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Palmitoylation but not the extreme amino-terminus of $G_{q\alpha}$ is required for coupling to the NK2 receptor

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Abstract $G_{q\alpha}$ and $G_{11\alpha}$ differ from other G protein α subunits in that they have unique, conserved 6 residue amino-terminal extensions. Wild-type and amino-terminal mutants of $G_{q\alpha}$ expressed in COS cells were analyzed for their ability to functionally couple with co-expressed neurokinin NK2 receptor. Wild-type, T2A and Δ2–7 $G_{q\alpha}$ were able to stimulate agonist driven phospholipase C (PLC) activity in identical manners. Other activities of these two amino-terminal mutants including aluminum fluoride stimulated PLC activity, palmitoylation, interaction with $G_{\beta\gamma}$ subunits and GTPγS-induced trypsin resistance are also similar to the wild-type α subunit. This demonstrates that the NK2 receptor is able to functionally interact with the α subunit of G_q and that the first seven amino-acids of $G_{q\alpha}$ are not required for any of the α subunit functions tested. In contrast to the T2A and Δ2–7 mutants, a C9,10A $G_{q\alpha}$ mutant was not able to couple to either the NK2 receptor or PLC, as assessed by high-affinity agonist binding and activation of PLC either in intact cells or in vitro. The C9,10A protein was able to assume a GTPγS-induced trypsin-resistant conformation and partitioned primarily to the pelletable fraction in a manner similar to the wild-type protein. However, it was not labeled with [^{3}H]palmitic acid. This suggests that blocking palmitoylation at the amino-terminus of $G_{q\alpha}$ results in a loss of functional activity which reflects an inability to interact with both the receptor and downstream signaling targets.

Key words: G protein; Palmitoylation; Neurokinin 2 receptor

1. Introduction

Amino-termini of the α subunits of heterotrimeric G proteins have been shown to be essential for both interaction with $G_{\beta\gamma}$ dimers and association with particulate cellular fractions [1–3]. The amino-termini of many, if not all, G protein α subunits also undergo lipid modification [4]. Palmitoylation of cysteine residues located close to the amino-termini of many G protein α subunits is thought to be important for interaction with receptors, effectors and membrane association [5–8]. It is a dynamic modification which can be regulated by G protein activation [9,10]. The amino-termini of both $G_{q\alpha}$ and $G_{11\alpha}$ differ from those of other G α subunits in that they contain a unique conserved six-amino acid extension. In this report we describe the effects of removing or modifying this extension and blocking palmitoylation on $G_{q\alpha}$ function.

2. Materials and methods

2.1. Plasmids and transfection

The genes for $G_{q\alpha}$, $G_{\beta 2}$ and $G_{\gamma 2}$, kindly provided by M. Simon, were amplified by PCR using primers which placed a consensus eukaryotic translation initiation sequence [11] at the 5′ end of the gene and cloned into pSG5 (Stratagene). The histidine-tagged G_β subunit, pSG5/$G_{\beta 2}$ 3′His, differs from the wild-type sequence by the addition of Asp(His)$_6$ to its carboxy-terminus. The NK2 receptor expression vector pCDL/NK2/Flag was derived from pCDL/NK2 [12] as described in [13]. Each of the mutants and all PCR amplified products were confirmed by DNA sequencing. COS-7 cells were transfected by electroporation.

