THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 272, No. 29, Issue of July 18, pp. 18015–18019, 1997

Printed in U.S.A.

Characterization of a Goα Mutant That Binds Xanthine Nucleotides*

(Received for publication, March 25, 1997, and in revised form, May 5, 1997)

Bo Yu, Vladlen Z. Slepak‡, and Melvin I. Simon§

From the Division of Biology, California Institute of Technology, Pasadena, California 91125

Several GTP binding proteins, including EF-Tu, Ypt1, rab-5, and FtsY, and adenylosuccinate synthetase have been reported to bind xanthine nucleotides when the conserved aspartate residue in the NKXD motif was changed to asparagine. However, the corresponding single Goα mutant protein (D273N) did not bind either xanthine nucleotides or guanine nucleotides. Interestingly, the introduction of a second mutation to generate the Goα subunit D273N/Q205L switched nucleotide binding specificity to xanthine nucleotide. The double mutant protein GoαD273N/Q205L (GoαX) bound xanthine triphosphate, but not guanine triphosphate. Recombinant GoαX (GoαD273N/Q205L) formed heterotrimers with βγ complexes only in the presence of xanthine diphosphate (XDP),[1] and the binding to βγ was inhibited by xanthine triphosphate (XTP). Furthermore, as a result of binding to XTP, the GoαX protein underwent a conformational change similar to that of the activated wild-type Goα. In transfected COS-7 cells, we demonstrate that the interaction between GoαX and βγ occurred only when cell membranes were permeabilized to allow the uptake of xanthine diphosphate. This is the first example of a switch in nucleotide binding specificity from guanine to xanthine nucleotides in a heterotrimeric G protein α subunit.

G proteins transduce receptor-generated signals across the plasma membranes of eukaryotic cells. They are heterotrimeric complexes composed of α, β, and γ subunits. Each of the subunits belongs to a multigene protein family, containing at least 18 distinct α, 5 β, and 11 γ subunits. Hundreds of seven-transmembrane receptors activated by a great variety of hormones, neuromediators, and growth factors are coupled to G proteins. Receptor-induced activation of a G protein leads to exchange of GDP for GTP bound to the α subunit. The GTP-bound α subunit is released from the αβγ trimeric complex, and both free α and βγ dimers are capable of modulating activities of effector enzymes and ion channels (1–3). G protein-mediated signaling is complicated; a single receptor can activate more than one kind of heterotrimer, and both the activated α and the βγ subunits can interact with multiple effectors. For example, the thrombin receptor is known to couple to G_{12}, Gi, and Gq family members (4), and physiological responses may be the result of contributions by both α and βγ subunits. Furthermore, cross-talk between these different G protein-regulated pathways makes the networks even more complex.

One way to analyze this complex network is to specifically activate a particular Gα *in vivo* to discern its function without interference from other G proteins. As a first step toward this goal, we used site-specific mutagenesis to switch the nucleotide specificity of Gα from guanine to xanthine nucleotides. In cells, xanothine monophosphate is an intermediate in the biosynthesis of GMP; however, the steady-state concentrations of XDP and XTP are relatively low (5). Thus, by subsequent introduction of XTP, we should be able to specifically activate the mutant protein. The α subunits of heterotrimeric G proteins belong to the GTPase superfamily that also includes factors involved in ribosomal protein synthesis, such as EF-Tu, and a large number of Ras-like small guanine nucleotide binding proteins (6, 7). Crystal structures of the α subunits of transducin and Gi have been recently solved (8–11). Both Gα structures had nearly identical binding pockets for the guanine nucleotide, which was similar to the guanine nucleotide binding pocket revealed in the crystal structures of Ras (12) and EF-Tu (13, 14). One of the conserved features was the interaction between a specific Gα amino acid residue and the guanine nucleotide ring, *i.e.* a hydrogen bond from the side chain of a conserved aspartic acid (Asp-268 in transducin) to the N-1 nitrogen and the N_2 amine of the guanine ring (see Fig. 1*a*). Asp-268 of transducin belongs to a conserved motif (NKXD) found in the GTPase superfamily. It has been shown that the characteristic hydrogen bond formed with the aspartic acid residue determines the specificity of guanine nucleotide binding in other GTP-binding proteins, such as EF-Tu and Ras (15, 16). A mutation of aspartate to asparagine at this position in several GTP binding proteins, including EF-Tu (17, 18), Ypt1 (19), rab-5 (20, 21), and FtsY (22) and adenylosuccinate synthetase (23), leads to active proteins regulated by xanthine nucleotides instead of guanine nucleotides. In this report, we studied the effect of the similar D273N mutation on nucleotide binding specificity of Goα.

MATERIALS AND METHODS

Mutagenesis and Expression of the Goα—Myristoylated recombinant mouse GoAα was expressed in *Escherichia coli*. Conditions for growth, induction, and lysis of the Goα-expressing cells were described previously (24). The D273N mutation was introduced in both wild-type Goα and the activated mutant GoαQ205L by oligonucleotide-directed mutagenesis. The oligonucleotide TTTCTAAACAAGAAAAATTTATTTGGCGAGAAGATTAAGAAGTC was annealed to uracil-containing single-stranded DNA from the plasmids pGoα and pGoαQ205L. The resulting vectors were designated as pGoαD273N and pGoαX.

