comparable, which suggested that the labeling of Cys347 in Chi6 prevented the labeling of Cys210.

**Functional Assays for LY-labeled and Unlabeled Chimera Proteins**—To determine whether the mutant proteins folded properly, had GDP bound, and could undergo GTP-dependent conformational changes, we measured the intrinsic fluorescence change of Trp207 after addition of AlF$_4^-$ (Table II). All the mutant proteins were able to undergo conformational change upon binding to AlF$_4^-$ (Table II). After LY labeling, the AlF$_4^-$-dependent intrinsic fluorescence change of Cys347-LY in Chi6a protein was compared to that of the unlabeled protein. The results showed that the efficiency of labeling at Cys347 was 6.5 times higher than that of Cys210. However, for mutants with one cysteine available for labeling (Chi6a and Chi6b), the efficiency of LY labeling at Cys210 and Cys347 was comparable, which suggested that the labeling of Cys347 in Chi6 prevented the labeling of Cys210.

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was 50% but Cys\textsuperscript{210}-LY in Chi\textsubscript{6b} protein showed significantly less (1.2%) fluorescence change (Fig. 4, Table II).

LY is a Fluorescent Reporter for Conformational Change in Switch II—There was only a 1.2% AlF\textsubscript{4}\textsuperscript{-}-dependent intrinsic Trp fluorescence change in Cys\textsuperscript{210}-LY (Table II). To determine whether there was an AlF\textsubscript{4}\textsuperscript{-}-dependent conformational change in Cys\textsuperscript{210}-LY, we measured the LY fluorescence change in Cys210-LY, which is within the switch II region, after addition of AlF\textsubscript{4}\textsuperscript{-}. The fluorescence of Cys\textsuperscript{210}-LY increased 220% in response to AlF\textsubscript{4}\textsuperscript{-} (Fig. 4, Table II). This fluorescence change can be reversed by 10 mM EDTA (data not shown).

Comparison of crystal structures of GDP and GTP\textsubscript{gS} bound Ga revealed decreased accessibility of Cys\textsuperscript{210} in the activated form. Therefore, the accessibility of cysteine residues to thiol-specific reagents (like LY) should decrease upon activation. Chi\textsubscript{6b} was labeled with LY in the presence or absence of AlF\textsubscript{4}\textsuperscript{-} and a decrease of 77.8% of LY labeling efficiency for Chi\textsubscript{6b} was observed in AlF\textsubscript{4}\textsuperscript{-} bound Chi\textsubscript{6b} (Fig. 5). Thus, in the active conformation, Cys\textsuperscript{210} is less accessible for LY labeling.

To further test the existence of a switch II conformational change in Cys\textsuperscript{210}-LY, the ability of the mutant to interact with the downstream effector enzyme, cGMP phosphodiesterase, was tested. Ga\textsubscript{g}-GTP binds to the inhibitory \(\gamma\) subunit (P\textsubscript{y}) with higher affinity than Ga\textsubscript{a}-GDP (1). The interaction of P\textsubscript{y} with Cys\textsuperscript{210}-LY Ga in the presence or absence of AlF\textsubscript{4}\textsuperscript{-} was monitored by LY fluorescence. There was only a small fluorescent change upon addition of increasing concentrations of P\textsubscript{y} in the absence of AlF\textsubscript{4}\textsuperscript{-}, while in the presence of AlF\textsubscript{4}\textsuperscript{-} there was a P\textsubscript{y} concentration-dependent increase in fluorescence (Fig. 6). Thus, the mutant that fluorescently labeled Ga\textsubscript{a} undergoes an activating conformational change that causes an increased affinity for PDE\textsubscript{g}, and association with PDE\textsubscript{g} further increases the fluorescence of Cys\textsuperscript{210}-LY.

Since the switch II region of Chi\textsubscript{6b}-LY undergoes a conformational change upon binding to AlF\textsubscript{4}\textsuperscript{-}, and this conformational change can be monitored by LY, we therefore concluded that targeted fluorophores could be used to monitor conformational changes in Ga subunits.

