Volume 287, number 1,2, 171–174 FEBS 10010 August 1991

ADONIS 0014579391007178

Widespread distribution of Gqα/G11α detected immunologically by an antipeptide antiserum directed against the predicted C-terminal decapeptide

Fiona M. Mitchell[1], Ian Mullaney[1], Philip P. Godfrey[3], Steve J. Arkinstall[3], Michael J.O. Wakelam[1] and Graeme Milligan[1,2]

Molecular Pharmacology Group, Departments of [1] Biochemistry and [2] Pharmacology, University of Glasgow, Glasgow, G12 8QQ, Scotland, UK and *[3] Department of Biological Chemistry, Glaxo Institute for Molecular Biology, Chemin des Aulx, 1228 Plan-les-Ouates, Geneva, Switzerland*

Received 28 May 1991

Antisera were raised to a synthetic peptide which represents the predicted C-terminal decapeptide of the α subunit of the G-proteins Gq and G11. Competitive ELISA indicated that antiserum CQ2 displayed strong reactivity against this peptide. Antiserum CQ2 identified an apparently single polypeptide of 42 kDa which was expressed widely. The mobility of this polypeptide in SDS-PAGE was not modified by pretreatment of cells with pertussis toxin, indicating that it was not a substrate for this toxin. Furthermore, the levels and mobility of this polypeptide were unaltered by treatment of cells with cholera toxin, defining that it was not related to $G_s\alpha$.

G-protein; Inositol phosphate; Transmembrane signalling

1. INTRODUCTION

Whilst the molecular identity of the G-proteins regulating both receptor-mediated stimulation (G_s) and inhibition (G_i2) of adenylyl cyclase are now firmly established it has been more difficult to identify positively the G-protein(s) responsible for receptor-linked phosphoinositide hydrolysis. The identification of G-proteins regulating adenylyl cyclase activity was promoted initially by the disruptive effect that both cholera and pertussis toxins had on receptor regulation of cyclic AMP generation [1] and subsequently confirmed by the production of antisera directed against the two G-proteins which were able to interfere with the interactions of these G-proteins and relevant receptors [2–5]. It was clear that receptors which cause an increase in the rate of production of water soluble inositol phosphates do so via a G-protein because of the effects both of poorly hydrolysed analogues of GTP on the affinity of agonist binding to such receptors, and of the synergistic effects of guanine nucleotides and receptor agonists on inositol phosphate production in permeabilized cells [6]. However, as both cholera and pertussis toxins have little or no effect on this process in the vast majority of cells and tissues alternate strategies have been required to identify this G-protein(s). The recent isolation of cDNA's corresponding to novel G-protein α subunits with unknown function and which do not have the sequence characteristics required for modification by pertussis toxin-catalysed ADP-ribosylation has provided a new impetus for studies in this field [7]. In this paper we report the generation and characteristics of an antiserum which shows high selectivity for the C-terminal decapeptide sequence of Gq/G11. Reconstitutive studies with G-protein containing fractions from either brain or liver have indicated that this G-protein(s) can interact with and activate phospholipase C β1 [8,9].

Correspondence address: G. Milligan, Department of Biochemistry, University of Glasgow, Glasgow, G12 8QQ, Scotland, UK. Fax: (44) (41) 330 4620.

2. MATERIALS AND METHODS

2.1. *Antisera*

Antiserum CQ2 was generated in a New Zealand White rabbit which was immunized with a conjugate of a synthetic peptide QLNLKEYNLV-COOH and keyhole limpet haemocyanin (KLH) (Calbiochem). An additional cysteine, to assist coupling of the peptide to the protein, was placed at the N-terminal sequence of the peptide, which is equivalent to the C-terminal decapeptide predicted for both Gq and G11. Coupling was achieved subsequent to the activation of KLH by treatment with *m*-maleimidobenzoylsulfo-succinimide ester (Pierce and Warringer, Chester, UK). Antiserum SG1 which identifies the C-terminal decapeptide of $G_i1\alpha$ and $G_i2\alpha$ and antiserum CS1 which identifies the C-terminal decapeptide of forms of $G_s\alpha$ have previously been described [10,11].