2.2. Intact cell phospholipase assay

COS-7 cells were assayed for phospholipase activity using a protocol modified from P. Godfrey [14]. Cells at 72 h post-transfection were harvested with PBS, 1 mM EDTA, washed twice with PBS, and resuspended in inositol-free DMEM (Gibco BRL) containing 20 μCi/ml [5-^{3}H]cytidine (NEN) at a density of 1×10^6 cells per ml. Aliquots of 0.3 ml were placed at 37°C. After 1 h, 15 μl of 210 mM LiCl was added. After an additional 15 min at 37°C, 15 μl of the NK2 selective agonist GR64349 [15] in 10 mM acetic acid or vehicle was added. The cultures were held at 37°C for 30 min. Radiolabeled CDP-diacylglycerol (CMP-phosphatidic acid) was measured by extracting the cells in 940 μl methanol/chloroform/HCl (200:100:1) on ice for 20 min. Chloroform, 300 μl, and water, 300 μl, were added to each sample. The samples were then vortexed and centrifuged at $2{,}000 \times g$ for 15 min. From the lower organic phase, 0.4 ml was taken and added to 1 ml of 0.5 M HCl, 50% methanol. The samples were vortexed and spun at $2{,}000 \times g$ for 15 min. Again from the lower phase, 0.2 ml was taken and placed in a scintillation vial. The chloroform was allowed to evaporate and the samples counted. Each point was assayed in triplicate and each assay was independently repeated at least 3 times.

2.3. Cell fractionation, immunoblotting and immunoprecipitation

Approximately 2×10^7 transfected cells were resuspended in 1 ml of buffer G (50 mM HEPES, 100 mM NaCl, 3 mM $MgCl_2$, 1 mM EDTA, pH 7.5) supplemented with 2 mM PMSF, 1 μg/ml antipain, 5 μg/ml aprotinin and 1 μg/ml leupeptin. Cells were lysed by homogenization in a glass homogenizer. Lysates were centrifuged at $1{,}000 \times g$ for 5 min, then at $100{,}000 \times g$ for 30 min. The $100{,}000 \times g$ pellet was washed twice more in G buffer. Antisera 92A and 93A were raised against peptides equivalent to mouse $G_{q\alpha}$ amino acids 350–359 and 119–134, respectively. For metabolic labelling of $G_{q\alpha}$, 5×10^5 transfected cells were placed in 0.3 ml DMEM F12 containing either 100 μCi/ml [9,10-^{3}H]palmitic acid (NEN) or 100 μCi/ml L-[^{35}S]methionine (Amersham). After 4 h at 37°C, the cells were pelleted, resuspended in 1.0 ml RIPA buffer and immunoprecipitated using antisera 92A.

2.4. NK 2 receptor binding and activation of recombinant PLCβ1

For binding, 125–250 μg of membrane protein was diluted in buffer N (25 mM Tris, 100 mM NaCl, 3 mM $MnCl_2$, 0.2% BSA, pH 7.4) and incubated for 2 h at room temperature with 0.5–1.0 nM of the NK2 receptor antagonist radioligand [^{3}H]SR48968 (Amersham) [16] and NKA. GTPγS was included at 100 μM where indicated. Binding was terminated by rapid filtration through glass fibre filters (GF/B) presoaked with 0.5% (v/v) polyethylenimine followed by 3 washes of 4.0 ml with ice-cold buffer N. Bovine PLCβ1 produced in *Schizosaccharomyces pombe* (S. Arkinstall and K. Maundrell, unpublished) was assayed as described [17].

2.5. Interaction with $G_{\beta\gamma}$ subunits

Particulate fractions from approximately 2×10^7 cells were resuspended in 0.5 ml G buffer. G buffer, 0.5 ml, containing 2% luberol PX, was added and the fractions were incubated on ice for 1 h. After centrifugation at $100{,}000 \times g$ for 20 min, solubilized polyhistidine-

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tagged $G_{\beta 2}$ subunits were bound by the addition of 0.2 ml 50% Ni-NTA slurry (Qiagen) in G buffer plus 1% luberol PX to the resulting supernatant. The mixtures were incubated at room temperature for 30 min with gentle agitation and then loaded into a small column. The resin was washed with 3.0 ml G buffer, 1% luberol PX, followed by 1.0 ml of the same buffer plus 10 mM imidazole. Bound proteins were eluted with 1.0 ml G buffer, 1% luberol PX, 250 mM imidazole.