Expression and Purification of His_6-tagged Goα—We subcloned wild type and mutant Goα cDNAs into the *E. coli* expression vector pET-15b (Novagen), which added a peptide of 20 amino acids MGSS(H_6)-SSGLVPRGSH containing the His_6 tag and a thrombin site upstream of the amino terminus of Goα. These clones were used to transform the *E. coli* strain BL21(DE3), and proteins were expressed. After harvesting the culture, cell extracts were resuspended in the binding buffer (5 mM imidazole, 0.5 M NaCl, 160 mM Tris-HCl, pH 7.9, 1 mM βMe). Binding to the Ni^{2+}-NTA resin was according to the protocol provided by Novagen.

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‡ Present address: Dept. of Molecular & Cellular Pharmacology, University of Miami School of Medicine, Miami, FL 33136.

§ To whom correspondence should be addressed: Division of Biology, California Institute of Technology, Pasadena, CA 91125. Tel.: 818-395-3944; Fax: 818-796-7066.

[1] The abbreviations used are: XDP, xanthine diphosphate; XTP, xanthine triphosphate; DTT, dithiothreitol; NDK, nucleotide diphosphate kinase; PAGE, polyacrylamide gel electrophoresis; PTX, pertussis toxin; PLCβ$_2$, phospholipase C β$_2$.

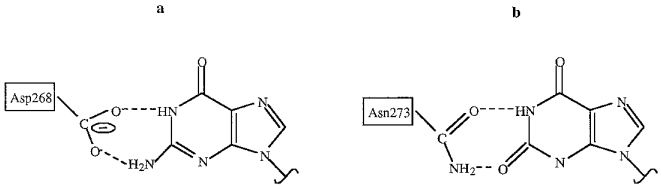

FIG. 1. *a*, interaction between the aspartic acid side chain at position 268 in the α subunit of transducin with the guanine ring of GTPγS, revealed by the solved crystal structure. *b*, a proposed model for the interaction between the substituted asparagine residue at position 273 in Goα and the xanthine ring.

The His_6-tagged protein was eluted with a gradient of imidazole concentration (5–500 mM). The Goα and various mutant proteins eluted at about 250 mM imidazole. Proteins were then transferred to TED buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM DTT) with 0.1 mM $MgCl_2$ and 0.1 mM nucleotide diphosphate (GDP or XDP as appropriate) by gel filtration. Purified proteins were stored in 50% glycerol at −70 °C.

Synthesis of XTPγS—XTPγS was synthesized from XDP and ATPγS with nucleotide diphosphate kinase (NDK) as described previously (25). To produce ^{35}S-labeled XTPγS, the reaction contained 10 μM XDP, 1 μM [^{35}S]ATPγS, and 10 units NDK (Sigma) in 100 μl of NDK buffer (1 mM $MgCl_2$, 5 mM DTT, 20 mM Tris-HCl, pH 8.0). The mixture was incubated at room temperature for 2 h. The resulting concentration of [^{35}S]XTPγS was about 1 μM (1 μCi/pmol). The radiochemical purity of XTPγS was monitored by thin layer chromatography on Avicel/DEAE plates (Analtech) in 0.07 N HCl.

Nucleotide Binding—Binding of [^{35}S]GTPγS and [^{35}S]XTPγS to the recombinant Goα and the mutant proteins was performed as described (24). The binding reaction contained 0.5 μg of purified protein or 200 μg of crude *E. coli* protein in TED buffer with 0.1 mM $MgCl_2$, 1 μM ATP, and 0.1 μM GTPγS or XTPγS (20,000 cpm/pmol). For the time course experiments, 20-μl aliquots were withdrawn from a 200-μl reaction, diluted 10-fold with ice-cold TED buffer containing 0.1 mM $MgCl_2$, filtered through a 0.45-μm nitrocellulose filter, washed, and dried. The amount of bound radioactivity was determined by scintillation counting.

Proteolysis with Trypsin—Approximately 0.1 μg of purified recombinant Goα was preincubated with nucleotide at room temperature for 30 min in the TED buffer. 10 ng of trypsin was then added to the mixture, and the reaction was terminated after 10 min by addition of an equal volume of 2 × SDS-PAGE sample buffer and heating for 3 min at 100 °C. The proteolytic pattern was subsequently analyzed by Western blot using antibodies against Goα.

ADP-ribosylation by Pertussis Toxin—Pertussis toxin-catalyzed ADP-ribosylation was performed as described (24). Briefly, 0.1 μg of recombinant Goα was mixed with 0.1 μg of purified retinal βγ subunit complex in the presence of the appropriate nucleotide and incubated for 10 min at room temperature before addition of the reaction mixture (final concentration of 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 2 mM $MgCl_2$, 2 mM DTT, 0.5 μM [^{32}P]NAD (20,000 cpm/pmol), and 10 μg/ml pertussis toxin (List Biologicals)). Reactions were incubated for 30 min at room temperature and terminated by the addition of 5 × SDS-PAGE sample buffer. Samples were resolved on SDS-PAGE. Gels were stained with Coomassie Blue, dried, and exposed to x-ray film.

