Specific antisera were produced to peptides representing the carboxyl terminus of \( \alpha_{13} \), a recently identified \( \alpha \) subunit of the heterotrimeric guanine nucleotide-binding proteins (G proteins). Immunodetection with these antisera indicated that the 43-kDa protein is expressed ubiquitously at low levels (0.005–0.05% of membrane protein) in tissues and cultured cells. A combination of conventional and immunoaffinity chromatographic techniques was used to purify small quantities of \( \alpha_{13} \) from bovine brain. Quantities of protein sufficient for biochemical analysis could be produced by concurrent expression of \( \alpha_{13} \) with G protein \( \beta_2 \) and \( \gamma_2 \) subunits using a baculovirus system.

The rate of dissociation of GDP from recombinant \( \alpha_{13} \) (\( r_{\alpha_{13}} \)) is slow (0.01–0.02 min\(^{-1}\) at 30°C), and relatively high concentrations of guanosine 5'-3-O-(thio)triphosphate (GTP\( \gamma \)S) are required to observe nucleotide binding. This binding was reduced significantly in the presence of 20 mM Mg\(^{2+}\). Rates of hydrolysis of GTP by \( \alpha_{13} \) were limited by nucleotide exchange; attempts to measure the intrinsic rate of hydrolysis indicate that it is greater than 0.2 min\(^{-1}\). Stoichiometric concentrations of \( \beta \gamma \) subunits inhibited binding of GTP\( \gamma \)S to and hydrolysis of GTP by \( \alpha_{13} \). By reconstitution, the purified \( \alpha_{13} \) did not affect the activity of several known effector enzymes. The availability of purified \( r_{\alpha_{13}} \), and knowledge of its biochemical properties will allow further characterization of its interactions with receptors and effectors.

EXPERIMENTAL PROCEDURES

Antisera—Peptides that correspond to predicted amino acid sequences of the \( \alpha_{13} \) cDNA from mouse brain (16) were synthesized and cross-linked to tuberculin-purified protein derivate (Statens Seruminstitut) with glutaraldehyde (17). The conjugates were used to immunize rabbits (18). Sera were collected and screened by immunoblot analysis of various membrane preparations that had been resolved by SDS-PAGE and transferred to nitrocellulose. Visualization of the immunoreactive proteins was achieved through the formation of a colored precipitate by goat anti-rabbit IgG cross-linked to alkaline phosphatase (Pierce) or production of a chemiluminescent reaction by donkey anti-rabbit IgG linked to horseradish peroxidase (Amersham Corp.). The specificity of sera was initially verified by comparison with preimmune serum or antigenicity with the appropriate antigenic peptides and later by interaction with purified proteins.

