

Two α subunits of the G_q class of G proteins stimulate phosphoinositide phospholipase C- $\beta 1$ activity

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Two G protein α subunits were detected in preparations of GTP γ S-dependent, phosphoinositide-specific phospholipase C-activating proteins from bovine liver membranes. Partial resolution of the two α subunits, of molecular mass 42 and 43 kDa, was achieved by Mono Q chromatography. Quantitation of the levels of each α subunit and reconstitution assays demonstrated that each possessed stimulatory activity towards the $\beta 1$ isozyme of phospholipase C. Immunoblot analysis showed that the 42 kDa protein was immunologically related to α_q , whereas the 43 kDa protein was related to α_{11} , another member of the G_q class. The data thus show that two different α subunits of the G_q class of G proteins stimulate phospholipase C- $\beta 1$ Activity.

G-protein; Phosphoinositide; Phospholipase C; G_q

1. INTRODUCTION

Biochemical and molecular cloning studies have revealed the existence of multiple isozymes of phosphoinositide-specific phospholipase C (PLC) [1,2] and at least two distinct mechanisms of receptor-mediated activation of this enzyme. The receptors for epidermal and platelet-derived growth factors form complexes with, and phosphorylate on tyrosine residues, the $\gamma 1$ isozyme of PLC in a ligand-dependent manner [3–5]. Translocation of PLC- $\gamma 1$ from cytosol to plasma membranes [6], relief of inhibitory constraint exerted by profilin [7], or activation of intrinsic catalytic activity [8] may account for growth factor-elicited Ca^{2+} mobilization and protein kinase C activation. A larger group of agonists, including several hormones, neurotransmitters, mitogens and autocrine agents, stimulate PLC activity via G protein-coupled pathways [9].

G protein-mediated PLC activation is either sensitive or insensitive to inhibition by pertussis toxin (PTX), indicating the existence of multiple stimulatory G proteins that are expressed in a tissue-specific manner. G_{α_o} subunits, which activate PLC in *Xenopus* oocytes [10], represent potential PTX-sensitive PLC-stimulatory α

subunits. The G_q class of α subunits [11] appears to be involved in PTX-insensitive PLC regulation, since activated α_q subunits stimulate PLC activity in vitro [12–14]. The $\beta 1$ isozyme of PLC is apparently the effector protein of this signal transduction pathway [12]. In this report we show that two α subunits of the G_q class activate PLC- $\beta 1$.

2. MATERIALS AND METHODS

2.1. Resolution of G protein α subunits

PLC-activating G protein α subunits (GPA) were purified through octyl Sepharose as previously described [12,15]. The leading half of the peak of GPA activity from octyl Sepharose was pooled, concentrated to 3 ml and diluted 1:4 in buffer C (25 mM HEPES, pH 7.25, 5 mM $MgCl_2$, 1 mM EGTA, 1% octyl glucoside, 0.5 mM dithiothreitol, 10 μ g/ml aprotinin, 10 μ g/ml leupeptin). The diluted pool was loaded onto a Mono Q HR 5/5 column equilibrated with buffer C containing 50 mM NaCl at a flow rate of 0.8 ml/min. The column was washed with buffer C containing 80 mM NaCl and then developed with a 24 ml gradient of 80–316 mM NaCl. Fractions of 0.6 ml were collected. GPA activity was divided into 2 pools representing the leading (pool A) and trailing (pool B) edges of the peak of activity. Each pool was subjected to PTX treatment [15] to eliminate G_{α_i} contamination. Briefly, Mono Q pools were diluted 1:1 into PTX buffer to give final concentrations of 25 mM HEPES, pH 7.5, 100 μ M ATP, 10 μ M GTP γ S, 2 mM NAD^+ , 10 μ g/ml leupeptin, 10 μ g/ml aprotinin and 10 μ g/ml dithiothreitol-activated PTX. Incubation was at 30°C for 20 min. Following dilution with an equal volume of buffer C, each PTX-treated pool was re-chromatographed on Mono Q under the above conditions. The peak of GPA activity from Mono Q(A) was symmetrically divided into 2 pools (A1: fractions 8–12 and A2: fractions 13–17) and the peak from Mono Q(B) was pooled in its entirety (B: fractions 11–15). Each pool was concentrated, using Centricon concentrators, to 0.3–0.4 ml.

2.2. Antisera

Antisera WO82, WO83, and X384, raised against peptides cor-

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Abbreviations: G protein, guanine nucleotide binding regulatory protein; PLC, phosphoinositide-specific phospholipase C; GPA, G protein phospholipase C activator; PTX, pertussis toxin; GTP γ S, guanosine 5'- γ -[3-thiotriphosphate]; HEPES, 4-[2-hydroxyethyl]-1-piperazineethane sulphonic acid.