Cell Culture and Transfection—COS-7 cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. 1×10^5 cells/well were seeded in 12-well plates 1 day before transfection. All transfection assays contained a total amount of 1 μg of DNA; the plasmid pCIS encoding β-galactosidase was used to maintain a constant amount of DNA. To each well, 1 μg of DNA was mixed with 5 μl of lipofectamine (Life Technologies, Inc.) in 0.5 ml of Opti-MEM (Life Technologies, Inc.), and five h later, 0.5 ml of 20% fetal calf serum in Dulbecco's modified Eagle's medium was added to the cells. After 48 h, cells were assayed for inositol phosphate levels as described previously (26, 27).

Permeabilization of COS-7 Cell Membranes—Transfected COS-7 cells were washed twice with phosphate-buffered saline and incubated in 200 μl of permeabilization solution consisting of 115 mM KCl, 15 mM NaCl, 0.5 mM $MgCl_2$, 20 mM Hepes-NaOH, pH 7, 1 mM EGTA, 100 μM ATP, 0.37 mM $CaCl_2$ (to give a free Ca^{2+} concentration of 100 nM), and 200 units/ml α-toxin with or without 0.1 mM XDP for 10 min at 37 °C. Then 2 μl of 1 M LiCl was added before the inositol phosphate assay.

RESULTS

To change the binding specificity of Goα from guanine nucleotides to xanthine nucleotides, we replaced Asp-273 by an asparagine residue, which was expected on the basis of structural analysis to coordinate with xanthine instead of guanine (Fig. 1*b*). This mutation was introduced into both the wild-type Goα subunit and the GTPase-deficient Goα mutant (Q205L). We chose Goα because myristoylated Goα can be expressed in *E. coli*, and it has been shown that many of the characteristics of the recombinant Goα protein are similar to those of the protein isolated from brain. To further characterize the function of XTP-bound Goα mutants, we purified these proteins in the form of non-myristoylated His_6-tagged Goα by affinity chromatography on a Ni^{2+}-NTA column. It has been shown that the non-myristoylated form of Goα has identical nucleotide binding properties compared with the myristoylated form, and it also forms trimeric complexes with βγ subunits although the affinity to βγ is much less than the myristoylated form (44).

Binding of GTPγS and XTPγS—The nucleotide binding of Goα, GoαD273N, and GoαX (GoαD273N/Q205L) was assayed with [^{35}S]GTPγS and [^{35}S]XTPγS. In *E. coli* crude extracts, Goα reached maximum binding of GTPγS in about 30 min (Fig. 2*a*). As expected, Goα showed no affinity for XTPγS. However, GoαX revealed a switch in nucleotide specificity. As shown in Fig. 2*b*, GoαX had high affinity for XTPγS but not for GTPγS. Interestingly, only the double mutant was active while GoαD273N did not bind either GTPγS or XTPγS (data not shown). Goα binds GTPγS very tightly in the presence of 1 μM Mg^{2+} (28, 29). Both Goα (Fig. 2*c*) and GoαX (Fig. 2*d*) did not exchange bound [^{35}S]NTPγS when excess non-radioactive nucleotides were subsequently added.

The purified His_6-tagged proteins in general retained the properties of the untagged myristoylated α subunits. However, we detected some differences in nucleotide binding. His_6-tagged Goα or GoαX bound GTPγS or XTPγS, respectively, but the binding was less stable than with the untagged myristoylated protein. In the case of His_6-tagged Goα, the bound GTPγS could be exchanged after excess non-radioactive GTPγS was added (Fig. 2*c*). Similar behavior was observed in the XTPγS binding of pure His_6-tagged GoαX, which also showed distinct nucleotide exchange after non-radioactive XDP or XTP were added to the binding reaction (Fig. 2*d*). The decrease in nucleotide affinity was apparently the result of the presence of the His_6-tag. Although the nucleotide binding of His_6-tagged proteins was less stable, the specificity of binding was clearly maintained, and the mutant bound the xanthine nucleotides rather than the guanine nucleotides. As expected, the purified single mutant GoαD273N did not show any nucleotide binding activity (data not shown).