Purification of \( \alpha_{13} \) from Bovine Brain—Bovine brain membranes were prepared according to the method of Sternweis and Robishaw (19) and stored at \(-80^\circ\) C until use. The membranes (10–15 g of protein) were washed and extracted for 60 min with buffer 1 (10 mM Tris-Cl, pH 8.0, 1 mM EDTA, 1 mM diithiostreitol, 10 mM GDP, 1% sodium cholate, and AMF (50 mM AlCl\(_3\), 5 mM MgCl\(_2\), and 10 mM NaF)) (19). The 100,000 x g supernatant (extract) was loaded onto a 1-liter column of DEAE-Sepharose which had been equilibrated with buffer 1. Bound protein was eluted with a 2-liter linear gradient of NaCl (0–300 mM) in buffer 1, followed by a 1-liter wash with 500 mM NaCl. Aliquots of fractions were analyzed for \(^{32}\)P(GTP\( \gamma \)S) binding and assayed for \( \alpha_{13} \) by immunoblot analysis. Immunoreactivity was detected as a broad peak between 1,400 and 2,100 ml; the majority of this \( \alpha_{13} \) eluted after the main peak of \(^{32}\)P(GTP\( \gamma \)S) binding activity. Fractions containing \( \alpha_{13} \) were concentrated.
to 50 ml by pressure filtration through an Amicon PM-30 membrane and loaded onto a 1.2-liter column of Ultrogel AcA 44 (Sepracor) which was equilibrated and subsequently eluted with 1.5 liters of buffer 1 containing 100 mM NaCl. Immunoreactivity and amino acid sequences of a13 (Table 1). The efficacy of the carboxyl-terminal and a lesser quantity of a13, eluted between 600 and 900 ml. The major peak of a13 was concentrated to 10 ml, then diluted to 50 ml with buffer 4 (10 mM Tris-Cl, pH 8.0, 1 mM dithiothreitol, 10 mM NaCl, 0.2% Lubrol, and AMF) to reduce the NaCl concentration, and reequilibrated with the same buffer. The a13 pool was added to the Mono Q step. The slurry was brought to 10 ml EDTA and mixed with 164°C. The resin was then washed with 20 volumes of buffer 5 containing 1.5% Lubrol, 15 volumes of buffer 5 containing 300 mM NaCl and 0.1% Lubrol, and 3 volumes of buffer 5 containing 0.2% cholate. Subsequently, a13 was eluted from the matrix with 5 volumes of buffer 5 containing 5% cholate, followed by 6 volumes of buffer 5 containing 1% cholate and AMF (10 mM MgCl2). The eluate containing a13 was concentrated to 0.5 ml. filtered through Sephadex G-50 resin into buffer 6 (90 mM NaHepes, pH 7.6, 1 mM EDTA, 1 mM dithiothreitol, and 0.1% Lubrol) containing 1 mM GDP, and stored at 8°C.

Miscellaneous Methods—Guanine nucleotide binding was quantified by the filter binding method (26), and GTase activity was measured as described (27). Two assays were performed at 30 °C and utilized buffer 6 containing 0.65% Lubrol, 0.20 or 20 mM MgSO4, and guanine nucleotide ([S-35]GTP-S or [3H]GTP, concentrations noted in figure legends). Free Mg2+ was calculated using a Kd of 1.3 mM for Mg2+ at pH 7.6 (28). Binding reactions were terminated by adding aliquots (10 ml) to 20 ml of 5% trichloroacetic acid (TCA) at 80°C. GTPase assays were terminated by adding aliquots (10 ml) to 780 ml of ice-cold 50 mM KH2PO4 containing 5% activated charcoal. [3H]GTPDip was prepared by cleavage of [3H]GTP with hexokinase and glucose (29). Dissociation of bound [3H]GTP from a13 was measured according to the method of Ferguson et al. (29). Rates of guanine nucleotide association and dissociation were obtained by fitting data to the equation: B = Bmax (1 - e^(-kt)) + B0 e^(-kt), respectively, where Bmax is equal to the maximum binding sites observed. Membranes were prepared from homogenized rat tissues as described (30), with final suspension in 20 mM NaHepes, pH 8.1, 1 mM EDTA, and 1 mM dithiothreitol. Cell membranes were prepared by Dounce homogenization (31) or N2 cavitation (20) and suspended in the same buffer. Bovine brain membrane subunits were purified as reported previously (19) and filtered through Sephadex G-50 resin into buffer 6. Analysis of proteins by SDS-PAGE was performed by the method of Laemmli (32). Protein content was determined by staining with Amido Black (33).

