Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 8268–8272, September 1992
Biochemistry

Attenuation of GTPase activity of recombinant $G_o\alpha$ by peptides representing sequence permutations of mastoparan

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Communicated by Robert Haselkorn, May 27, 1992 (received for review April 2, 1992)

ABSTRACT **There is convincing evidence that the cytoplasmic domains of multispanning receptors interact with guanine nucleotide-binding proteins (G proteins). What are the rules governing these interactions? In an attempt to answer this question, we focused our attention on mastoparan, an amphiphilic tetradecapeptide from wasp venom, and on nine of its variants, produced by sequence permutation, which have altered amphiphilicity or no amphiphilicity at all. Mastoparan enhances the GTPase activity of recombinant $G_o\alpha$ 5-fold in phospholipid vesicles. Like mastoparan, four of the synthetic variants can form amphiphilic α-helices and two of them indeed stimulate the GTPase activity of the G protein, whereas the other two have no effect. This confirms that the activation of certain G proteins by a number of peptides is mainly due to their cationic amphiphilicity. However, this structural feature is clearly not sufficient. The relative orientation of the positively charged residues as well as that of the hydrophobic side chains appear to be of fundamental importance. The other five peptides are not amphiphilic and do not enhance the rate of GTP hydrolysis. Surprisingly, three of them almost completely inhibit the G protein's intrinsic GTPase activity. This finding is of interest because of the possible role differential regulation of G protein activity can play in cellular functions.**

The guanine nucleotide-binding proteins (G proteins) that link cell surface receptors to cytosolic effectors in signal transduction comprise a family of $\alpha\beta\gamma$ heterotrimers associated with the plasma membrane. Upon activation by a liganded receptor the α-subunit binds GTP, dissociates from $\beta\gamma$, and interacts with one or more effectors (for recent reviews see refs. 1–4). Most known G protein-coupled receptors are characterized by seven hydrophobic transmembrane domains (multispanning receptors), but there is increasing evidence that indicates that certain receptors for growth factors, with a single transmembrane domain, also interact with G proteins (5).

The G protein coupling sites of the multispanning receptors presumably are located in the second and third cytoplasmic loops (6–8). Synthetic peptides with sequences corresponding to segments of the cytoplasmic loops of the β_2-adrenergic receptor have been tested for their effect on adenylate cyclase in erythrocyte membranes. Results suggest that parts of the second, third, and fourth intracellular loops interact with the G protein (9). In contrast, two peptides, comprising the N- and C-terminal 15 amino acids of the third intracellular loop of the β_2-adrenergic receptor, stimulate the GTPase activity of a recombinant α-subunit of G_s (where G_s indicates stimulatory G protein) (10). Most recently, Okamoto *et al.* (11) have reported stimulation of guanosine 5′-[γ-thio] triphosphate (GTP[γS]) binding and GTPase activity of heterotrimeric G_s by a pentadecapeptide corresponding to the C-terminal sequence of the third cytoplasmic loop of the same receptor.

On the whole it appears that several receptor segments are involved in G protein activation in different coupling systems. What are the rules governing the interaction between cytoplasmic receptor segments and the α-subunits of G proteins?

We decided to focus on mastoparan (MP), a tetradecapeptide amide that is a major component of wasp venom. MP activates certain G proteins [G_i and G_o in particular (where G_i indicates inhibitory G protein and G_o indicates G protein of unknown function)] by increasing the rate of GDP/GTP exchange in a way that emulates liganded receptors (12). Because of its amphiphilic nature, MP assumes an α-helical structure when bound to phospholipids, with the positively charged residues exposed to the aqueous medium (13). Presumably, the multispanning receptor regions that interact with G proteins also form cationic amphiphilic α-helices (14).

Are there interactions that do not cause stimulation of G protein activity and, instead, result in its attenuation? So far the attenuation of G protein activity has only been envisaged in *Saccharomyces cerevisiae*, where genetic analysis indicates that the *CDC39* gene product may down-regulate G protein activity (15).

In the present work we report that peptides corresponding to MP permutations exert differential effects on the activity of a recombinant α-subunit of G_o ($rG_o\alpha$): although some of them stimulate or have no effect on the GTP hydrolysis by this protein, three peptides have been found to be strongly inhibitory. These inhibitory peptides may constitute additional tools for the investigation of the role of G proteins in signal transduction pathways.