Activation Conformational Change as Assessed by Limited Proteolysis—Guanine nucleotides protect G protein α subunits, including Goα, from complete proteolytic degradation (30–32). The pattern of fragments derived from partial tryptic digestion can be used as an indicator of the conformation of the protein. In the presence of GDP, Goα is hydrolyzed by trypsin resulting in two products, a stable 25-kDa and an unstable 17-kDa peptide. Binding of non-hydrolyzable analogs of GTP can induce an active conformation of the Goα subunit, which is resistant to proteolytic degradation, and protects a stable 37-kDa polypeptide from further degradation. In the case of the activated mutant GoαQ205L, GTP can also protect the remaining 37-kDa polypeptide from complete proteolytic digestion by trypsin because GoαQ205L lacks GTPase activity. Fig. 3*a* shows that XTP protects GoαX from proteolysis by trypsin (*lanes 4* and *5*), whereas in the control experiment, GTPγS protected wild-type Goα (*lane 8*). This experiment indicates that GoαX binds XTP without hydrolyzing it. After binding to XTP, GoαX must have assumed a conformation similar to that of GTPγS-bound wild-type Goα. In this experiment, wild-type

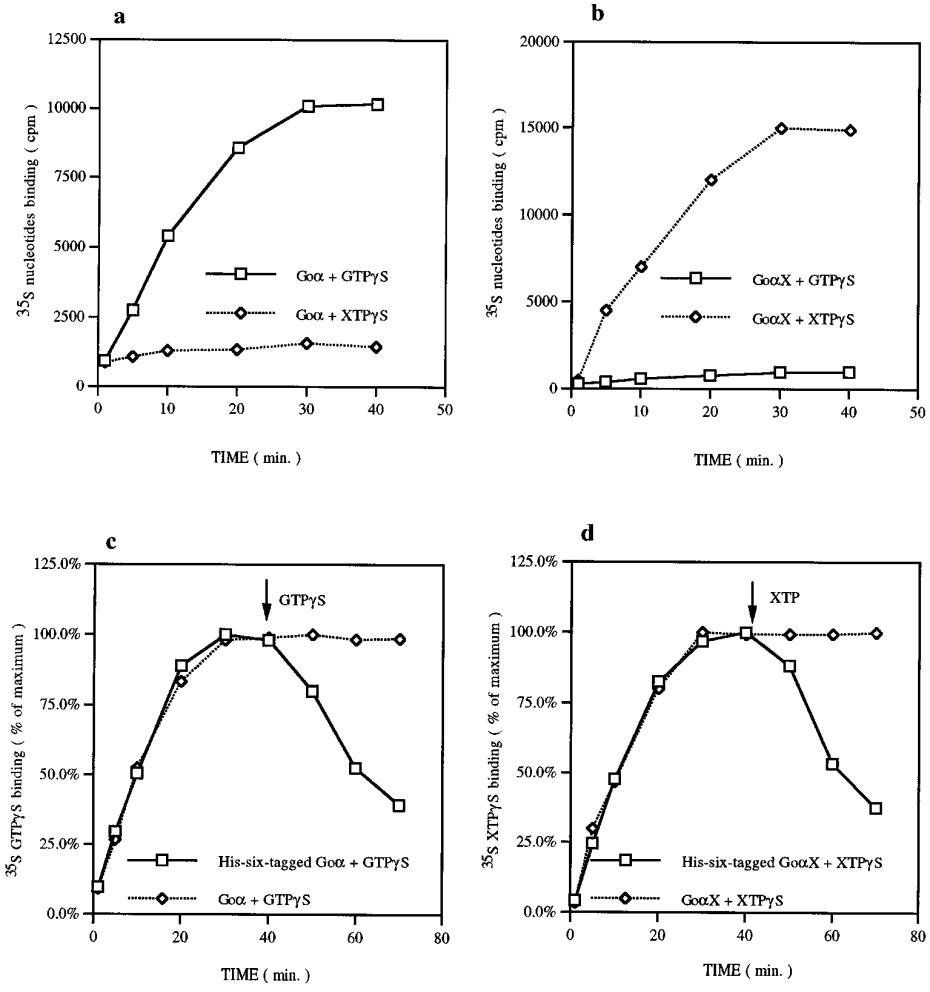

FIG. 2. **GoαX binds XTPγS but not GTPγS.** 20 μl of the *E. coli* extract containing wild-type Goα (*a*) or GoαX (*b*) was diluted 10-fold with TEDM buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM DTT, 1 mM $MgCl_2$) containing either 0.1 μM [^{35}S]GTPγS or [^{35}S]XTPγS (20,000 cpm/pmol) and incubated at room temperature. At the indicated times, 20-μl aliquots were withdrawn and assayed for the bound nucleotides. GTPγS binding of the purified His_6-tagged Goα (*c*) and XTPγS binding of the purified His_6-tagged GoαX (*d*) were compared with those of untagged Goα and GoαX in the *E. coli* extract. After 40 min, 1 mM unlabeled GTPγS (*c*) or XTP (*d*) was introduced into the reaction.

Goα needed only 1 μM GTPγS to prevent complete proteolysis. Similarly, GoαX was sufficiently protected in the presence of 1 μM XTP. It is noteworthy that GTPγS, but not GTP, was also able to protect GoαX from complete tryptic digestion although this protection required GTPγS concentrations above 100 μM (*lanes 1, 2*, and *3*). Thus, GoαX has a much lower affinity for GTPγS than for XTP. We did not detect any of GTPγS binding activity of GoαX in our nucleotide binding assay because the highest concentrations of [^{35}S]GTPγS used in the reaction were micromolar. Consistent with the results of the nucleotide binding experiments, the single mutant GoαD273N was not protected by any nucleotides including GTP, GTPγS, and XTP up to millimolar concentrations (data not shown).