RESULTS

Polyclonal antibodies were raised to synthetic peptides corresponding to the carboxyl-terminal and two internal amino acid sequences of a13 (Table 1). The efficacy of the carboxyl-terminal antisera was established initially by several criteria. First, the antisera recognizes a protein with the expected mo-
Isolation and Characterization of \(G_{\alpha}^{\text{ol}}\)

**Fig. 1. Immunological detection of \(G_{\alpha}^{\text{ol}}\) in membranes from tissues and cell lines.** Membrane protein (40 \(\mu\)g) obtained from the indicated tissues and cell lines were resolved by SDS-PAGE through 9% polyacrylamide gels, transferred onto nitrocellulose, and processed with alkaline phosphatase reagents. The \(G_{\alpha}^{\text{ol}}\) derived from mouse fibroblasts; A431, a human epidermal carcinoma cell line; cyc-, a mutant of the murine S49 lymphoma cell line deficient in \(G_{\alpha}^{\text{ol}}\); and NG108, a mouse neuron-rat glioma hybrid cell line.

**Fig. 2. Specificity of B860 antiserum and recognition of \(G_{\alpha}^{\text{ol}}\) by E733 and E734 antisera in immunoblots.** Purified \(G_{\alpha}^{\text{ol}}\) (20 ng) were resolved by SDS-PAGE and visualized by silver staining or analyzed by immunoblotting with a 1:1,000 dilution of the indicated sera (see Table I). Visualization of the immunoblots was accomplished with Enhanced Chemiluminescence reagents. Recombinant \(G_{\alpha}^{\text{ol}}, G_{\alpha}^{\text{ol}}, G_{\alpha}^{\text{ol}}, G_{\alpha}^{\text{ol}}, G_{\alpha}^{\text{ol}}, G_{\alpha}^{\text{ol}}\) and \(G_{\alpha}^{\text{ol}}\) were expressed in SF9, and the purified subunits (25, 36) were the gift of Tohru Kazasa and John Hepler. Recombinant \(G_{\alpha}^{\text{ol}}, G_{\alpha}^{\text{ol}}, G_{\alpha}^{\text{ol}}, G_{\alpha}^{\text{ol}}, G_{\alpha}^{\text{ol}}, G_{\alpha}^{\text{ol}}\) and \(G_{\alpha}^{\text{ol}}\) were expressed in Escherichia coli, and the purified subunits (37–39) were the gift of Suzanne Mumby.

Purification of \(G_{\alpha}^{\text{ol}}\) — Bovine brain was selected as the starting source for the purification of \(G_{\alpha}^{\text{ol}}\); this choice was based on a relatively high content of immunoreactivity and experience with purifying other G proteins from this tissue (19, 41, 42). AMF was included in the purification buffers to produce activation of \(G_{\alpha}^{\text{ol}}\) and thus facilitate its stability and its separation from \(G_{\beta}\) subunits (43, 44). Purification utilized at least three conventional chromatographic steps and one affinity matrix (Table IIA). Estimates of the -fold purification at each step are based on quantification of \(G_{\alpha}^{\text{ol}}\) by comparative immunoreactivity. Analysis of fractions from the third conventional step indicated that \(G_{\alpha}^{\text{ol}}\) coeluted with other \(G_{\alpha}\) subunits and comprised only a minor portion (<1%) of the \(G_{\alpha}\) subunit pool. Therefore, some selective affinity step was required. This was developed by immobilization of antibodies specific for the carboxy terminus of \(G_{\alpha}^{\text{ol}}\). The \(G_{\alpha}^{\text{ol}}\) subunit bound to such a matrix and could be specifically eluted by competition with the antigenic peptide. An example of this purification step is shown in Fig. 3A. In some instances, eluates from the immunoaffinity resin were contaminated with some other polypeptides; these could be removed by affinity chromatography with \(G_{\beta}\)-agarose (21, 22). Unfortunately, the amount of pure protein acquired by these methods proved insufficient for detailed biochemical characterization.