3. Results

3.1. Characterization of wild-type and mutant $G_{q\alpha}$ proteins

The predicted amino acid sequences of both $G_{q\alpha}$ and $G_{11\alpha}$ differ from those of most other G protein α subunits in that they contain a unique six-amino acid extension preceding the methionine codon used in the initiation of most other G proteins. Mutant $G_{q\alpha}$ genes encoding T2A, Δ2–7, C9,10A or Δ2–27 proteins were created to test the role of this extension, and also potential palmitoylation sites located at positions 9 and 10, in $G_{q\alpha}$ function. The first two mutants, T2A and Δ2–7, were used to test the role of the unique N-terminal extension. The T2A mutant was designed in analogy with a similar G2A mutant of $G_{s\alpha}$, which was shown to decrease the affinity of the $G_{s\alpha}$ subunit for $G_{\beta\gamma}$ and adenylylcyclase [18]. The third mutant, C9,10A, was designed to prevent addition of palmitic acid to the amino-terminal cysteine residues of $G_{q\alpha}$. The final mutant, Δ2–27, was designed to mimic a tryptically cleaved G_{α} subunit and to serve as a control for loss of amino-terminal function. Fig. 1 shows an immunoblot of soluble and particulate fractions from COS cells that were transiently expressing each of these α subunits. Each of the proteins can be seen to fractionate to both the particulate and soluble fractions.

Proteins from cells expressing the C9,10A mutant or other forms of $G_{q\alpha}$ were labelled in vivo with either [^{3}H]palmitic acid or [^{35}S]methionine and then immunoprecipitated with anti-$G_{q\alpha}$ antiserum 92A (data not shown). Wild-type, T2A and Δ2–7 $G_{q\alpha}$ were all labelled with palmitic acid. However, Δ2–7 $G_{q\alpha}$ was seen to be labelled with palmitic acid to a somewhat lesser extent than wild-type and T2A $G_{q\alpha}$ proteins. As expected [8], the C9,10A and Δ2–27 mutants failed to become labelled with palmitic acid.

3.2. Interaction of $G_{q\alpha}$ and $G_{q\alpha}$ mutants with the NK2 receptor

Fig. 2A shows that when the NK2 receptor is transiently expressed in COS cells it is able to stimulate production of

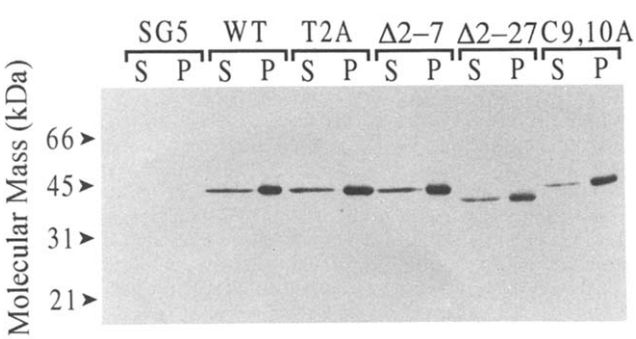

Fig. 1. Fractionation properties of $G_{q\alpha}$ subunits expressed in COS-7 cells. Equal numbers of COS-7 cells transfected with vectors encoding wild-type or mutant $G_{q\alpha}$ were lysed and separated into soluble (S) and particulate (P) fractions by centrifugation at 100,000 × g for 20 min. Equal proportions of the extracts were assayed for $G_{q\alpha}$ content by immunoblotting with anti-$G_{q\alpha}$ antiserum 93A. Similar results were obtained in six independent experiments.