MATERIALS AND METHODS

Cloning and Expression of $G_o\alpha$ in *Escherichia coli*. To express $G_o\alpha$ in *E. coli*, we chose the T7 promoter-based expression system (16). Plasmid pT7-7 and *E. coli* strain K38 were provided by S. Tabor (Harvard Medical School). Experimental details for all procedures described below are given by Maniatis *et al.* (17). Plasmid pGEM-2 with the inserted rat brain $G_o\alpha$ cDNA (18) was a gift from R. Reed (Johns Hopkins University). A 1325-base-pair *Xho* I and *Eco*RI restriction fragment, containing the rat brain $G_o\alpha$ cDNA, was end-filled with Klenow enzyme and ligated to *Sma* I-digested, dephosphorylated pT7-7 plasmid DNA. Upon transformation of *E. coli* strain MC 1061, plasmid DNA was prepared from ampicillin-resistant colonies and the presence and orientation of the $G_o\alpha$ cDNA were determined by

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Abbreviations: MP, mastoparan; G_s, stimulatory G protein; G_i, inhibitory G protein; G_o, G protein of unknown function; DTT, dithiothreitol; $rG_o\alpha$, recombinant α-subunit of G_o.

restriction mapping. *E. coli* strain K38 harboring plasmid pGP1-2, which encodes T7 RNA polymerase, was transformed with the construct DNA and the double transformants expressing the recombinant $G_o\alpha$ were selected (19).

SDS/PAGE and Immunoblotting. SDS/PAGE of proteins was performed on slab gels according to Laemmli (20) using 12.5% acrylamide in the separating gel. Protein concentrations were determined as described by Bradford (21). After electrophoresis, the gels were stained with Coomassie blue. Immunoblotting of proteins transferred to nitrocellulose sheets was performed using a rabbit antiserum produced in response to the C-terminal decapeptide of rat brain $G_o\alpha$ (22), provided by G. Milligan (University of Glasgow), and anti-antibody conjugated to alkaline phosphatase (Promega) according to Blake *et al.* (23).

Purification of $rG_o\alpha$. Three liters of a culture of *E. coli* K38 harboring the pT7-7 expression vector that contains the $G_o\alpha$ cDNA and plasmid pGP1-2 were grown as described (19). The bacteria were harvested by centrifugation in a Beckman JS-4.2 rotor (4100 rpm, 30 min) at 4°C and stored frozen at −20°C. All subsequent steps were carried out at 4°C. The cells were suspended in 300 ml of a buffer containing 50 mM Tris·HCl (pH 8), 1 mM EDTA, 1 mM dithiothreitol (DTT), and 0.1 mM phenylmethylsulfonyl fluoride and then lysed for 40 min after adding 0.1 mg of DNase I per ml and 0.5 mg of lysozyme per ml. Cell debris was removed by centrifugation in a Beckman JA-14 rotor (14,000 rpm, 1 hr). The $rG_o\alpha$ was purified from the supernatant according to a published procedure (24). After each chromatographic step the protein was detected by assaying aliquots (10 μl) of the collected fractions for GTP[γS] binding and for immunoreactivity following SDS/PAGE. The purified protein was stored at −20°C in a buffer containing 50 mM Hepes·KOH (pH 7.6), 1 mM EDTA, 1 mM DTT, and 40% (vol/vol) glycerol.

Peptides. MP was a commercial product from Sigma. All other peptides were synthesized as described (25). The freeze-dried powders were kept at −20°C. Before experiments the peptides (including MP) were purified on a semi-preparative (10 × 250 mm) LiChrospher 300 RP_{18} HPLC column (Merck) using a linear acetonitrile gradient (45–50%, 20 min, at 2 ml/min) in 0.1% trifluoroacetic acid. Subsequent analysis of each peptide on an Ultrasphere ODS HPLC column (4.6 × 250 mm, Beckman), applying the same gradient (at 1 ml/min), gave a single absorbance peak at 230 nm. The concentration of peptides in aqueous solution was determined by their absorbance at 205 nm (26).