Synthetic peptides corresponding to the C-terminal decapeptides of G_i1/G_i2, G_i3, G_o and G_z which were used to assess potential cross-reaction of antiserum CQ2 with these G-proteins are lsited in Table I and were obtained either from Biomac (Glasgow, Scotland) or as gifts from Dr C.G. Unson, Department of Biochemistry, Rockefeller University, New York, NY, USA.

Table I

The C-terminal decapeptides of the α subunits of a range of G-proteins

Gq/G11	QLNLKEYNLV-COOH
Gz	QNNLKYIGLC-COOH
G_i1	KNNLKDCGLF-COOH
G_i2	KNNLKDCGLF-COOH
G_i3	KNNLKECGLY-COOH
G_o	ANNLRGCGLY-COOH

The above peptides, with in the case of Gq/G11 an additional N-terminal cysteine were used in competitive ELISA's with antiserum CQ2. (see Fig. 1 for results). Analysis of the full length predicted amino acid sequences of the various G-proteins identified no other peptides in the primary amino acid sequences likely to cross-react with antiserum CQ2.

2.2. *Immunoblotting*

Was performed as in [5] using a horseradish peroxidase (HRP)-linked donkey anti-rabbit IgG fraction (Scottish Antibody Production Unit, Carluke, Scotland) as the secondary antiserum. Visualization of the immunologically detected polypeptide was achieved using *o*-dianisidine (Sigma) as substrate for HRP.

2.3. *ELISA*

The reactivity of antiserum CQ2 against 1 μg of the various synthetic peptides was assessed by using *o*-phenylenediamide dihydrochloride as substrate for HRP-linked secondary antibody. Colour production was measured at 492 nm on a Titertek Multiscan.

2.4. *Cells*

Neuroblastoma × glioma hybrid NG108-15, Glioma C6 BU1, Rat 1 fibroblast and human monocytic U937 cells were grown in tissue culture as described previously [12–14]. In some cases cells were treated with either pertussis toxin (25 ng/ml, 16 h) or cholera toxin (1 μg/ml, 16 h) prior to harvest of the cells. Membranes from these cells and from rat cerebral cortex were prepared as in [12].

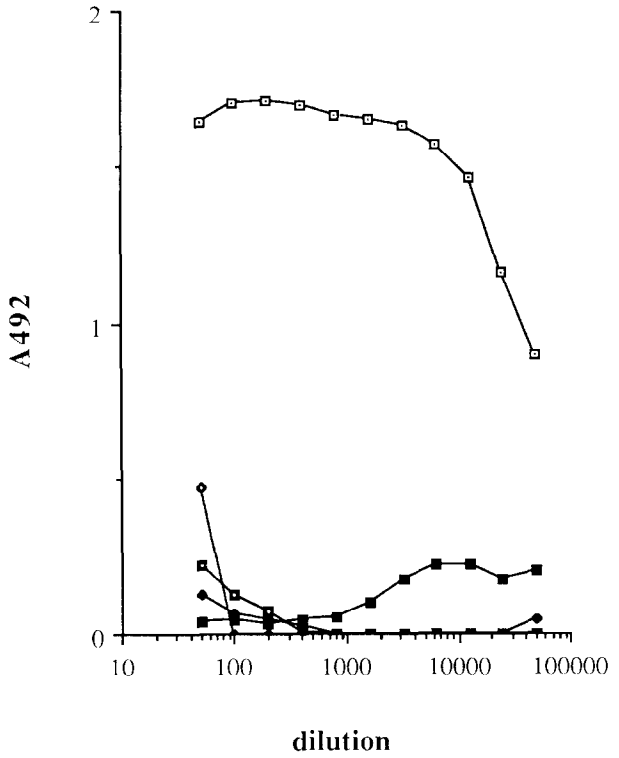

Fig. 1. ELISA assay of the reactivity of antiserum CQ2 against peptides which correspond to the C-terminal decapeptides of the α subunits of a range of G-proteins. ELISA assays were performed using varying dilutions of antiserum CQ2 and the peptides listed in Table I. These represent the predicted C-terminal decapeptides of Gq/G11 (⊡), G_i1/G_i2 (■), G_i3 ( ), G_o (◆) and Gz (◇).