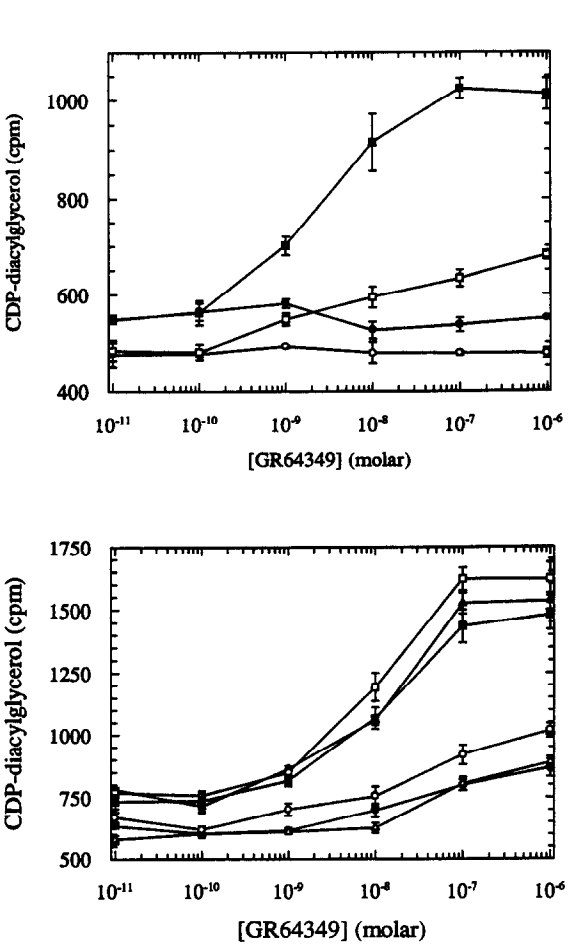

Fig. 2. Agonist-stimulated phospholipase activity by $G_{q\alpha}$ and $G_{q\alpha}$ mutants. Transfected COS-7 cells were assayed for their ability to produce CDP-diacylglycerol (CMP-phosphotidic acid) [14] in response to addition of increasing amounts of the NK2 selective agonist GR64349. (A) NK2 receptor (□), $G_{q\alpha}$ T2A (●), NK2 receptor plus $G_{q\alpha}$ T2A (■), vector without insert (○). (B) Wild-type $G_{q\alpha}$ (▲), $G_{q\alpha}$ T2A (□), $G_{q\alpha}$ Δ2–7 (■), $G_{q\alpha}$ C9,10A (●), $G_{q\alpha}$ Δ2–27 (△), vector without insert (○). Error bars represent standard errors of the mean from triplicate assays from a single experiment. Comparable results were obtained in three similar experiments.

[^{3}H]CDP-DAG, a measure of phospholipase C activation [14], when treated with the NK2-selective agonist GR64349 [15]. Cells transfected with plasmids encoding both the NK2 receptor and $G_{q\alpha}$ show a marked increase in agonist-dependant phospholipase activity. Excess GR100679, an NK2 selective antagonist [19], was able to block agonist-induced $G_{q\alpha}$-dependent phospholipase activity (data not shown). From this we conclude that $G_{q\alpha}$ is able to interact with the NK2 receptor to stimulate phospholipase activity in COS-7 cells.

As shown in Fig. 2B, addition of agonist to cells expressing the NK2 receptor and either the T2A or the Δ2–7 mutants stimulates CDP-diacylglycerol accumulation to the same extent as was observed for cells expressing the wild-type G_{α} subunit. However, the C9,10A and Δ2–27 mutants both fail to enhance agonist-stimulated phospholipase activity, indicating that the C9,10A and Δ2–27 proteins are unable to interact with receptor and/or effector in this assay. These results show that the first 26 residues of $G_{q\alpha}$ are required for normal protein function, as

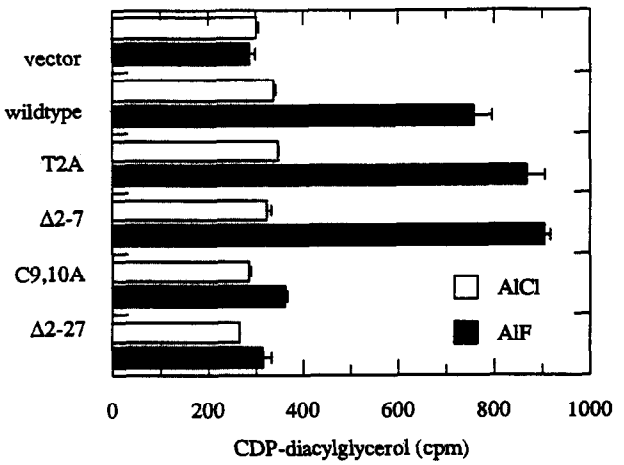

Fig. 3. AlF-induced stimulation of phospholipase activity by $G_{q\alpha}$ mutants. COS-7 cells transfected with vectors expressing G_q α subunits were assayed for their ability to produce CDP-diacylglycerol [14] in response to the addition of 2.5 μM $AlCl_3$ together with 2.5 mM NaF or NaCl. Error bars represent standard errors of the mean from triplicate assays from a single experiment. Comparable results were obtained in three similar experiments.