Pertussis Toxin-induced ADP-ribosylation—The interaction of Goα with the βγ complex can be assayed by ADP-ribosylation of the α subunit induced by pertussis toxin (PTX) because ADP-ribosylation requires the formation of the heterotrimeric complex (33, 34). Modification (by ADP-ribosylation) of recombinant Goα catalyzed by PTX is the same in the presence of GTP or GDP because of the GTPase activity of Goα. However, GTPγS strongly inhibits the modification since Goα cannot hydrolyze GTPγS. GTPγS binding thus promotes the dissociation of the trimeric αβγ complex and prevents the ADP-ribosylation of the Goα subunit. The activated GoαQ205L mutant lacks GTPase activity, and the effect of GTP on ADP-ribosylation is similar to that of GTPγS on the wild-type Goα. Therefore, PTX labeling can be used not only to examine βγ binding but also GTPase activity. Fig. 3*b* shows that purified Goα was ADP-ribosylated by pertussis toxin (*lane 7*), and the labeling was strongly inhibited by GTPγS (*lane 6*). In contrast, GoαX was modified by pertussis toxin only in the presence of XDP (*lane 4*) but not with GDP (*lane 5*), and as expected, the reaction was strongly inhibited by XTP (*lane 2*), whereas GTP had no effect (*lane 3*). Therefore, only XDP-bound GoαX can form trimeric complexes with βγ, and binding of XTP induces dissociation of the trimeric complex. As a control, we did not detect any ADP-ribosylation of GoαX when GTPγS, GTP, or XTP alone was present (data not shown). Consistent with the results of trypsin digestion, this experiment indicated that XTP was not hydrolyzed by GoαX. The quantitation of [^{32}P]ADP-ribose incorporation revealed that the labeling of GoαX was proportional to the amount of βγ used and reached a maximum at a GoαX:βγ ratio of 1:1, similar to wild-type Goα (data not shown). Interestingly, high concentrations (over 100 μM) of GTPγS also inhibited the ADP-ribosylation of GoαX (Fig. 3*b*, *lane 1*), offering further evidence that GoαX was able to bind GTPγS with low affinity. As expected, GoαD273N did not interact with βγ and was not modified by pertussis toxin in the presence of either GDP or XDP (data not shown).

XDP-dependent βγ Interaction in Transfected COS-7 Cells—In transfected COS-7 cells, $\beta_1\gamma_2$ is able to activate PLCβ_2, and the activation of PLCβ_2 can be inhibited by cotransfection with Goα because of competition for βγ (35). We cotransfected COS-7 cells with PLCβ2, β1, γ2, and GoαD273N or GoαX and found that both Goα mutants did not inhibit PLCβ_2 activity, whereas wild-type Goα did. This experiment indicates that both mutants do not bind βγ in COS-7 cells and is consistent with the *in vitro* experiments on PTX-induced ADP-ribosylation. GoαX bound βγ only in the presence of XDP, and because XDP concentration is negligible inside the cell, the