A much greater yield of purified \(G_{\alpha}^{\text{ol}}\) was obtained through expression of the recombinant protein in SF9 cells. The protein was coexpressed with \(G_{\beta}\) and \(G_{\gamma}\) subunits of G proteins. In contrast to expression in the absence of \(G_{\beta}\), a greater portion of \(G_{\alpha}^{\text{ol}}\) was present in the membrane fraction of the cells compared with the cytosolic fraction. This is similar to experiments with \(G_{\alpha}^{\text{ol}}\) and \(G_{\alpha}^{\text{ol}}\) in SF9 cells, where enhanced yields of functional proteins were purified from membranes following coexpression with \(G_{\beta}\) (25, 36). When cells were infected simultaneously with recombinant baculoviruses directing the expression of the three subunits, a 43-kDa protein recognized by \(G_{\alpha}^{\text{ol}}\) antisera accumulated to concentrations of approximately 1 mg/liter of culture after 48 h. The immunoreactive protein was not detected in uninfected cells. After lysis and centrifugation, two-thirds of the \(G_{\alpha}^{\text{ol}}\) was localized in the particulate fraction, which then served as the source for purification. The two initial conventional chromatographic steps were the same as those for purification of \(G_{\alpha}^{\text{ol}}\) (25). At this stage other \(G_{\alpha}\) subunits had been removed, and there was no need for an antibody affinity step. Therefore, subsequent chromatography with immobilized \(G_{\beta}\) subunits provided a final procedure for purification and ensured that the isolated \(G_{\alpha}^{\text{ol}}\) was in a functional conformation. The efficiency of this step is demonstrated in Fig. 3B and Table IIB. Table IIB represents a typical purification from 4 liters of cells and demonstrates the more robust yield of purified protein.
Association of Guanine Nucleotides with \( \alpha_{13} \)—Bovine brain \( \alpha_{13} \) was initially found to be protected against cleavage by trypsin in the presence of AMF and GTP\( \gamma \)S (data not shown), which is indicative of conversion of an \( \alpha \) subunit to the activated state (45). However, measurement of GTP\( \gamma \)S binding to the purified protein yielded only low stoichiometries, and low yields of purified protein prevented optimization of the process. This problem was alleviated by production of the recombinant protein.

The time and concentration dependence of GTP\( \gamma \)S binding to \( \alpha_{13} \) at 30 °C is shown in Fig. 4. In the presence of 0.5 mM Mg\( \text{SO}_4 \), about 1 \( \mu \)m free Mg\( \text{g}^{2+} \), a maximal rate of binding was reached with 10 and 20 \( \mu \)M GTP\( \gamma \)S. Although saturation of sites was not yet attained after 150 min, the data could be fit to single exponential saturation isotherms with maximal binding declining with decreased concentrations of nucleotides. Therefore, rates of GTP\( \gamma \)S binding were similar (about 0.01 min\(^{-1} \)) over the entire concentration range shown. The maximal stoichiometry achieved at the highest concentration of GTP\( \gamma \)S was 0.5 mol of nucleotide/mol of \( \alpha_{13} \). At high concentrations of Mg\( \text{SO}_4 \) (20 mM, 19 mM free Mg\( \text{g}^{2+} \)), lower stoichiometries of binding which were also dependent on the concentration of GTP\( \gamma \)S were clearly observed. The apparent rate of binding was increased slightly to about 0.02 min\(^{-1} \) at all concentrations of nucleotide. At both 1 \( \mu \)m and 19 \( \mu \)m free Mg\( \text{g}^{2+} \), the rate of dissociation of GDP was essentially identical to the rate of association of GTP\( \gamma \)S (0.009 min\(^{-1} \) and 0.023 min\(^{-1} \), respectively). Therefore, it appears that the dissociation of GDP is the major limitation on the rate of nucleotide exchange on \( \alpha_{13} \) and accounts for the independence of rates of association and GTP\( \gamma \)S concentrations. This is similar to observations with other G protein \( \alpha \) subunits.