are cysteines and presumably palmitate modification at residues 9 and/or 10. In contrast, neither threonine-2 nor the first 6 amino acids of the protein are required for full activity.

3.3. *Aluminum fluoride activation of mutant G_q α subunits*

To localize the lesion in the transduction chain created by the C9,10A and Δ2–27 mutations, we have assayed receptor-independent phospholipase activity by direct activation using aluminum fluoride. As shown in Fig. 3, wild-type $G_{q\alpha}$ stimulated phospholipase activity in a manner similar to the T2A and Δ2–7 mutants, while the C9,10A and Δ2–27 mutants completely failed to stimulate [^{3}H]CDP-diacylglycerol accumulation over background. This pattern is nearly identical to that observed for receptor stimulated phospholipase activation. This suggests that the lesion in receptor-linked [^{3}H]CDP-DAG generation (Fig. 2) is due, at least in part, to a decrease in the ability of the C9,10A and Δ2–27 mutant G proteins to functionally interact with endogenous phospholipase(s).

3.4. *Detergent solubilization and interaction with $G_{\beta\gamma}$*

Because the first 18–28 amino acids of several different G_α subunits have been shown to be necessary for tight association with $G_{\beta\gamma}$ subunits and a G2A $G_{s\alpha}$ mutant has been shown to reduce $G_{\beta\gamma}$-dependant $G_{s\alpha}$ activity [18], we sought to test each $G_{q\alpha}$ mutant for their ability to interact with βγ subunits. We began by solubilizing the G α subunits from particulate fractions using detergents. Luberol PX (1%) was found to solubilize endogenous $G_{q\alpha}$ and most of the over-expressed wild-type, T2A and Δ2–7 α subunits. However, the amount of protein solubilized from particulate fractions of cells expressing C9,10A or Δ2–27 proteins was never greater than that extracted from cells transfected with vector alone. Similar results were obtained with 1% sodium cholate, Triton X-100, Triton X-114, or *n*-octylglucoside (data not shown). Interaction of G_α with βγ subunits was assayed by co-transfecting COS-7 cells with expression vectors encoding: wild-type, T2A or Δ2–7 G_α subunits, $G_{\gamma 2}$, and a $G_{\beta 2}$ protein which was modified to include an additional six carboxy-terminal histidine residues. Solubilized G protein α, β and γ subunits were loaded onto a Ni-NTA column, washed extensively, and then eluted with buffer containing 250 mM imidazole. Fig. 4 shows an immunoblot of unbound and eluted material from cells expressing $G_{q\alpha}$ alone, $G_{\beta 2}$-His and $G_{\gamma 2}$, or $G_{\beta 2}$-His, $G_{\gamma 2}$ plus each of the three active G_q α subunits. Wild-type, T2A and Δ2–7 $G_{q\alpha}$ can all be seen to elute from the column in a manner dependent on the presence of the poly-histidine-tagged $G_{\beta 2}$. This assay demonstrates that each of the three G_α subunits is capable of interacting with $G_{\beta\gamma}$ dimers containing $G_{\beta 2}$. Thus, the T2A and Δ2–7 mutations do not block association of $G_{q\alpha}$ with $G_{\beta\gamma}$ dimers.

