Volume 286, number 1,2, 214–216 FEBS 09948 July 1991

ADONIS 0014579391006574

Two α subunits of the G_q class of G proteins stimulate phosphoinositide phospholipase C-β1 activity

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Received 7 May 1991

Two G protein α subunits were detected in preparations of GTPγS-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 β1 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-β1 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 γ1 isozyme of PLC in a ligand-dependent manner [3–5]. Translocation of PLC-γ1 from cytosol to plasma membranes [6], relief of inhibitory constraint exterted 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. $G\alpha_o$ 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 β1 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-β1.

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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; GTPγS, guanosine 5'-*o*-[3-thiotriphosphate]; HEPES, 4-[2-hydroxyethyl]-1-piperazineethane sulphonic acid.

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 μg/ml aprotinin, 10 μg/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 $G\alpha_i$ contamination. Briefly, Mono Q pools were diluted 1:1 into PTX buffer to give final concentrations of 25 mM HEPES, pH 7.5, 100 μM ATP, 10 μM GTPγS, 2 mM NAD^+, 10 μg/ml leupeptin, 10 μg/ml aprotinin and 10 μg/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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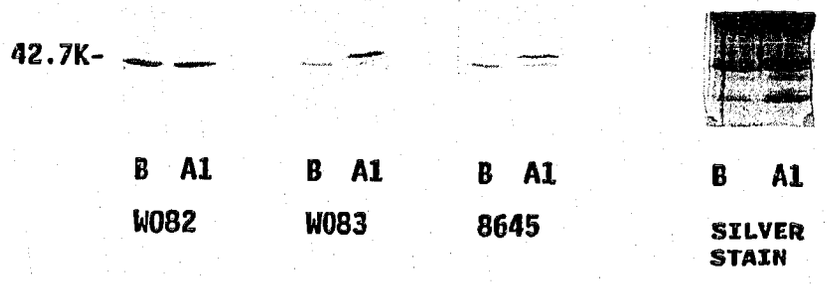

Fig. 1. Silver-stained gel and immunoblot analysis of partially resolved GPA-42 and GPA-43. 8 μl aliquots of Mono Q pools A1 and B were subjected to SDS-PAGE (13% acrylamide) and immunoblotted against 1/500 dilutions of the indicated antisera or silver stained as previously described [15].

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 Ultroscan coupled to a Varian 650 data analysis system. The β1 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 GTPγS 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-β1 (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-β1 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-β1, is not without precedent. All three forms of $G\alpha_i$ stimulate K^+ channel activity [19], while at least three of the four possible splice variants of $G\alpha_s$ 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 aribitrary units of absorbance.

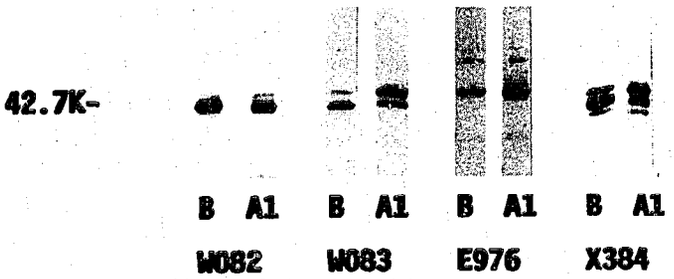

Fig. 2. Immunoblot analysis of partially resolved GPA-42 and GPA-43. 8 μl aliquots of Mono Q pools A1 and B were subjected to SDS-PAGE (13% acrylamide) and immunoblotted against 1/1000 dilutions of the antisera WO82, WO83 and X384 and 1/200 dilution of E976, as described previously [15].

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-β1 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 GTPγS-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-β1, 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.

Acknowledgements: We thank Ron Wange for performing the densitometric analysis and Annette Ross for performing the immunoblot analysis shown in Fig. 2. We are grateful to Drs. Sue Goo Rhee for providing PLC-β1, Paul Sternweis for supplying antisera WO82, WO83, and X384, and David Manning for making available antiserum 8645. Special thanks go to Judy Childs for preparation of the manuscript.

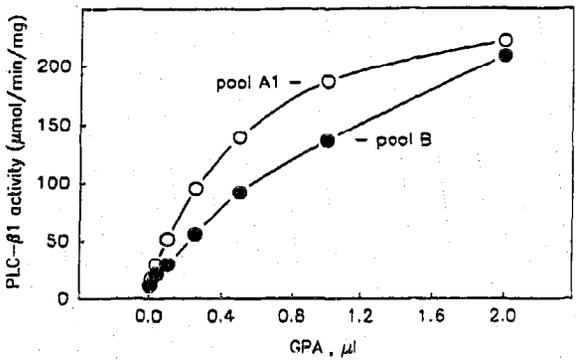

Fig. 3. Activation of PLC-β1 by partially resolved GPA-42 and GPA-43. The indicated volumes of pools A1 and B (diluted in Mono Q column buffer) were tested for GPA activity against 2 ng of PLC-β1, as previously described [12]. The results are means of two assays.

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