### Table II

<table>
<thead>
<tr>
<th>Protein</th>
<th>Unlabeled peak</th>
<th>Labeled peak 1 (1:1)</th>
<th>Labeled peak 2 (1:1)</th>
<th>Labeled peak 3 (2:1)</th>
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<tr>
<td>Chi\textsubscript{6}-LY</td>
<td>10.46</td>
<td>12.12</td>
<td>13.23</td>
<td>15.37</td>
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<td>Chi\textsubscript{6a}-LY</td>
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<td>10.5</td>
<td>11.8</td>
<td>10.28</td>
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<tr>
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<td>13.01</td>
<td>13.01</td>
<td>13.06</td>
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<tr>
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<td>10.5</td>
<td>12.0</td>
<td>10.5</td>
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<td>Chi\textsubscript{6ab}-LY (labeled in the presence of AlF\textsubscript{4}\textsuperscript{-})</td>
<td>11.0</td>
<td>20.9</td>
<td>22.3</td>
<td>80.5</td>
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<tr>
<td>Chi\textsubscript{6b}-LY (labeled in the presence of AlF\textsubscript{4}\textsuperscript{-})</td>
<td>11.0</td>
<td>20.9</td>
<td>22.3</td>
<td>80.5</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Retention time (min)</th>
<th>Total peak area (%)</th>
<th>Total protein (%)</th>
<th>AlF\textsubscript{4}\textsuperscript{-}-dependent fluorescence change (%)</th>
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<tbody>
<tr>
<td>Trp (280 nm, 340 nm)</td>
<td>LY (430 nm, 520 nm)</td>
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<td>Chi\textsubscript{6}-LY</td>
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<td>41.7</td>
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<td>5.8</td>
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<td>Labeled peak 2 (1:1)</td>
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<td>43.2</td>
<td>30.0</td>
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<td>9.3</td>
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<td>66.5</td>
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<td>33.5</td>
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<tr>
<td>Label peak 1’ (1:1)</td>
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<td>33.4</td>
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<td>49.3</td>
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<td>7.4</td>
<td>1.2</td>
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</table>

*This peak (peak 1’) has different retention time when compared to that of peak 1 observed in Chi\textsubscript{6}-LY and Chi\textsubscript{6b}-LY.

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FIG. 3. Proteolytic analysis of labeled peak of Chi\textsubscript{6} and Chi\textsubscript{6a}-LY. The proteins represented by labeled peak 3 of Chi\textsubscript{6}-LY (upper left panel) and labeled peak of Chi\textsubscript{6b}-LY (upper right panel) were treated with trypsin (weight ratio protein:trypsin 5:16.6:1). Trypsin fragments were resolved by polyacrylamide gel electrophoresis and each gel contains protein samples with trypsin treatment time of 0, 5, 15, and 30 min before the reactions were stopped by TLCK. A UV transilluminator was used to observe the LY-labeled fragments on the gel. Simplified scheme for limited proteolytic digestion pattern of Chi\textsubscript{6} is shown on the lower panel. The cleavage sites in Chi\textsubscript{6} protein for trypsin are shown with a hollow arrow (under Lys18, Arg204, and Arg310). See “Experimental Procedures” for more details.
that LY labeled at Cys210 is a sensitive fluorescent reporter for the conformation of the switch II region.

The COOH Terminus of Go Undergoes a Conformational Change Upon AlF4-dependent Activation—The COOH terminus of Go, is not ordered in crystal structure (2, 11–13). To assess its conformation and whether it changes conformation during activation, we labeled Cys347 with LY in Chi6a (Cys347-LY). As shown in Table II and Fig. 4B, an 91% LY fluorescence increase was observed upon addition of AlF4 in Cys347-LY protein. This observation suggests that upon binding of AlF4, the COOHOH terminus of Go undergoes a conformational change. To understand and confirm the existence of this COOH-terminal conformational change, we determined the accessibility of Cys347 in the activated protein. Before LY labeling, the latter group was activated by addition of AlF4 (10 mM sodium fluoride and 50 μM AlCl3) for 10 min and followed by measurements of AlF4-dependent Tryp207 intrinsic fluorescence change in both groups. Labeling efficiency for two chimera proteins, Chi6a and Chi6b, were shown in the figure. Total amount of labeled protein in control group was set to 100%. The labeling efficiency of LY to Chi6a (Cys347-LY) was reduced by 58.6% in preactivation group while a 77.8% reduction was observed in preactivated Chi6b (Cys210-LY).