3.5. *Pelletable C9,10A protein is not active*

The inability of the C9,10A and Δ2–27 proteins to activate phospholipase in intact cells (Figs. 2 and 3), together with the observation that they could not be solubilized by mild detergent extraction, suggests that they are not associated with the particulate fraction in the same manner as wild-type $G_{q\alpha}$. We further assayed the pelletable C9,10A protein for function using two in vitro assays: (i) formation of a GTPγS-sensitive high-affinity agonist-binding state in the NK2 receptor, and (ii) in vitro activation of recombinant PLCβ1. Fig. 5A shows that a clear increase in high affinity neurokinin A binding is detectable at low agonist concentrations in particulate fractions prepared from cells co-expressing the NK2 receptor and T2A $G_{q\alpha}$. No such change was observed with the C9,10A mutant or control cells transfected with the vector alone. Importantly, increased high affinity agonist binding with T2A $G_{q\alpha}$ was abolished in the presence of GTPγS. Fig. 5B shows that while T2A $G_{q\alpha}$ (used here instead of wild-type) in COS cell particulate fractions activated PLCβ1 following preincubation with GTPγS, particulate fractions containing the C9,10A mutant were totally inactive under identical conditions. Together, these data show that the pelletable C9,10A protein is unable to functionally interact with receptor or effector.

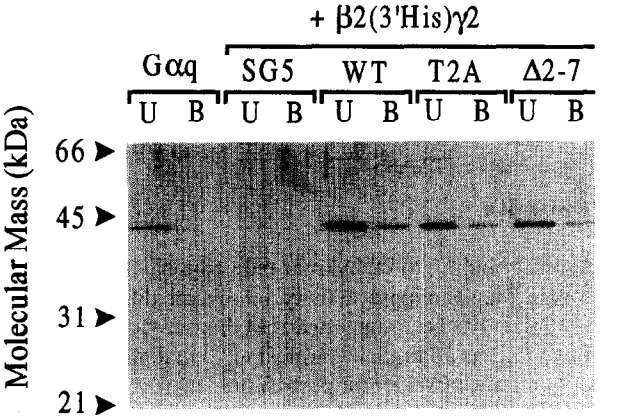

Fig. 4. Interaction of G_q α subunits with $G_{\beta 2}$-His_6. Luberol extracts from particulate fractions of COS-7 cells expressing $G_{q\alpha}$ and/or $G_{\gamma 2}$ plus a $G_{\beta 2}$ modified to contain a carboxy-terminal extension of six histidine residues, were mixed with Ni-NTA agarose and loaded into a small column. Flow-through was collected, the column was washed with 10 vols. of buffer followed by an additional 5 vols. of buffer with 10 mM imidazole, and then eluted with buffer containing 250 mM imidazole. Equal amounts of flow-through (U) and eluted (B) material were analysed for $G_{q\alpha}$ content by immunoblotting anti-$G_{q\alpha}$ antiserum 93A. Data shown is representative of three independent experiments.