GTP[γS] Binding. GTP[^{35}S] binding was determined as published (27). Reaction mixtures (50 μl) contained 50 mM Hepes·KOH (pH 8), 1 mM DTT, 1 mM EDTA, 0.1% Lubrol, 1.1 mM $MgCl_2$, and 0.3 μM GTP[^{35}S] (22,000 cpm/pmol) and included aliquots (10 μl) of the collected fractions. They were incubated at 20°C for 30 min, then diluted with 1 ml of an ice-cold buffer containing 20 mM Tris·HCl (pH 8), 100 mM NaCl, and 25 mM $MgCl_2$, and filtered through cellulose nitrate membranes (0.45-μm pore size, Whatman) under weak vacuum. The filters were washed three times with 1 ml of the same buffer and dried, and the retained radioactivity was measured by scintillation spectroscopy.

GTP Hydrolysis. The GTP hydrolysis was measured according to a standard method (28). The $rG_o\alpha$ was incubated at 20°C for 5 min in 50 μl of a reaction mixture containing 50 mM Hepes·KOH (pH 8), 1 mM EDTA, 1 mM DTT (HED), 0.1% Lubrol, 1.1 mM $MgCl_2$, and 0.4 μM [γ-^{32}P]GTP (10,000–30,000 cpm/pmol). Alternatively, 5 volumes of the G protein solution in HED (containing 0.02% Lubrol) were mixed with 1 volume of the same buffer containing 0.84% sodium cholate, 0.05% dimyristoyl-L-α-phosphatidylcholine, 0.05% bovine brain phosphatidylethanolamine, and 0.067% bovine brain phosphatidylserine and kept at 4°C overnight. A 32-μl sample of this solution was incubated at 20°C for 5 min in a final volume of 50 μl of HED containing 1.1 mM $MgCl_2$ and 0.4 μM [γ-^{32}P]GTP as well as the respective peptide at the indicated final concentration. The reactions were terminated by adding 750 μl of a 5% charcoal suspension in 20 mM phosphoric acid. After vigorous shaking and centrifugation (Beckman Microfuge, 13,000 rpm for 3 min) the radioactive phosphate in 400 μl of the supernatant was determined by liquid scintillation spectroscopy.

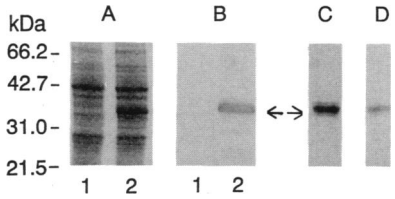

FIG. 1. SDS/PAGE of lysed bacteria expressing $rG_o\alpha$ and of the purified $rG_o\alpha$. (*A* and *B*) Pellets of induced *E. coli* K38 cells, harboring plasmids pGP1-2 and pT7-7 (lane 1) or pGP1-2 and the pT7-7 expression vector for $rG_o\alpha$ (lane 2), obtained from 200-μl cell cultures, were suspended at room temperature in 20 μl of Laemmli's sample buffer (20), sonicated, denatured at 95°C for 5 min, and applied to SDS/12.5% polyacrylamide slab gels as described (20). (*C* and *D*) Purified $rG_o\alpha$ (20 μg per lane). The gels were stained with Coomassie blue (*A* and *C*) or the electrophoresed proteins were transfered to nitrocellulose sheets and immunoblotted with a 1:10,000 dilution of OC1 antiserum specific for $G_o\alpha$ (*B* and *D*).

RESULTS

Expression and Purification of $rG_o\alpha$. A variant of rat $G_o\alpha$, in which the N-terminal 11 amino acids were replaced by 5 different ones, was expressed upon cloning of the corresponding cDNA into the vector pT7-7 and transforming of *E. coli* strain K38, which harbored a plasmid containing the T7 RNA polymerase gene.

rat $G_o\alpha$: NH_2–MGCTLSAEERA————————COOH (354 amino acids)

$rG_o\alpha$: NH_2–MARIR————————COOH (348 amino acids)

The expression of the recombinant protein was detected by Coomassie blue staining of gels after SDS/PAGE of the lysed cells (Fig. 1*A*). The $rG_o\alpha$ was identified by immunoblotting with an antiserum specific for $G_o\alpha$ (Fig. 1*B*).