3. RESULTS

Competitive ELISA of the reactivity of antiserum CQ2 against the peptide used for its generation indicated strong reactivity with half maximal interaction recorded at a 1:30 000 to 1:50 000 dilution of the crude antiserum (Fig. 1). By contrast, equivalent ELISAs with this antiserum demonstrated no cross-reactivity with synthetic peptides representing the C-terminal decapeptides of the α subunits of G_i1, G_i2, G_i3, G_o or G_z (Fig. 1), confirming the specificity of this antiserum for the C-terminal region of the α subunit of Gq/G11.

As one of the first tissue sources used for purification of these G-proteins was brain [15], we used antiserum CQ2 in immunoblots of rat brain cortical membranes. An apparently single polypeptide of 42 kDa was identified by this antiserum (Fig. 2). An equivalent polypeptide was detected by antiserum CQ2 in membranes of neuroblastoma × glioma hybrid NG108-15 cells, glioma C6 BU1 cells and Rat 1 fibroblasts (Fig. 2), all cell lines which are widely used in studies on pertussis toxin-insensitive agonist regulation of the hydrolysis of inositol containing phospholipids. Immunoblotting of membranes of U937 cells with antiserum CQ2 also identified a single 42 kDa polypeptide. This polypeptide was present, however, at a considerably lower level on a protein basis than in the other cell lines tested (Fig. 2). No difference in levels of the polypeptide identified by antiserum CQ2 was noted between undifferentiated U937 cells and U937 cells differentiated by exposure to dimethylsulphoxide (1.25% (v/v), 72 h) (Fig. 2).

It has been noted previously that G-proteins which are substrates for pertussis toxin-catalysed ADP-ribosylation migrate more slowly in SDS-PAGE following this covalent modification (e.g. [16]). Furthermore, treatment of cells with cholera toxin substantially downregulates the population of $G_s\alpha$ in cells [17–19]. To confirm that the polypeptide identified by antiserum CQ2 was not a substrate for pertussis toxin-catalysed ADP-ribosylation, or a form of $G_s\alpha$, we separated membranes of untreated and either pertussis toxin or cholera toxin-treated rat glioma C6 BU1 cells. These were subsequently immunoblotted with either antiserum CQ2 or with antisera SG1 which identifies only $G_i2\alpha$ in these cells, or CS1 which identifies the C-terminal region of forms of $G_s\alpha$. Immunoreactivity corresponding to $G_i2\alpha$ migrated more slowly following pertussis toxin-treatment of the cells but the mobility of the polypeptide identified by antiserum CQ2 was unaf-