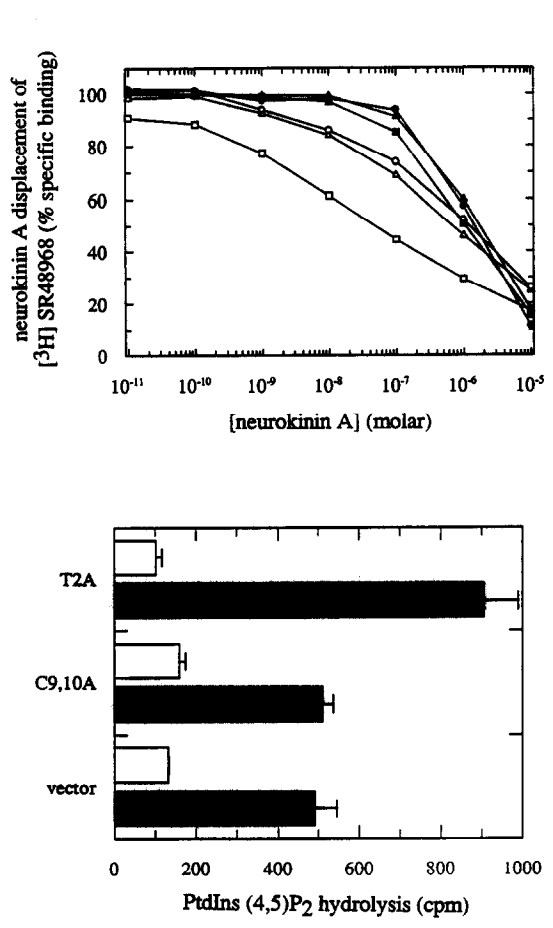

Fig. 5. Interaction of NK2 receptor with $G_{q\alpha}$ in the particulate fraction. Particulate fractions from transfected COS-7 cells were assayed for (A) GTPγS-induced changes in agonist affinity or (B) GTPγS-stimulated activation of PLCβ1. (A) NKA competition with [^{3}H]SR48968 for binding to NK2 receptor in the presence (filled symbols) and absence (open symbols) of 100 μM GTPγS. $G_{q\alpha}$ T2A (squares), $G_{q\alpha}$ C9,10A (circles), vector (triangles). (B) In vitro activation of recombinant PLCβ1. Particulate fractions preincubated with (filled bars) or without (open bars) 100 μM GTPγS were assayed for their ability to activate PLCβ1 in crude *S. pombe* extracts. Data points are means from triplicate measurements at each point. Data shown are from a single experiment which is representative of two (antagonist displacement) or three (PLC activation) independent experiments.

4. Discussion

Agonist binding to tachykinin receptors has been shown to stimulate phospholipase activity in several different systems [12,20,21]. It has been suggested that this activity is transduced through $G_{q\alpha}$, as addition of purified $G_{q/11}$ to a partially purified NK1 (substance P) receptor preparation augments high affinity agonist binding [22]. In this report we have shown that $G_{q\alpha}$ is capable of coupling to the NK2 receptor when the two proteins are co-expressed in COS cells.

The $G_{q\alpha}$ and $G_{11\alpha}$ genes are predicted to encode proteins with amino-terminal extensions of 6 amino acids relative to the amino-termini of most other G protein α subunits. This extension is predicted to occur in mouse and *Xenopus* $G_{q\alpha}$ subunits, and also in mouse, human, cow and turkey $G_{11\alpha}$ subunits. We have assayed T2A and Δ2–7 $G_{q\alpha}$ for their ability to transduce signals from the NK2 receptor, to directly stimulate PLC, to interact with $G_{\beta\gamma}$ dimers, and to become palmitoylated. These amino acids are not required for any of these functions. The only difference we have detected is a decrease in the palmitoylation of the Δ2–7 mutant. This could reflect a reduced affinity for the putative palmitoyl transferase, an increase in nucleotide exchange rate [9,10], or a reduction in the amount of properly folded material.

Wedegaertner et al. [8], working in HEK cells, have published an elegant series of experiments which demonstrate that lipidation of $G_{q\alpha}$ is required for signal transduction via the α2-adrenoceptor and activation of PLC. Their results differ from ours primarily in that non-palmitoylated $G_{q\alpha}$ was not pelletable in their system. Such a result is consistent with the demonstrated role of palmitoylation in membrane association of H-ras and GAP-43 [23,24]. Despite this, we and others [6,7,25] have not found significant differences in the fractionation properties of either palmitoylation-defective or amino-terminally truncated mutant α subunits compared to the wild-type protein when expressed in COS cells. The pelletable, non-palmitoylated $G_{q\alpha}$ is unable to interact with either the NK2 receptor or PLCβ1 (Fig. 5), nor could it be solubilized by detergent extraction (data not shown). This suggests that the pelletable protein may be present in an inactive, possibly aggregated, form. However, immunoblots of soluble $G_{q\alpha}$ from cells expressing C9,10A and Δ2–27 proteins show that both are able to attain GTPγS-dependant trypsin-resistant conformations (data not shown), indicating that some of the soluble protein is properly folded. Thus, we can not rule out the possibility that the protein is associated with some other cellular component, such as the cytoskeleton, in such a manner as to render it unable to stimulate phospholipase activity or interact with the receptor.