The G protein was purified to near homogeneity from the soluble cell lysate (Table 1). Stoichiometry of GTP[γS] binding indicated a 70% purity for the $rG_o\alpha$, whereas according to Coomassie blue-stained gel analyses the protein appeared to be >80% pure (Table 1, Fig. 1*C*).

GTP[γS] Dissociation Constant. The $rG_o\alpha$ binds GTP[γS] to saturation at 0.1 mM free Mg^{2+} (Fig. 2). Under these conditions the apparent dissociation constant (K_d) for GTP[γS] was 2.9 nM at 20°C, as calculated from the double reciprocal plot of the binding isotherm data (Fig. 2 *Inset*). This value agrees well with a K_d of 6 nM, reported by Sternweis and Robishaw (30), for $G_o\alpha$ purified from bovine brain in the absence of Mg^{2+}. In contrast, Huff *et al.* (31) determined a K_d of 20 nM at 5 mM free Mg^{2+} with the same bovine brain protein.

Table 1. Purification of $rG_o\alpha$

Fraction	Volume, ml	Protein, mg	GTP[γS] binding	
			nmol	nmol/mg of protein
Soluble lysate	300	1800	137	0.076
DEAE-Sephacel	275	800	75	0.094
Hydroxylapatite I	95	14	27	1.93
Mono Q	3	2.6	22	8.46
Hydroxylapatite II	0.4	1.1	20	18.2

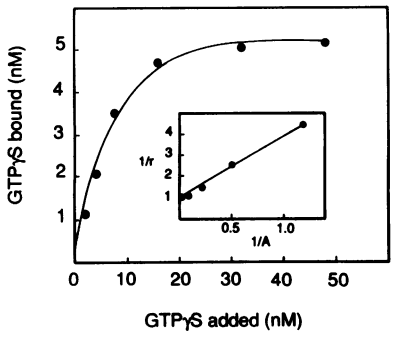

FIG. 2. GTP[γS] binding isotherm of $rG_o\alpha$. The $rG_o\alpha$ (8 nM) was incubated with increasing concentrations of GTP[^{35}S] at 20°C for 30 min and the bound radiolabeled nucleotide was quantitated. (*Inset*) Double reciprocal plot of the protein fraction with bound nucleotide (r) against the concentration of free nucleotide (A), according to Klotz and Hunston (29), from which the dissociation constant was calculated.

Stimulation of GTP Hydrolysis by MP. The basal GTPase activity of the $rG_o\alpha$ was measured in the presence of detergent (0.1% Lubrol), a mixture of phospholipids, or empty phospholipid vesicles (formed by preincubating the phospholipids at 4°C overnight). The molar turnover number for the G protein's GTP hydrolysis was 0.09–0.10 min^{-1} at 20°C and 0.1 mM free Mg^{2+} (Table 2). Reconstituting the $rG_o\alpha$ into phospholipid vesicles (by overnight preincubation of the protein with phospholipids at 4°C) decreased its basal GTPase activity to 0.02 min^{-1} (Table 2). Under these conditions the α-subunit seems to abide in a resting state similar to that of the membrane-bound heterotrimeric G protein, which hydrolyzes GTP only at a very slow rate. A higher $rG_o\alpha$ concentration (56 nM instead of 18 nM) was necessary for the detection of this low level of GTPase activity. The addition of MP to reaction mixtures containing detergent, phospholipids, or phospholipid vesicles (formed in the presence of MP by overnight preincubation at 4°C) enhanced the GTPase activity of $rG_o\alpha$ 2.2- to 2.9-fold (Table 2). The effect started to manifest itself at 100 μM peptide (Fig. 3*A*) but a concentration of 1 mM was necessary to obtain full stimulation. When $rG_o\alpha$ had been reconstituted into phospholipid vesicles, the addition of MP stimulated GTP hydrolysis more markedly. At 1 mM peptide, where the dose–response curve started leveling off (Fig. 3*A*), the basal GTPase activity was enhanced 5-fold (Table 2). This indicates that the α-subunit is more responsive toward presumed receptor mimetic activating agents like MP in a vesicle environment. MP alone, or MP