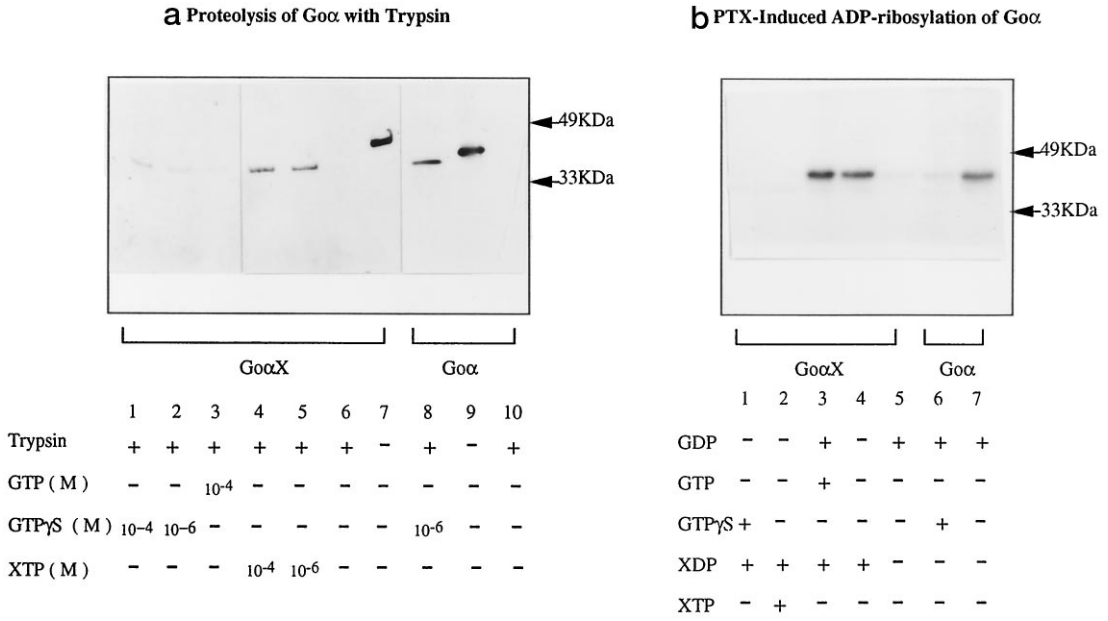

FIG. 3. **Functional Regulation of Goα by Xanthine Nucleotides.** *a*, XTP protects the proteolysis of GoαX with trypsin. 0.1 μg of purified recombinant Goα or GoαX was incubated with indicated nucleotides at room temperature for 30 min. 10 ng of trypsin was then added to the mixture, and the reaction was terminated by addition of an equal volume of 2 × SDS-PAGE sample buffer. The proteolytic pattern was visualized by Western blot using an antibody against a C-terminal peptide of Goα. *b*, PTX-induced ADP-ribosylation of GoαX requires XDP and is inhibited by XTP. 0.1 μg of purified recombinant Goα or GoαX was mixed with 0.1 μg of purified bovine retinal βγ complex in the presence of indicated nucleotides (100 μM each, including the carry-over GDP or XDP from the protein storage buffer) and incubated for 10 min at room temperature. Then the reaction mixture containing 10 μg/ml pertussis toxin, 0.5 μM [^{32}P]NAD (20,000 cpm/pmol), and other necessary components were added. Reactions were incubated for 30 min at room temperature and terminated by the addition of 10 μl of 5 × SDS-PAGE sample buffer. The samples were then resolved on a 10% SDS-polyacrylamide gel and visualized by autoradiography. The *arrows* indicate the positions of molecular mass markers.

interaction did not occur. To deliver XDP into cells, we tried to permeabilize COS-7 cells by several methods including digitonin treatment, electroporation, and α-toxin (36). We found that only α-toxin gave us consistent results and had no effect on the PLCβ2 activities stimulated by βγ. After incubating cells with α-toxin in the presence of XDP, we found that GoαX inhibited PLCβ2 activity, whereas GoαD273N was not affected by XDP (Fig. 4). In the control experiments, we found that adding GDP or GTP to the permeabilization buffer had no effect on the PLCβ2 activity of cells transfected with the Goα mutants (data not shown). This experiment shows that the Goα mutants behave similarly *in vitro* and in cultured cells; GoαX binds βγ only when exogenous XDP is available.

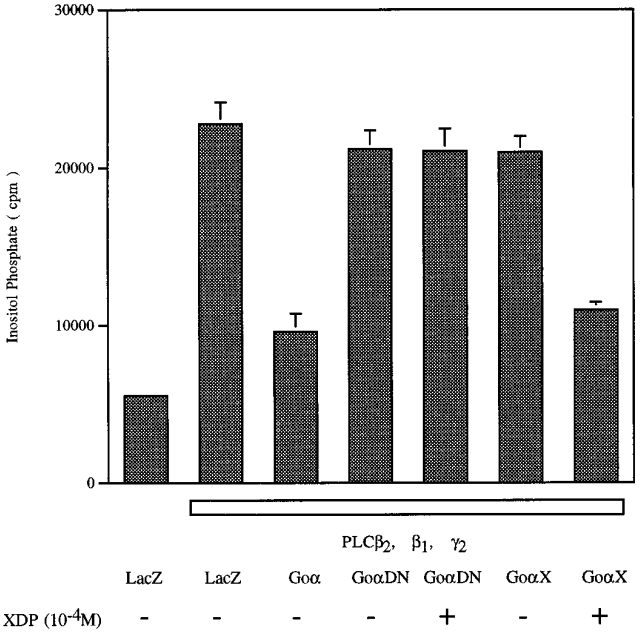

FIG. 4. **The interaction of GoαX with βγ in transfected COS-7 cells is XDP-dependent.** 1 × 10^5 cells/well were seeded in a 12-well plate and then were transfected with cDNAs encoding the indicated proteins the next day. The total amount of cDNA for each well was adjusted to 1.0 μg by addition of CMV-LacZ cDNA. Cells were labeled with [^{3}H]inositol, and the levels of inositol phosphates were determined after incubating cells with 200 units/ml α-toxin with or without 10^{-4} M XDP.

DISCUSSION

We engineered a mutant of Goα that switched nucleotide binding activity from guanine nucleotides to xanthine nucleotides. The mutation (D273N) was at a conserved residue of the NKXD motif that appears in all GTPase superfamily proteins. Crystal structures of transducin and Gi showed that this aspartic acid residue participated in hydrogen bonding to the guanine ring (Fig. 1*a*). The proposed interaction between the mutagenized Asn and the xanthine ring is shown in Fig. 1*b* in which the hydrogen bond is "flipped" when compared with wild-type Gα. Similar single Asp→Asn mutations have been made in other GTP binding proteins, including EF-Tu (17, 18), Ypt1 (19), rab-5 (20, 21), and FtsY (22), and *E. coli* adenylosuccinate synthetase (23), resulting in active proteins regulated by xanthine nucleotides instead of guanine nucleotides. However, the similar D119N mutant of H-Ras induced transformation of NIH-3T3 cells with efficiency indistinguishable from wild-type H-Ras (16, 37). Although the mutant D119N Ras exhibited decreased affinity for GTP and increased affinity for XTP (by 2 to 3 orders of magnitude), the high intracellular concentration of GTP (millimolar) probably ensures that the protein is still bound to the guanine nucleotides in the cell. Interestingly, we found the corresponding D273N mutation in Goα did not result in binding of either GTPγS or XTPγS, whereas the D273N/Q205L double mutant, GoαX, switched nucleotide binding ability. When examining the crystal structure of transducin, it is not clear why the