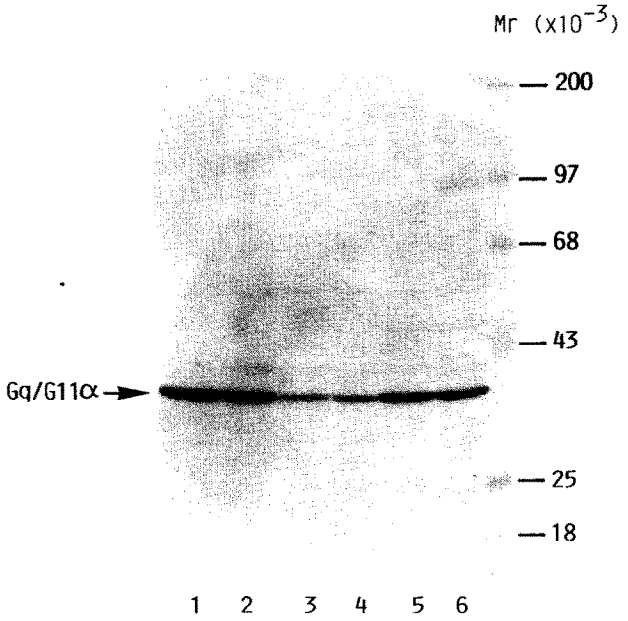

Fig. 2. Immunological detection of Gqα/G11α by immunoblotting membrane fractions of cells and tissues with antiserum CQ2. Membranes (150 μg) from Rat 1 fibroblasts transfected with the human α2-C10 adrenergic receptor (clone 1C) [13] (1), glioma C6 BU1 cells (2), DMSO-differentiated U937 cells (3), undifferentiated U937 cells (4), neuroblastoma × glioma hybrid NG108-15 cells (5) and rat cerebral cortex (6) were resolved on SDS-PAGE (10% (w/v) acrylamide) and immunoblotted using antiserum CQ2 (1:200 dilution) as the primary antiserum.

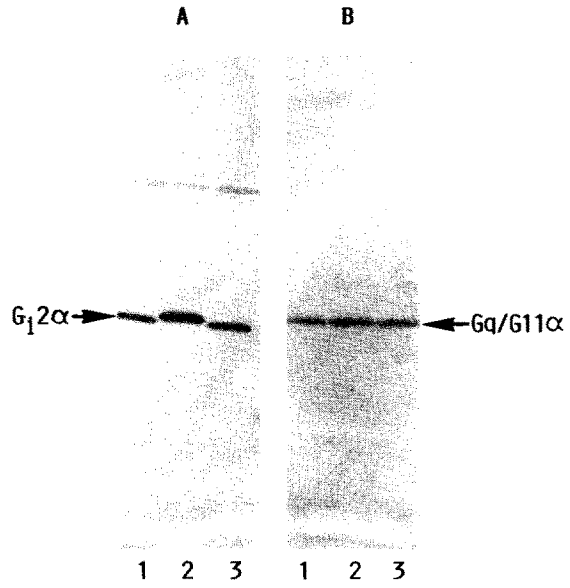

Fig. 4. CQ2 immunoreactivity in U937 cells is not due to cross-reactivity of the antiserum with a pertussis toxin-sensitive G-protein. Membranes from U937 cells which were either untreated (1 and 3) or pretreated with pertussis toxin (25 ng/ml, 16 h) (2) were resolved by SDS-PAGE and immunoblotted with either antiserum SG1 (panel A) or antiserum CQ2 (panel B) (see legend to Fig. 3 for details). The mobility of the CQ2 immunoreactive polypeptide was not modified by pertussis toxin treatment of the cells whilst all of the SG1 immunoreactive polypeptide migrated more slowly in the gel following this treatment.

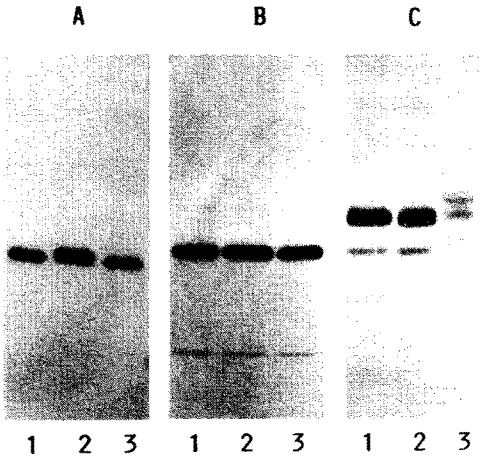

Fig. 3. The CQ2 immunoreactive polypeptide of rat glioma C6 BU1 cells is not a substrate for pertussis toxin-catalysed ADP-ribosylation and is not downregulated by cholera toxin treatment of the cells. Membranes (panel A, 20 μg, panel B, 100 μg, panel C, 40 μg) from rat glioma C6 BU1 cells which were either untreated (1) or had been pretreated with either pertussis toxin (25 ng/ml, 16 h) (2) or with cholera toxin (1 μg/ml, 16 h) (3) were resolved on SDS-PAGE and immunoblotted with antiserum SG1 (panel A) to detect $G_i2\alpha$, with antiserum CQ2 (panel B) to detect Gqα/G11α or with antiserum CS1 (panel C) to detect $G_s\alpha$.