Table 2. Effect of MP on the GTPase activity of $rG_o\alpha$

Experiment	Activity, min^{-1}, × 10^{-3}		Stimulation, fold
	Basal	With MP	
1	90	198	2.2
2	95	256	2.7
3	100	290	2.9
4	20	100	5

Experiments 1 and 2: 18 nM $rG_o\alpha$ was incubated with 0.1% Lubrol (experiment 1) or with a mixture of phospholipids (experiment 2) for the duration of the reaction. Experiment 3: a mixture of phospholipids was preincubated at 4°C overnight in the absence or presence of peptide and $rG_o\alpha$ was added at the start of the reaction to a final concentration of 18 nM. Experiment 4: $rG_o\alpha$ was preincubated with the same mixture of phospholipids at 4°C overnight and this protein was added to the reaction mixture to a final concentration of 56 nM. Protein concentrations are based on the amount of bound GTP[γS]. The final concentration of MP was 1 mM. Activities are expressed as molar turnover numbers (mol of phosphate × min^{-1} × mol of $protein^{-1}$).

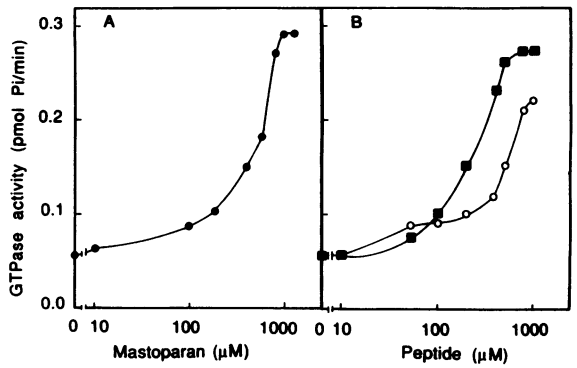

FIG. 3. Stimulation of the GTPase activity of $rG_o\alpha$. (*A*) MP. (*B*) Peptide 4 (○) or peptide 8 (■) was added to $rG_o\alpha$ preincubated at 4°C overnight in a mixture of phospholipids and subsequently the GTP hydrolysis was assayed. The final protein concentration was 58 nM, as determined by GTP[^{35}S] binding. The data represent the mean of duplicate measurements of three separate experiments.

together with bovine serum albumin, did not hydrolyze GTP at all.

Effect of MP Variants on GTP Hydrolysis. Nine peptides representing sequence permutations of MP (Table 3) were synthesized and purified by reverse-phase HPLC. Their effects on the GTPase activity of $rG_o\alpha$, reconstituted into phospholipid vesicles as described above, were examined. Peptides 4 and 8 stimulated GTP hydrolysis in a concentration-dependent manner (Fig. 3*B*), the former enhancing the GTPase activity nearly 4-fold and the latter enhancing activity nearly 5-fold at 1 mM. Peptide 8 showed 50% stimulation at about 250 μM, whereas the concentration of peptide 4 and MP needed for the same level of stimulation was 500 μM. In the absence of $rG_o\alpha$ there was no GTP hydrolysis above background level. The basal GTPase activity of the G protein was inhibited by peptides 1, 2, and 7 (Fig. 4). Peptide 2 was the most potent inhibitor, with an IC_{50} of 130 μM and >90% inhibition at 1 mM. The IC_{50} values for the less inhibitory ones, peptides 1 and 7, were 215 μM and 600 μM, respectively. At 1 mM the extent of inhibition of the latter peptides was 75% and 63%, respectively. Peptides 3, 5, 6, and 9 had a negligible effect or no effect on $rG_o\alpha$'s GTPase activity in the same concentration range—i.e., up to 1 mM.

DISCUSSION

There is convincing evidence that the cytoplasmic domains of multispanning receptors interact with G proteins (32). To investigate the rules governing this interaction we made use of MP and a series of its variants.

Activation of $rG_o\alpha$ by MP. The GTPase activity of $rG_o\alpha$ is strongly enhanced by MP when the G protein is reconstituted into phospholipid vesicles. The stimulatory effect is less pronounced in the presence of detergent (0.1% Lubrol). The

Table 3. MP and peptides representing sequence permutations

Peptide	Sequence
MP'	INLKALAALAKKIL
1	ALAIKLILNLKAKA
2	LKIALNLKALIAAK
3	NAALIAKLLKAKLI
4	INLAALKKLAAKIL
5	INLAKAALKALKIL
6	KILINLKALAALAK
7	LNAKLKAIALALIK
8	NILALAKALIKALK
9	NAKILALLALIKAK

Amino acids are indicated by the single-letter code.