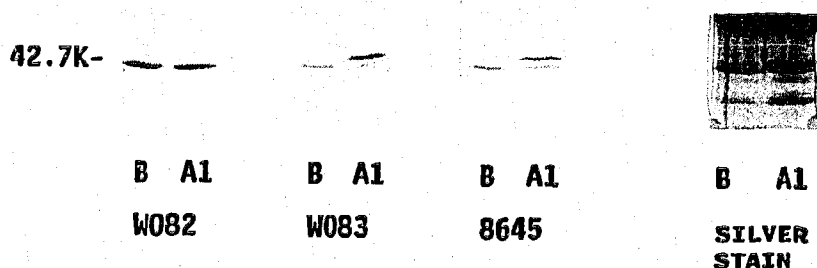


Fig. 1. Silver-stained gel and immunoblot analysis of partially resolved GPA-42 and GPA-43. 8 μ l aliquots of Mono Q pools A1 and B were subjected to SDS-PAGE (13% acrylamide) and immunoblotted against 1/500 dilutions of the indicated antisera or silver stained as previously described [15].

responding to amino acids 115-133, 283-300 and 348-359 of α_q [13,16], respectively, were provided by Dr P.C. Sternweis, University of Texas, Southwestern Medical Center, Dallas, TX. Antiserum 8645 [17] was provided by Dr D.R. Manning, University of Pennsylvania, Philadelphia, PA. Antiserum 588 was generated and affinity-purified as previously described [15]. Antiserum E976 was raised against a peptide corresponding to amino acids 160-172 of α_{11} [11].

2.3. Miscellaneous

PLC activity was assayed as described previously using [3 H]phosphatidylinositol 4,5-bisphosphate as substrate [12,15]. Electrophoresis and immunoblotting were performed as in [15]. Relative quantitation of GPA-42 and GPA-43 was achieved by scanning laser densitometry of a silver-stained gel using an LKB 2202 Ultrosan coupled to a Varian 650 data analysis system. The β_1 isozyme of PLC, purified from bovine brain cytosol [18], was kindly provided by Dr S.G. Rhee, National Institutes of Health, Bethesda, MD. The sources of all other materials have been previously detailed [15].

3. RESULTS

The yield of the 42 kDa and 43 kDa protein α subunit PLC activators (GPA-42 and GPA-43) from bovine liver membranes [15] was increased by the inclusion of the Ca^{2+} -mobilizing agonist 8-arginine vasopressin during GTP γ S treatment (data not shown). Presumably, this occurred because of greater binding of the nucleotide and consequent dissociation of the G proteins. Although the two α subunits co-chromatographed extensively during their purification, substantial resolution was obtained by selective pooling and successive Mono Q chromatographic runs (see section 2). This is shown by the fact that polyacrylamide gel electrophoresis followed by silver staining of pools A1 and B from Mono Q revealed that pool A1 was enriched in the 43 kDa α subunit (GPA-43) while pool B consisted primarily of GPA-42 (Fig. 1 and Table I). Immunoblot analysis of these two pools (Figs. 1 and 2) demonstrated immunoreactivity of GPA-42 with antisera WO82, WO83, X384 raised against peptides corresponding to sequences within α_q [11,13,16] and with antiserum 8645 [17], directed against the most homologous region of α subunits. GPA-43 was specifically recognized by antiserum E976, raised to a unique sequence in α_{11} , and by antisera 8645, X384 and WO83, but not by WO82 (Figs. 1 and 2). Neither α

subunit was detected by antiserum 588 (' α common' antiserum) (not shown).

Both pools A1 and B from Mono Q chromatography were capable of markedly stimulating the activity of purified PLC- β_1 (Fig. 3). However, as noted above, their contents of GPA-42 and GPA-43 were very different (Table I) making it unlikely that only one of the proteins has PLC-stimulatory activity. Furthermore, it can be seen from Fig. 3, that the volume of pool A1 causing half-maximal activation of PLC- β_1 was approximately half that of pool B. Since the total concentration of GPA-42 plus GPA-43 in pool A1 was about 2-fold that in pool B (Table I), these data confirm the view that both GPA-42 and GPA-43 possess PLC-stimulatory activity, and suggest that they are approximately equipotent.