FIG. 5. LY labeling efficiency of Cys210 and Cys347 in the presence or absence of AlF4. Equal amounts of chimera proteins from the same preparation were taken and divided into control and AlF4 preactivated group. Before LY labeling, the latter group was activated by addition of AlF4 (10 mM sodium fluoride and 50 μM AlCl3) for 10 min and followed by measurements of AlF4-dependent Tryp207 intrinsic fluorescence change in both groups. Labeling efficiency for two chimera proteins, Chi6a and Chi6b, were shown in the figure. Total amount of labeled protein in control group was set to 100%. The labeling efficiency of LY to Chi6a (Cys347-LY) was reduced by 58.6% in preactivation group while a 77.8% reduction was observed in preactivated Chi6b (Cys210-LY).

FIG. 6. Binding of Chi6b-LY protein to Pγ subunit in the presence or absence of AlF4. The relative increase in LY fluorescence change (% of initial) was measured after addition of increasing concentrations of Pγ to 50 nM Chi6b-LY (square) or AlF4-preactivated Chi6b-LY (triangle). The kinetic parameters calculated from the best fit to the four parameter logistic (sigmoidal) equation were: $K_a = 100 \pm 11$ nM, $\Delta F_{MAX} 99\%$, and Hill slope 1.2.

To determine whether this fluorescence change had a similar rate as the AlF4-dependent fluorescence change at Tryp207, the kinetics of the AlF4-dependent Trp and LY fluorescence at Cys347-LY were measured. The rates of the intrinsic fluorescence change of Trp207 and LY fluorescence at Cys347-LY proteins were very similar, with a $K_{act}$ of 0.462 and 0.364, respectively (Fig. 4C). This result suggested that upon binding of AlF4, the COOH terminus of Go undergoes a fast conformational change that follows the slow AlF4-dependent conformational change in the switch II region. Alternatively, the change in conformation of the switch II region could lead to an increased contact with the COOH terminus that resulted in the increase of LY fluorescence.
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DISCUSSION

Knowledge of the functional structure of heterotrimeric G proteins has been greatly advanced with the solution of the crystal structure of all their subunits (2, 11–18). A comparison of the three-dimensional structure of the α subunits in their GDP, GTPγS, and transition state analogue forms has revealed the molecular principles of nucleotide binding, hydrolysis, and the nature of the conformation changes upon protein activation. The COOH-terminal region of Ga, known to be important for receptor interaction (19–24), was not ordered in crystal structures (2, 11–13). Thus, other biochemical or biophysical methods are needed to further define the structural features of this region and understand its role in mediating receptor interaction and receptor induction of GDP release.

In this report, we have developed an approach of sensitive real-time monitoring of the structural changes in Ga using targeted fluorescent labeling of single surface-exposed cysteine residues. Our studies have identified the labeling sites of LY on Gaα/Gαq chimera (Chi6), characterized each labeled protein and, most importantly, shown the existence of a previously unsuspected conformational change in the COOH terminus of Ga upon activation.

The existence of two negative charges in LY and the mild conditions used for labeling Chi6 with LY allowed us to resolve differently modified proteins by anion-exchange chromatography (Fig. 2). The labeling sites on Ga identified in this report, Cys210 and Cys347, confirms the studies of Dhanasekaran et al. (6) who found that Cys347 is highly accessible and Cys210 is partially accessible to 125I-ACTP, when this cross-linking reagent was used to label Gaα.

All mutants were properly folded and functional as judged by several criteria: first, all mutants had an increased Trp fluorescence upon addition of AlF4⁻ indicating that they all have GDP bound and undergo conformational changes. Second, all mutants activated with AlF4⁻ had similar affinity to Pγ when compared with Chi6 (1), which was in the range of 1 μM. Third, the cleavage patterns of tryptic digestion were identical in Chi6 and Chi6 and therefore it can be concluded the mutant chimeras were properly folded during expression.