fected by such treatment (Fig. 3). Furthermore no reduction in levels of the polypeptide identified by antiserum CQ2 was noted following cholera toxin treatment of the cells whilst levels of $G_s\alpha$ were substantially reduced (Fig. 3) as has previously been recorded [19]. To confirm that the polypeptide identified by antiserum CQ2 in U937 cells was not a pertussis toxin-sensitive G-protein to which the antiserum displayed weak cross-reactivity we immunoblotted membranes of untreated and pertussis toxin-pretreated U937 cells with either antiserum SG1 to identify $G_i2\alpha$ or with antiserum CQ2. $G_i2\alpha$ migrated more slowly through SDS-PAGE following pertussis toxin treatment (Fig. 4) but the mobility of the CQ2 immunoreactive polypeptide in U937 cells was not altered by this treatment (Fig. 4) confirming the presence of Gq/G11 in these cells and conclusively demonstrating that this reactivity was not due to interaction with one of the pertussis toxin-sensitive G-proteins identified previously in these cells.

4. DISCUSSION

The recent use of polymerase chain reaction (PCR) technology [20] and the isolation of cDNA's [7] has identified a new subfamily of G-protein α subunits

which are unlikely to be substrates for either pertussis or cholera toxin-catalysed ADP-ribosylation. This has led to intense speculation as to whether one or more of these G-proteins corresponds to G_p, the proposed G-protein responsible for pertussis toxin-insensitive receptor regulation of a phosphoinositidase C. These G-proteins migrate as 42 kDa polypeptides on SDS-PAGE and partial sequence analysis indicated that they may well correspond to the product of the Gqα and/or the G11α gene [8,9,15]. These two G-proteins are 88% identical in overall primary sequence but differ in only 4 of the C-terminal 144 amino acids. As this C-terminal region is believed to define both receptor and effector interactions with the G-protein it would not be surprising if these polypeptides had very similar functions. The availability of antisera with defined specificity against these proteins will be of considerable use in the definitive characterization of the role(s) of these G-proteins.

Antiserum CQ2 showed no detectable cross-reactivity against peptides corresponding to the equivalent region of a wide variety of other G-proteins (Table I), including those which are substrates for the ADP-ribosyltransferase activity of pertussis toxin, indicating that this antiserum is highly specific for Gq/G11. Furthermore, based on the predicted C-terminal heptapeptides of G12, G13, G15 and G16 [21] we predict that antiserum CQ2 will also fail to cross-react with any of these polypeptides.

We note that Gq/G11 immunoreactivity is widely expressed as predicted by analysis of the presence of their mRNA's [7]. This is consistent with predictions for G-proteins involved in receptor regulation of phosphoinositidase C. Moreover, immunodetection with antiserum CQ2 demonstrates clearly that Gq/G11 do not display any mobility shift in SDS-PAGE following treatment of cells with pertussis toxin (Figs. 3 and 4). By immunoblotting with antiserum CQ2 we have detected a 42 kDa polypeptide in a range of cell types including the neuroblastoma × glioma hybrid NG108-15, glioma C6BU1, Rat 1 fibroblasts and also human U937 cells. Stimulation of inositol phosphate production in U937 cells by the chemotactic peptide *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) is prevented by prior exposure of the cells to pertussis toxin [14] indicating that the relevant G-protein is a substrate for pertussis toxin-catalysed ADP-ribosylation. As such, Gq/G11 presumably cannot be involved in transducing the effects of FMLP. However, phosphoinositide metabolism in U937 cells stimulated by platelet activating factor is not sensitive to treatment of the cells with pertussis toxin [22] and as such Gq/G11 may be important for this process.