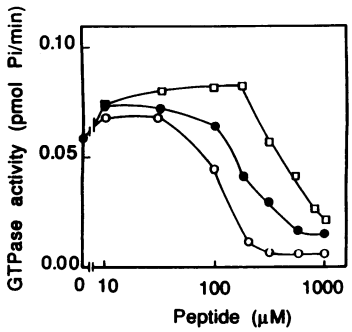

FIG. 4. Inhibition of the basal GTPase activity of $rG_o\alpha$. Peptide 1 (●), peptide 2 (○), or peptide 7 (□) was added to $rG_o\alpha$ as described in the legend to Fig. 3.

association with phospholipids seems to induce a protein conformation that facilitates activation by the peptide. However, the basal GTPase activity of $rG_o\alpha$ reconstituted into phospholipid vesicles is only detectable at higher protein concentrations (56 nM as compared to 18 nM in detergent). In the phospholipid vesicles the $rG_o\alpha$ possibly adopts a conformation with a lower affinity for MP than the heterotrimeric protein. This may partly explain the high concentration (1 mM) of peptide needed for maximum stimulation of GTP hydrolysis.

In a recent report (33) the cross-linking of a synthetic MP analogue to recombinant $G_o\alpha$ at Cys-3 is described. Furthermore, MP no longer activates this G protein when the N-terminal region (a 2-kDa fragment, ref. 34) is removed by tryptic proteolysis. These observations suggest that the N terminus of $G_o\alpha$ is part of the MP-binding site. Our results indicate that the N-terminal 11 amino acids of $G_o\alpha$ are not crucial for its activation by MP, since the recombinant protein, in which 5 different amino acids have been inserted instead, is still activated by MP, albeit at high peptide concentrations. It is likely that the N terminus of the G protein contributes to its affinity for MP.

MP Variants. Higashijima *et al.* (35) have tested a number of naturally occurring and synthetic (MP-related) peptides for their ability to activate G_o and G_i. In general, it appears that peptides with cationic amphiphilic properties stimulate the GDP release, or GTP hydrolysis through the GDP release, by these G proteins. To gain a more detailed insight into the specificity and the mode of action of MP, we permuted its sequence to produce peptides with altered amphiphilicity or no amphiphilicity at all. The sequences of MP and of the nine peptides that were synthesized are listed in Table 3.

Two of the synthetic peptides, peptide 4 and peptide 8, strongly enhance the GTPase activity of $rG_o\alpha$. Peptide 4 is quite similar to MP: K4 and A7 have been switched, as have A8 and K11. It is evident from the helical wheel projection that this peptide can form a cationic amphiphilic α-helix like the one formed by MP (Fig. 5). Peptide 8 is twice as efficient as peptide 4 and MP in stimulating the GTPase activity of $rG_o\alpha$ (in terms of the concentration needed for 50% stimulation). It has conserved amino acids in only four positions with respect to MP. However, the helical wheel projection reveals that the three positively charged side chains are clustered together on the left face (with respect to the N terminus) of the presumed α-helix; its amphiphilicity is obvious (Fig. 5).

Peptides 5 and 6, which in their helical wheel projections display a hydrophobic face on one side and a positively charged hydrophilic face on the other, do not activate $rG_o\alpha$. In peptide 5 the three lysines are clustered together on the right of the N terminus (Fig. 5). This may constitute a "wrong" orientation of the charged residues with respect to the N terminus, which renders the peptide inactive. Peptide