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References

[1] Conklin, B.R. and Bourne, H.R. (1993) Cell 73, 631–641.
[2] Navon, S.E. and Fung, B.K.-K. (1988) J. Biol. Chem. 263, 489–496.
[3] Denker, B.M., Neer, E.J. and Schmidt, C.J. (1992) J. Biol. Chem. 267, 6272–6277.
[4] Casey, P.J. (1994) Curr. Opin. Cell Biol. 6, 219–225.
[5] Linder, M.E., Middleton, P., Hepler, J.R., Taussif, R., Gilman, A.G. and Mumby, S.M. (1993) Proc. Natl. Acad. Sci. USA 90, 3675–3679.
[6] Parenti, M., Vigano, M.A., Newman, C.M.H., Milligan, G. and Magee, A.I. (1993) Biochem. J. 291, 349–353.
[7] Degtyarev, M.Y., Spiegel, A.M. and Jones, T.L.Z. (1993) Biochem. 32, 8057–8061.
[8] Wedegaertner, P.B., Chu, D.H., Wilson, P.T., Levis, M.J. and Bourne, H.R. (1993) J. Biol. Chem. 268, 25001–25008.
[9] Degtyarev, M.Y., Spiegel, A.M. and Jones, T.L.Z. (1993) J. Biol. Chem. 268, 23769–23772.
[10] Wedegaertner, P.B. and Bourne, H.R. (1994) Cell 77, 1063–1070.
[11] Kozak, M. (1987) Nucleic Acids Res. 15, 8125–8148.
[12] Arkinstall, S., Emergy, I., Church, D., Chollet, A. and Kawashima, E. (1994) FEBS Lett. 338, 75–80.

[13] Guan, X.-M., Kobilka, T.S. and Kobilka, B.K. (1992) J. Biol. Chem. 267, 21995–21998.
[14] Godfrey, P.P. (1989) Biochem. J. 258, 621–624.
[15] Hagan, R.M., Ireland, S.J., Jordan, C.C., Beresford, J.M., Deal, M.J. and Ward, P. (1991) Neuropeptides 19, 127–135.
[16] Advenier, C., Rouissi, N., Nguyen, Q.T., Emonds-Alt, X., Breliere, J.-C., Neliat, G., Naline, E. and Regoli, D. (1992) Biochem. Biophys. Res. Commun. 184, 1418–1424.
[17] Wu, D., Lee, C.H., Rhee, S.G. and Simon, M.I. (1992) J. Biol. Chem. 267, 1811–1817.
[18] van der Neut, R., Pantaloni, C., Nebout, I., Bockaert, J. and Audigier, Y. (1993) J. Biol. Chem. 268, 436–441.
[19] Smith, P.W., McElroy, A.B., Pritchard, J.M., Deal, M.J., Ewan, G.B., Hagen, R.M., Ireland, S.J., Ball, D., Beresford, I., Sheldrick, R., Jordan, C.C. and Ward, P. (1993) Bioorg. Med. Chem. Lett. 3, 931–936.
[20] Gerard, N.P., Bao, L., He, X.-P. and Gerard, C. (1993) Reg. Peptides 43, 21–35.
[21] Eistetter, H.R., Church, D.J., Mills, A., Godfrey, P.P., Capponi, A.M., Brewster, R., Schulz, M.-F., Kawashima, E. and Arkinstall, S.J. (1991) Cell Reg. 2, 767–779.
[22] Kwatra, M.M., Schwinn, D.A., Schreurs, J., Blank, J.L., Kim, C.M., Benovic, J.L., Krausse, J.E., Caron, M.G. and Lefkowitz, R.J. (1993) J. Biol. Chem. 268, 9161–9164.
[23] Liu, Y., Fischer, D.A. and Storm, D.R. (1993) Biochem. 32, 10714–10719.
[24] Hancock, J.F., Paterson, H. and Marshall, C.J. (1990) Cell 63, 133–139.
[25] Juhnn, Y.-S., Jones, T.L.Z. and Spiegel, A.M. (1992) J. Cell Biol. 119, 523–530.