The decrease in maximal binding at lower concentrations of nucleotide could be due to a relatively high affinity of \( \alpha_{13} \) for GDP or low affinity for GTP\( \gamma \)S. In the former case, competition for GTP\( \gamma \)S binding might come from free GDP (0.07 \( \mu \)m) present in the binding assays and GDP which could dissociate from the \( \alpha_{13} \) (0.5 \( \mu \)m) during incubations. This did not seem to be the case, however, since the IC\(_{50}\) for GDP following a 30-min incubation with 5 \( \mu \)M GTP\( \gamma \)S was much higher, approximately 15 and 30 \( \mu \)m in the presence of 1 \( \mu \)m and 19 \( \mu \)m free Mg\( \text{g}^{2+} \), respectively (data not shown).

It does not appear that low affinity binding of GTP\( \gamma \)S contributes to the changing stoichiometry either. Once bound, GTP\( \gamma \)S dissociates very slowly from \( \alpha_{13} \) (Fig. 5). At the two Mg\( \text{g}^{2+} \) concentrations described above, approximately 90% of bound \( ^{35} \text{S}\)GTP\( \gamma \)S remained bound to the protein 90 min after the addition of unlabeled nucleotide. This is consistent with the formation of essentially nondissociable \( \alpha \)-GTP\( \gamma \)S-Mg\( \text{g}^{2+} \) complexes which have been described for other G protein \( \alpha \) subunits (28). A significant increase in the rate of dissociation of the \( \alpha_{13} \)-GTP\( \gamma \)S complex was observed in the absence of Mg\( \text{g}^{2+} \) (\( k_{	ext{off,GTP\gammaS}} \approx 0.015 \text{ min}^{-1} \)), which is comparable to the off rate for GDP in the presence of Mg\( \text{g}^{2+} \). A similar relationship between the dissociation of GTP\( \gamma \)S in the absence of Mg\( \text{g}^{2+} \) and the off rate for GDP has been observed for \( \alpha_{13} \), but both rates were about 20-fold faster than those observed for \( \alpha_{13} \). The \( \alpha_{13} \) is not protected against trypsin cleavage by GTP\( \gamma \)S in the absence of Mg\( \text{g}^{2+} \) (data not shown), which suggests that the metal ion is required to lock the GTP\( \gamma \)S-bound protein into an activated conformation.

The ability of various nucleotides to compete for the nucleotide binding site of \( \alpha_{13} \) was also examined (data not shown). Simultaneous incubation of the protein with 10 \( \mu \)m \( ^{35} \text{S}\)GTP\( \gamma \)S and a 100-fold excess of GDP, GTP, or Gpp(NH)p reduced binding to undetectable levels, whereas ATP, UTP, and CTP were unable to compete for binding. ITP (1\( \mu \)m) reduced binding.
by about 50%, which conforms with its ability to compete for the guanine nucleotide binding site on other G protein α subunits (19, 26, 39).

Hydrolysis of GTP by ra13—Fig. 6 demonstrates that ra13 possesses an intrinsic GTPase activity. The release of phosphate was linear for at least 60 min, and the steady-state rate of hydrolysis was 0.007 mol/min/mol of ra13 in the presence of 1 μM free Mg²⁺ and 10 μM GTP. Under these conditions, the low rate of hydrolysis reflects the rate of nucleotide exchange. A slower rate of hydrolysis was observed at 20 mM Mg²⁺; this does not match an increased rate of GDP dissociation at the higher Mg²⁺ concentration but does reflect the decreased number of binding sites observed under these conditions.