Our data clearly show that LY at Cys210 is a reporter of conformational change of the switch II region. The fluorescence of LY attached to Cys210 (Chi6α-LY) increases significantly (220%) in the presence of AlF4⁻. However, the intrinsic Trp fluorescence of Cys210-LY (Chi6β-LY) under similar conditions underwent only 1.2% change, which is much less than 50% change in Trp fluorescence for the unlabeled mutant. But Chi6β-LY protein does indeed undergo switch II region conformational change upon binding of AlF4⁻ as judged by acquisition of high affinity for Pγ. An explanation for a decreased Trp fluorescence change in Cys210-LY may be that the fluorescent group at Cys210 increases local hydrophobicity in the environment of Trp207 that causes an increase in Trp fluorescence in the GDP-bound state of Ga. Upon addition of AlF4⁻, switch II moves and adopts the conformation of active Ga subunit, as Trp207 does. However, the increased hydrophobicity of Trp207 in the new environment is not much more than that created by the LY group, so that it does not result in a regular change of intrinsic fluorescence.

The solvent-accessible surface area of Cys210 decreased 68.6% in the activated form of Gaα. This calculation matches well with our finding that the labeling efficiency at Cys210 dropped 77.8% in the presence of AlF4⁻. Thus, Cys210 of Chi6β becomes partially protected from LY labeling in the presence of AlF4⁻, reflecting these changes in accessibility. The method is thus clearly useful for monitoring known conformational changes.

The conformational changes seen at the COOH terminus of Ga are the first indications that the GTP-dependent or activation-dependent conformational switch may extend to this region of the molecule. Several facts support the existence of those environmental changes around the COOH terminus. First, the fluorescence of LY at Cys347-LY (Chi6α-LY) increases by more than 91% in the presence of AlF4⁻ and can be reversed by 10 mM EDTA. There is no specific interaction between LY and AlF4⁻ since in the presence of free LY, no fluorescence change was observed by addition of sodium fluoride and AlCl3 (data not shown). Second, the efficiency of Cys347 labeling with LY for Chi6α is significantly reduced with addition of AlF4⁻, suggesting that surface exposure and reactivity of this residue is decreased as a result of protein activation. To test whether a fluorescent tag on a cysteine in a part of the molecule that is known to have no conformation change upon activation, Chi6β, with both reactive cysteines changed to serines, was mutated to replace Val301 with cysteine. There was no AlF4⁻-dependent fluorescent change in the resultant mutant, Chi6β-Cys301-LY (data not shown). Thus the fluorescence change is not seen in regions known not to change conformation. Third, these effects are very similar to those we observed for Cys210 in Chi6β, which is an indicator of the known conformational change of switch II region. Thus, we can conclude that it is likely that a change in conformation does exist at or near the COOH terminus of Ga upon activation or more precisely in the GDP-AlF4⁻ form. The comparison of the kinetics of fluorescence changes monitored by LY to the fluorescence changes monitored by Trp207 for Chi6α-LY upon addition of AlF4⁻ shows that they have a similar time course (Fig. 4C). This analysis implies that the structural change at the COOH terminus is rate limited by the relatively slow movement of the switch II region upon binding of AlF4⁻. Dhanasekaran et al. (1988) also proposed that conformational changes could be transmitted between domains containing Cys347 and Cys210 in Gaα. As seen in the crystal structure of all Gaα subunits (2, 11–18), the COOH-terminal residues 343–349 form a binding pocket for the COOH terminus of Gaβ and can be reversed by 10 mM EDTA. There is no nonspecific interaction between LY and AlF4⁻ since in the presence of free LY, no fluorescence change was observed by addition of sodium fluoride and AlCl3 (data not shown).