We have previously generated a selection of antipeptide antisera against the predicted C-terminal decapeptide sequences of G_i2, G_o and G_s and used these antisera to prevent receptor/G-protein contacts [3,5,23,24]. We hope that the antiserum described in this study will be useful to define conclusively if Gq and/or G11 regulate receptor control of the generation of lipid-derived secondary messengers in permeabilized cells and native membrane systems as well as in reconstitutive assays.

REFERENCES

[1] Milligan, G. (1988) Biochem. J. 255, 1–13.
[2] Simonds, W.F., Goldsmith, P.K., Woodward, C.J., Unson, C.G. and Spiegel, A.M. (1989) FEBS Lett. 249, 189–194.
[3] Nair, B.G., Parikh, B., Milligan, G. and Patel, T.B. (1990) J. Biol. Chem. 265, 21317–21322.
[4] Simonds, W.F., Goldsmith, P.K., Codina, J., Unson, C.G. and Spiegel, A.M. (1989) Proc. Natl. Acad. Sci. (USA) 86, 7809–7813.
[5] McKenzie, F.R. and Milligan, G. (1990) Biochem. J. 269, 391–398.
[6] Fain, J.N., Wallace, M.A. and Wojcikiewicz, R.J.H. (1988) FASEB. J. 2, 2569–2574.
[7] Strathmann, M. and Simon, M.I. (1990) Proc. Natl. Acad. Sci. USA, 87, 9113–9117.
[8] Smrcka, A.V., Helper, J.R., Brown, K.O. and Sternweis, P.C. (1991) Science 251, 804–807.
[9] Taylor, S.J., Chae, H.Z., Rhee, S.G. and Exton, J.H. (1991) Nature 350, 516–518.
[10] McKenzie, F.R. and Milligan, G. (1990) J. Biol. Chem. 265, 17084–17093.
[11] Milligan, G. and Unson, C.G. (1989) Biochem. J. 260, 837–841.
[12] Milligan, G. (1987) Biochem. J. 245, 501–505.
[13] Milligan, G., Carr, I.C., Gould, G.W., Mullaney, I. and Lavan, B.E. (1991) J. Biol. Chem. 266, 6447–6455.
[14] Pollock, K., Creba, J., Mitchell, F. and Milligan, G. (1990) Biochim. Biophys. Acta 1051, 71–77.
[15] Pang, I.H. and Sternweis, P.C. (1990) J. Biol. Chem. 265, 18707–18712.
[16] Goldsmith, P., Gierschik, P., Milligan, G., Unson, C.G., Vinitsky, R., Malech, H. and Spiegel, A. (1987) J. Biol. Chem. 262, 14683–14688.
[17] Milligan, G., Unson, C.G. and Wakelam, M.J.O. (1989) Biochem. J. 262, 643–649.
[18] Chang, F.H. and Bourne, H.R. (1989) J. Biol. Chem. 264, 5352–5357.
[19] Carr, C., Loney, C., Unson, C., Knowler, J. and Milligan, G. (1990) Eur. J. Pharmacol. Mol. Pharm. Section 188, 203–209.
[20] Strathmann, M., Wilkie, T.M. and Simon, M.I. (1989) Proc. Natl. Acad. Sci. USA 86, 7407–7409.
[21] Simon, M.I., Strathmann, M.P. and Gautam, N. (1991) Science 252, 802–808.
[22] Barzaghi, G., Sarau, H.M. and Mong, S. (1989) J. Pharmacol. Exp. Ther. 248, 559–566.
[23] McClue, S.J. and Milligan, G. (1990) FEBS Lett. 269, 430–434.
[24] McFadzean, I., Mullaney, I., Brown, D.A. and Milligan, G. (1989) Neuron 3, 178–182.