Attempts to determine the \( k_{\text{cat}} \) for hydrolysis of GTP by ra13 by three separate methods were unsuccessful. First, the slow exchange of GTP for GDP on ra13 hindered attempts to bind \( [\gamma-\text{32P}]\text{GTP} \) in the absence of Mg²⁺ (which retards hydrolysis of GTP in other α subunits). Thus the rate of a single burst of hydrolysis following the addition of Mg²⁺ (27) could not be measured. Second, binding of \( [\gamma-\text{32P}]\text{GTP} \) to ra13 was not detectable, presumably because hydrolysis was much more rapid than rates of exchange (values for \( k_{\text{cat}} \) can be extrapolated from both the apparent \( k_{\text{cat}} \) of \( [\gamma-\text{32P}]\text{GTP} \) and the extent of binding of \( [\gamma-\text{32P}]\text{GTP versus } [\gamma-\text{32P}]\text{GTP} \) (39)). Finally, there was no measurable lag in the approach to steady-state GTP hydrolysis (see Fig. 6), which is equal to \( (k_{\text{cat}} + k_{\text{GDp}})^{-1} \) (27, 39). However, by assuming a lag of less than 5 min and using \( k_{\text{GDp}} = 0.01 \text{ min}^{-1} \), a lower limit for the hydrolytic rate can be calculated. Therefore, the \( k_{\text{cat}} \) for ra13 at 1 μM free Mg²⁺ is predicted to be >0.2 min⁻¹.

Interaction of ra13 with βγ Subunits—The use of βγ chromatography for the purification of ra13 (Fig. 2B) and bovine brain α13 (data not shown) demonstrated that the subunits can form a heterotrimer. Formation of the heterotrimer is known to stabilize the GDP-bound form of other α subunits (28). Therefore, the effect of purified bovine brain βγ subunits on GTPγS binding and hydrolysis of GTP by ra13 was examined (Fig. 7). In the presence of 1 μM free Mg²⁺, both processes were potently inhibited by the βγ subunits, with half-maximal inhibition observed at a ratio of βγ to ra13 of 0.5:1. Maximal reductions in rates of greater than 90% were obtained at about 1:1 ratios of βγ to α13.

Higher ratios of βγ to α13 were required to observe similar inhibitions of the binding of GTPγS and hydrolysis of GTP in the presence of 20 mM MgSO₄ (data not shown), which suggests that association of the subunits is reduced at higher concentrations of Mg²⁺. The βγ preparation used in these experiments contains a mixture of different β and γ subtypes (19, 46). The possibility that α13 prefers interaction with a specific βγ complex has not yet been explored.

Interaction with Effectors—The availability of purified α13 and knowledge of its requirements for activation with GTPγS allowed testing for its potential action on known effector molecules. So far, the purified α13 has not demonstrated an effect on ARF-sensitive phospholipase D (47), βγ-sensitive phosphatidylinositol 3-kinase (8), adenylyl cyclase subtypes I, II, and III expressed in S9 membranes (12, 48, 49), or three β subtypes of phospholipase C (20).

**DISCUSSION**

We have purified α13 to determine its biochemical properties and to search for its functions. Initial purification of native α13 from bovine brain was facilitated through recognition of the protein with specific carboxyl-terminal antisera. Immunoblot analysis revealed that α13 is distributed ubiquitously in various tissues, but its abundance is somewhat lower than many other α subunits. Estimates based on quantitative immunoblotting indicate that the protein makes up 0.005–0.02% of the total membrane protein in bovine brain, which is 100-fold lower than...
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Fig. 7. Effect of βγ on the binding of GTPγS and hydrolysis of GTP by ra13. The ra13 (1 μM) was incubated on ice for 15 min with the indicated molar ratio of purified bovine brain βγ in 20 μl of solution containing 50 mM NaHepes, pH 7.6, 1 mM EDTA, 1 mM dithiothreitol, 140 mM GDP, and 0.1% Lubrol. The mixtures were then diluted 2-fold with a solution containing 1 mM MgSO4 and 20 mM [35S]GTPγS (left panel) or 20 μM [γ-32P]GTP (right panel), such that the final concentration of buffer components was the same as described in the legend to Fig. 4. The reaction mixtures were incubated for 60 min at 30 °C, then binding of GTPγS (left panel) and hydrolysis of GTP (right panel) were measured as described in "Experimental Procedures.”

αs (19) and 10-fold lower than αs and αy (22, 50). The combination of low abundance and copurification with the more abundant α subunits resulted in poor yields of purified bovine brain Gα13.