Gln→Leu mutation (position 200 in transducin α), which is at the opposite side of the nucleotide binding pocket from the Asp→Asn mutation (position 268 in transducin α), rescued the xanthine nucleotide binding of GoαD273N. It is interesting to note that GoαX binds GTPγS at concentrations higher than 100 μM. In our nucleotide binding experiments, we could not observe this binding because the affinity was weak, requiring concentrations higher than 1 μM [^{35}S]GTPγS, which was the highest concentration that we could use. The P–S bond of the γ phosphate in GTPγS is longer than the P–O bond in GTP, which not only prevents nucleotide hydrolysis when binding to G protein α subunits, it also results in qualitatively different interactions and different affinities.

In vitro experiments using limited trypsin digestion and PTX-induced ADP-ribosylation showed that GoαX retained the characteristic properties of wild-type Goα in the presence of XDP or XTP. In addition, our data confirm the assumption that diphosphate nucleotides are required for the interaction of G protein α subunits with βγ subunits. XTP-bound GoαX assumed a trypsin-resistant conformation similar to that of the activated wild-type Goα and stimulated βγ dissociation from the trimeric complex, suggesting that GoαX can be activated by XTP. In transfected COS-7 cells, PLCβ2 is activated by G protein βγ subunits, and the activity is inhibited when cotransfecting with Goα because of the competition for βγ. To study βγ binding of the mutant GoαX *in vivo*, we looked for inhibition of PLCβ2 activity as an indication of βγ binding. We found that GoαX did not affect βγ-stimulated PLCβ2 activity because of the absence of XDP. To turn on βγ binding, we used α-toxin to make cell membranes permeable to XDP, and indeed under these conditions, GoαX attenuated PLCβ2 activity. G protein-derived βγ subunits are shown to be able to bind many proteins other than Gα, and may be involved in many signal transduction pathways. We demonstrated that XDP can be delivered into cells and GoαX may be used as a βγ quencher that can make the cellular βγ pool unavailable to other βγ effectors. The ability to turn on and off βγ *in vivo* could be useful to better understand the physiological function of βγ.

Goα is one of the G protein α subunits whose functions are not well understood although there is some evidence supporting a role in the regulation of calcium channels (38–42). Since βγ subunits are also proposed as regulators of calcium channels (43), it is difficult to differentiate the activities of Goα and βγ in some situations when activated receptors release both Goα and βγ subunits. This is one of the problems that the GoαX mutant might be used to address. The channel may be activated directly by adding XTP without releasing free βγ in cells that have been transfected with cDNA expressing the mutant protein. Cross-talk between the different G protein-mediated signaling pathways has been well demonstrated. Activating GoαX directly and instantly by XTP would avoid the interference of other pathways and help us to differentiate individual pathways. Introducing this mutation into other G protein α subunits may be used to study their functions as well.

Acknowledgments—We thank members of the Simon laboratory for helpful discussions and Drs. Lorna Brundage and Tau-Mu Yi for comments on the manuscript.