4. DISCUSSION

The present finding that two related G protein α subunits are capable of modulating the activity of a single effector protein, in this case PLC- β_1 , is not without precedent. All three forms of $\text{G}\alpha_i$ stimulate K^+ channel activity [19], while at least three of the four possible splice variants of $\text{G}\alpha_s$ activate both Ca^{2+} channels and adenylate cyclase [20]. The exact relationship between the two GPA α subunits described here remains unknown since sequence data are not available. It is clear, however, that both are immunologically

Table I
Concentrations of GPA-42 and GPA-43 in Pools from Mono Q chromatography

		Peak Area ^a (Arbitrary Units)
Pool A1	GPA-42	11.2
	GPA-43	36.1
	Total	47.3
Pool B	GPA-42	20.7
	GPA-43	3.8
	Total	24.5

^a The 42 and 43 kDa bands of the silver-stained gel shown in Fig. 1 were quantitated by scanning laser densitometry. Results are expressed as arbitrary units of absorbance.

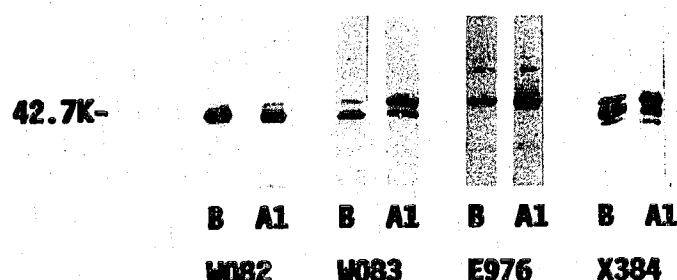


Fig. 2. Immunoblot analysis of partially resolved GPA-42 and GPA-43. 8 μ l aliquots of Mono Q pools A1 and B were subjected to SDS-PAGE (13% acrylamide) and immunoblotted against 1/1000 dilutions of the antisera WO82, WO83 and X384 and 1/200 dilution of E976, as described previously [15].

related, and belong to the G_q class of α subunits [11]. Since the antisera used to identify the GPA α subunits were generated against peptides derived from α_q or α_{11} sequences [11,16], it is possible that GPA-42 represents α_q and GPA-43 represents α_{11} . Both α_q and α_{11} are widely expressed and present in mammalian liver [11], while other members of this class appear to possess a more restricted tissue distribution [21]. If GPA-42 and GPA-43 do indeed represent α_q and α_{11} , it is perhaps not surprising that both stimulate PLC- β 1 activity, since these subunits are virtually identical in their C-terminal region, a domain implicated in effector (and receptor) interaction [22].

The results presented here are supported by other observations. First, purification of heterotrimeric G proteins from bovine liver which stimulate PLC activity in a GTP γ S-dependent manner, results in the purification of two α subunits, with associated $\beta\gamma$ subunits, of identical M_r and immunologic reactivity to GPA-42 and GPA-43 [14]. Second, the Ca^{2+} -mobilizing agonist vasopressin stimulates labelling, with a photoreactive GTP analogue, of two α subunits of 42 and 43 kDa which are immunoprecipitated by an antiserum (X384) raised against the common C-terminal dodecapeptide of α_q and α_{11} [23]. Since both these α subunits also couple to other receptors for Ca^{2+} -mobilizing agonists [23] and both apparently activate PLC- β 1, one may question why more than one G protein α subunit is involved in the same receptor-effector coupling pathway. It is possible that GPA-42 and GPA-43 possess different GTP binding or hydrolyzing activities, or that they differentially interact with $\beta\gamma$ subunits or other regulatory components. In any case, it will be of interest to determine whether other α subunits of the G_q class are involved in PLC regulation, perhaps in a tissue- or isozyme-specific manner.

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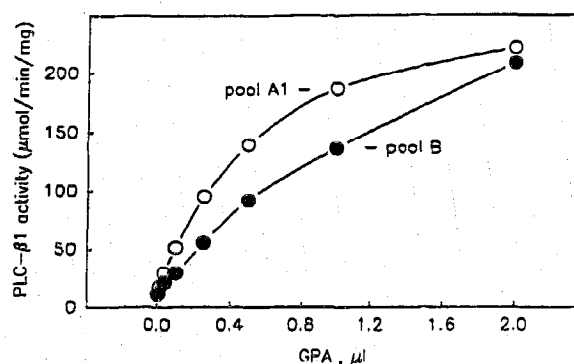


Fig. 3. Activation of PLC- β 1 by partially resolved GPA-42 and GPA-43. The indicated volumes of pools A1 and B (diluted in Mono Q column buffer) were tested for GPA activity against 2 ng of PLC- β 1, as previously described [12]. The results are means of two assays.

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