Much greater quantities of purified α13 were obtained from SF9 cell membranes following coexpression with βγ and γy subunits. Purification utilizing three chromatographic steps was relatively rapid and yielded approximately 500 μg of protein from 4 liters of cultured cells. The purified ra13 appears to be active based on its ability to interact with βγ subunits, bind GTPγS, and hydrolyze GTP. Purification of the membrane-bound ra13, following coexpression with βy and γ subunits also maximizes the possibility that the protein is able to interact functionally with effectors. G protein α subunits expressed in SF9 cells are found in both cytosolic and membrane fractions. Coexpression of α13 with the βγ and γ subunits increases the percentage of protein associated with the membrane, as has been shown for the αy, α11, and α9 (25, 36). The membrane-bound form of the latter three proteins was far more active than the cytosolic form in stimulating β isomers of phospholipase C. Interestingly, the membrane-bound pool of α subunits expressed in SF9 cells contained palmitoylated subunits, whereas the cytosolic pool did not (25, 51). A similar observation has been made for α13 in crude extracts of SF9 cells following expression in the absence of βy and γ subunits (52). The α13 in platelets has also recently been shown to be labeled with both palmitate and arachidonate (53). It will be important to assess the effects of these or other modifications on the properties and functions of purified ra13.

Purified ra13 interacts with guanine nucleotides in a manner that is distinct from other α subunits. Its basal rate of guanine nucleotide exchange is very slow (k = 0.01 min⁻¹ at 30 °C). This value is about an order of magnitude lower than reported for αy and α9 (37, 38, 54) and 2–3-fold lower than values for αy, αν, and αz (37, 39). Dissociation of GTPγS from ra13 is also very slow, even in the absence of Mg²⁺. Despite the apparent high affinity interaction of ra13 with GTPγS, much higher concentrations of its nucleotide are required for effective exchange into the binding site of ra13 compared with other α subunits; an exception is αy (25). These data could be explained by the unliganded nucleotide site rapidly becoming refractory to further occupancy; therefore, high concentrations of GTPγS would be required to reenter the site before it becomes inaccessible. The reduced Fmax observed at low concentrations of nucleotide may reflect denaturation of the protein in its unliganded state. This process appears to be more pronounced in the presence of higher concentrations of Mg²⁺. This contrasts with other α subunits, except αν (39), which display maximal binding at the higher Mg²⁺ concentrations. Overall, it seems that both the occupied and unoccupied nucleotide binding site of α13 are relatively obstructed. It is likely that the interaction of α13 with an agonist-bound receptor or guanine nucleotide exchange factor would increase both kcat, and the effective kcat for GTP. This hypothesis could be tested through in vitro reconstitutions of purified ra13 with receptors. Potential candidates for reconstitutions include the thrombin and thromboxane A2 receptors, which have been shown to interact with α13 in platelet membranes (55). A receptor could also be used to load the protein rapidly with GTP for the direct measurement of kcat. Initial results indicate that the intrinsic kcat for α13 is at minimum faster than that of αy, which displays the slowest rate (0.05 min⁻¹) among α subunits (39).

The effect regulated directly by α13 has not yet been identified. Expression of α13 stimulates the activity of the Na+/H+ exchanger (56), and the carboxyl-terminal antibodies described here block the inhibition of a voltage-dependent Ca²⁺ current by bradykinin, but both of these could be downstream effects. Expression of an activated mutant of α13 did not stimulate inositol phosphate or cAMP synthesis in 293 cells (56). Initial reconstitutions reported here suggest that α13 does not act through adenyl cyclase, phospholipase Cβ, and at least one type each of phospholipase D (47) and phosphatidylinositol 3-kinase (8). The availability of ra13 coupled with the knowledge of its mechanistic properties will facilitate further studies of its possible involvement in other described and evolving signaling systems.

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