REFERENCES

1. Neer, E. J. (1995) *Cell* **80,** 249–257
2. Simon, M. I., Strathman, M. P., and Gautam, N. (1991) *Science* **252,** 802–808
3. Gilman, A. G. (1987) *Annu Rev. Biochem.* **56,** 615–649
4. Grand, R. J. A., Turnell, A. S., and Grabham, P. W. (1996) *Biochem. J.* **313,** 353–368
5. Henderson, J. F., and Paterson, A. R. P. (1973) in *Nucleotide Metabolism*, p. 97–169, Academic Press, New York
6. Bourne, H. R., Sanders, D. A. and McCormick, F. (1990) *Nature* **348,** 125–132
7. Bourne, H. R., Sanders, D. A., and McCormick, F. (1991) *Nature* **366,** 654–663
8. Noel, J., Hamm, H. E., and Sigler, P. B. (1993) *Nature* **366,** 654–663
9. Lambright, D. G., Noel, J. P., Hamm, H. E., and Sigler, P. B. (1994) *Nature* **369,** 621–628
10. Sondek, J., Lambright, D. G., Noel, J. P., Hamm, H. E., and Sigler, P. B. (1994) *Nature* **372,** 276–279
11. Coleman, D. E., Berghuis, A. M., Lee, E., Linder, M. E., Gilman, A. G., and Sprang, S. R. (1994) *Science* **265,** 1405–1412
12. De Vos, A. M., Tong, L., Milburn, M. V., Maniatis, P. M., Jancarik, J., Noguchi, S., Nishimura, S., Muira, K., Ohtsuka, E., and Kim, S.-H. (1988) *Science* **239,** 888–893
13. Jurnak, F. (1988) *Science* **230,** 32–36
14. La Cour, T. F. M., Nyborg, J., Thirup, S., and Clark, B. F. C. (1985) *EMBO J.* **4,** 2385–2388
15. Der, C. J., Pan, B.-T., and Cooper, G. M. (1986) *Mol. Cell. Biol.* **6,** 3291–3294
16. Feig, A. L., Pan, B.-T., Roberts, T. M., and Cooper, G. M. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 4607–4611
17. Hwang, Y. W., and Miller, D. L. (1987) *J. Biol. Chem.* **262,** 13081–13085
18. Weijland, A., and Parmeggiani, A. (1993) *Science* **259,** 1311–1314
19. Jones, S., Litt, R. J., Richardson, C. J., and Seger, N. (1995) *J. Cell Biol.* **130,** 1051–1061
20. Rybin, V., Ullrich, O., Rubino, M., Alexandrov, K., Simon, I., Seabra, M., Goody, R., and Zerial, M. (1996) *Nature* **383,** 266–269
21. Hoffenberg, S., Nikolova, L., Pan, J. Y., Daniel, D. S., Wessling-Resnick, M., Knoll, B. J., and Dickey, B. F. (1995) *Biochem. Biophys. Res. Commun.* **215,** 241–249
22. Powers, T., and Wolter, P. (1995) *Science* **269,** 1422–1424
23. Kang, C., Sun, N., Honzatko, R. B., and Fromm, H. J. (1994) *J. Biol. Chem.* **269,** 24046–24049
24. Slepak, V. Z., Wilkie, T. M., and Simon, M. I. (1993) *J. Biol. Chem.* **268,** 1414–1423
25. Goody, R. S., Eckstein, F., and Schrimer, H. (1972) *Biochim. Biophys. Acta* **276,** 155–161
26. Wu, D., Lee, C. H., Rhee, S. G., and Simon, M. I. (1992) *J. Biol. Chem.* **267,** 1811–1817
27. Wu, D., Katz, A., Lee, C. H., and Simon, M. I. (1992) *J. Biol. Chem.* **267,** 25798–25802
28. Higashijima, T., Ferguson, K. M., Sternweis, P. C., Smigel, M. D., and Gilman, A. G. (1987) *J. Biol. Chem.* **262,** 762–766
29. Higashijima, T., Ferguson, K. M., Sternweis, P. C., Ross, E. M., Smigel, M. D., and Gilman, A. G. (1987) *J. Biol. Chem.* **262,** 752–756
30. Hurley, J. B., Simon, M. I., Teplow, D. B., Robishaw, J. D., and Gilman, A. G. (1984) *Science* **226,** 860–862
31. Graziano, M. P., and Gilman, A. G. (1989) *J. Biol. Chem.* **264,** 15475–15482
32. Denker, B. M., Neer, E. J., and Schmidt, C. J. (1992) *J. Biol. Chem.* **267,** 6272–6277
33. Bokoch, G. M., Katada, T., Northup, J. K., Hewlett, E. L., and Gilman, A. G. (1983) *J. Biol. Chem.* **258,** 2072–2075
34. Casey, P. J., Graziano, M. P., and Gilman, A. G. (1989) *Biochemistry* **28,** 611–616
35. Slepak, V. Z., Katz, A., and Simon, M. I. (1994) *J. Biol. Chem.* **270,** 4037–4041
36. Ahnert-Hilger, G., Mach, W., Fohr, K. J., Gratzl, M. (1989) *Methods Cell Biol.* **31,** 63–90
37. Zhong, J.-M., Chen-Hwang, M.-C., Hwang, Y.-W. (1995) *J. Biol. Chem.* **270,** 10002–10007
38. Kleuss, C., Hescheler, J., Ewel, C., Rosenthal, W., Schultz, G., and Wittig, B. (1991) *Nature* **353,** 43–48
39. Taussig, R., Sanchez, S., Rifo, M., Gilman, A. G., and Belardetti, F. (1992) *Neuron* **8,** 799–809
40. Lledo, P. M., Homburger, V., Bockaert, J., and Vincent, J.-D. (1992) *Neuron* **8,** 455–463
41. Kalkbrenner, F., Degtiar, V. E., Schenker, M., Brendel, S., Zobel, A., Heschler, J., Wittig, B., and Schultz, G. (1995) *EMBO J.* **14,** 4728–4737
42. Charpentier, N., Prezeau, L., Carrette, J., Bertorelli, R., Le Cam, G., Manzoni, O., Bockaert, J., and Homburger, V. (1993) *J. Biol. Chem.* **268,** 8980–8989
43. Ikeda, S. R. (1996) Nature **380,** 255–258
44. Denker, B. M., Neer, E. J., and Schmidt, C. J. (1992) *J. Biol. Chem.* **267,** 6272–6277