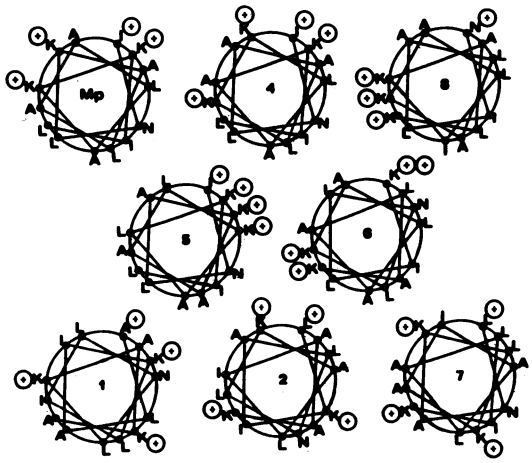

FIG. 5. Helical wheel diagrams of MP and peptides representing sequence permutations of MP. Upper row, from left to right: MP and peptides 4 and 8, which stimulate the $rG_o\alpha$'s GTPase activity. Middle row, from left to right: peptides 5 and 6, which have no effect on the GTPase activity of the protein. Bottom row, from left to right: peptides 1, 2, and 7, which inhibit the basal GTPase activity of the $rG_o\alpha$.

6 carries two positive charges at the N terminus (the N-terminal amino acid being a lysine), a fact that possibly accounts for its inertness in the GTP hydrolysis reaction. In the light of the results obtained by others (35), who found varying stimulatory responses with G_o and G_i for a large number of peptides, it is interesting that peptides 5 and 6 have absolutely no effect on the GTPase activity of our G protein. Hence these peptides are ideal controls and underscore the selective activation of $rG_o\alpha$ by peptides 4 and 8. Our results confirm that those tetradecapeptides, like MP, which are able to form cationic amphiphilic α-helices, can activate G proteins. This structural feature, however, is necessary but not in itself sufficient. The orientation of the positively charged amino acids relative to the N terminus also seems to play an important role.

In all other permuted MP variants the lysines and the N-terminal amino acid are not clustered together in such a way as to form a positively charged face on the putative α-helices. Two of these peptides (3 and 9) are inactive in the reaction catalyzed by the $rG_o\alpha$. Surprisingly though, three peptides (1, 2, and 7) almost completely inhibit the basal GTPase activity of the G protein. A common feature that distinguishes them from the other peptides tested in this study is the absence in their sequences of palindromes composed of at least three consecutive amino acids.

Attenuation of G Protein Activity. The attenuation of G protein activity may constitute a basic mechanism controlling cellular functions. The peptides we tested, which have an inhibitory effect on $rG_o\alpha$, bear no sequence homologies to intracellular regions of known G protein-coupled receptors. It is not certain, therefore, that their effect mimicks a physiological process. However, their specificity is remarkable, since four other peptides with an identical amino acid composition are completely ineffective.

The finding that certain tetradecapeptides representing sequence permutations of MP inhibit the GTPase activity of a recombinant $G_o\alpha$ *in vitro* is unexpected. It is possible that the various G protein subtypes respond differently to the same peptide (10). Further studies will form an important basis for the search of agents that can specifically block distinct signal transduction pathways at the G protein level.

Note Added in Proof. The inhibition of the GTPase activity of bovine brain $G_o\alpha$ (but also G_s and G_i from rabbit liver) by the 33-kDa protein

named phosducin has just been described (36). However there is no significant sequence homology between that protein and the peptides that inhibit GTP hydrolysis by our $rG_o\alpha$. On the other hand, we have found a sequence in the human neurofibromatosis related protein (NF1, a putative GTPase activating protein) that is highly homologous to (inhibitory) peptide 1. Nine of the peptide's 14 amino acids are identical in the noncatalytic N-terminal region of NF1 comprising amino acids 451–465 (37).

We express our gratitude to Dr. R. Reed (Johns Hopkins University, Baltimore) for kindly providing plasmid pGEM-2 containing the rat brain $G_o\alpha$ cDNA, Dr. S. Tabor (Harvard Medical School, Boston) for generously supplying plasmid pT7-7 and *E. coli* strain K38 pGP1-2, as well as Dr. G. Milligan (University of Glasgow, Glasgow, Scotland) for the magnanimous gift of the anti-$G_o\alpha$ antiserum. Our special thanks go to Dr. R. Matteoni for helping us with computer search programs and for many valuable discussions. This work was in part supported by the Consiglio Nazionale delle Ricerche, Progetto Finalizzato Ingegneria Genetica. C. O. and T. W. have contributed equally to this work.

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