The conformational changes seen at the COOH terminus of Ga are the first indications that the GTP-dependent or activation-dependent conformational switch may extend to this region of the molecule. Several facts support the existence of those environmental changes around the COOH terminus. First, the fluorescence of LY at Cys347-LY (Chi6α-LY) increases by more than 91% in the presence of AlF4⁻ and can be reversed by 10 mM EDTA. There is no specific interaction between LY and AlF4⁻ since in the presence of free LY, no fluorescence change was observed by addition of sodium fluoride and AlCl3 (data not shown). Second, the efficiency of Cys347 labeling with LY for Chi6α is significantly reduced with addition of AlF4⁻, suggesting that surface exposure and reactivity of this residue is decreased as a result of protein activation. To test whether a fluorescent tag on a cysteine in a part of the molecule that is known to have no conformation change upon activation, Chi6β, with both reactive cysteines changed to serines, was mutated to replace Val301 with cysteine. There was no AlF4⁻-dependent fluorescent change in the resultant mutant, Chi6β-Cys301-LY (data not shown). Thus the fluorescence change is not seen in regions known not to change conformation. Third, these effects are very similar to those we observed for Cys210 in Chi6β, which is an indicator of the known conformational change of switch II region. Thus, we can conclude that it is likely that a change in conformation does exist at or near the COOH terminus of Ga upon activation or more precisely in the GDP-AlF4⁻ form. The comparison of the kinetics of fluorescence changes monitored by LY to the fluorescence changes monitored by Trp207 for Chi6α-LY upon addition of AlF4⁻ shows that they have a similar time course (Fig. 4C). This analysis implies that the structural change at the COOH terminus is rate limited by the relatively slow movement of the switch II region upon binding of AlF4⁻. Dhanasekaran et al. (1988) also proposed that conformational changes could be transmitted between domains containing Cys347 and Cys210 in Gaα. As seen in the crystal structure of all Gaα subunits (2, 11–18), the COOH-terminal residues 343–349 form a binding pocket for the COOH terminus of Gaβ and can be reversed by 10 mM EDTA. There is no nonspecific interaction between LY and AlF4⁻ since in the presence of free LY, no fluorescence change was observed by addition of sodium fluoride and AlCl3 (data not shown). Thus the fluorescence change is not seen in regions known not to change conformation. Third, these effects are very similar to those we observed for Cys210 in Chi6β, which is an indicator of the known conformational change of switch II region. Thus, we can conclude that it is likely that a change in conformation does exist at or near the COOH terminus.
change indicates an important communication between the GDP binding pocket and the COOH terminus of the protein.

The COOH-terminal conformational change that was detected in this study is of significant interest because of the implications for receptor interaction mechanisms. The COOH terminus of Ga is known to be a key determinant of the fidelity of receptor activation (19, 23). Since known receptor-binding regions on Ga are distant from the GDP-binding site, it is likely that an allosteric mechanism triggers GDP release (24). It appears from a number of studies that mutations at the COOH terminus of Ga proteins can both regulate specific receptor interaction and affect GDP affinity (24–28). COOH-terminal peptides from Ga can both block receptor-G protein interaction and stabilize the active conformation of G protein-coupled receptors (21, 29–32). These data suggest that the COOH terminus may be a key relay for communication between the activated receptor and the GDP binding pocket. The activation-dependent conformational change reported here thus suggests that an allosteric mechanism triggers GDP release (24). It appears from a number of studies that mutations at the COOH terminus of Ga might lead to a lowered ternary complex (29, 30).

It is possible that an activation-dependent conformational change at the COOH terminus of Ga might lead to a lowered receptor affinity and dissociation from the ternary complex. Currently, it is thought that dissociation of activated G protein from receptor is secondary to the GDP-dependent dissociation of Ga from Gβγ. This concept has not been rigorously tested, however. Future studies will test the notion that the COOH-terminal conformational change after GDP binding is a component of Ga protein dissociation from an activated receptor.

Site-specific Cys-directed fluorescent groups could be reporters for conformation changes in various regions of Ga. For example, they could probe conformational changes in other regions of the Ga subunit like the NH₂ terminus, which was not resolved in crystal structures of either the free Ga GDP form (11) or the GTPγS form (12, 14). Also, they could be used to monitor protein-protein interaction, such as Ga interaction with receptors, Gβγ, and effectors. Future studies will explore these possibilities.

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