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# Immunological identification of the α subunit of G13, a novel guanine nucleotide binding protein

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An antiserum (13CB) was generated against a synthetic peptide, HDNLKQLMLQ, which is predicted to represent the C-terminal decapeptide of the α subunit of the novel G-protein, G13. Competitive ELISA indicated that the antiserum reacted with this peptide but that it showed minimal ability to recognize peptides which represent the equivalent regions of the pertussis toxin-insensitive G-proteins, Gq + G11, G12, G15 + G16, $G_L1$ (also called G14) as Gz, and well as other G-proteins. Immunoblots of human platelet membranes with antiserum 13CB identified a single 43-kDa polypeptide, and while this immunoreactivity was abolished by the presence of the cognate peptide it was not modified by the presence of peptides corresponding to the equivalent region of other G-proteins. Immunoreactivity corresponding to G13α was detected in a range of cell types with human platelets having the highest levels of this polypeptide.

Guanine nucleotide binding protein; Signal transduction

## 1. INTRODUCTION

The family of heterotrimeric guanine nucleotide binding proteins (G-proteins) consists of a large number of individual members in which the molecular nature of the α subunit defines the identity of the G-protein [1]. Recent application of polymerase chain reaction (PCR) technology has considerably expanded the number of G-protein α subunits which are known to be expressed [2]. In a number of cases the G-protein α subunit is, to date, known only from the isolation of a corresponding cDNA. One G- protein α subunit which falls into this class has been named G13α [3]. This G-protein is somewhat distantly related at the primary sequence levels to other identified G-proteins, and along with another G-protein (G12) of unidentified function has been proposed to define a separate class of G-proteins from those which had been identified previously [3]. We have taken advantage of the known cDNA sequence to generate a specific antipeptide antiserum (13CB) against the α subunit of this G-protein. In this report we show the specificity of this antiserum for G13α and demonstrate, as predicted from the distribution of relevant mRNA [3], the widespread occurence of this polypeptide.

## 2. MATERIALS AND METHODS

### 2.1. *Antisera*

Antiserum 13CB was generated in a New Zealand White rabbit which was immunized with a conjugate of a synthetic peptide, HDNLKQLMLQ-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 end of the peptide, which is equivalent to the C-terminal decapeptide predicted for G13α. This peptide was the kind gift of Dr. S.J. Arkinstall, Glaxo Institute for Molecular Biology, Geneva, Switzerland. Coupling of the peptide and protein was achieved subsequent to the activation of KLH by treatment with *m*-maleimidobenzoylsulpho-succinimide ester [4] (Pierce and Warringer, Chester, UK). Antiserum CQ2, which identifies the C-terminal decapeptide of Gqα and G11α [4], SG1, which identifies the C-terminal decapeptide of $G_i1\alpha$ and $G_i2\alpha$ [5], and CS1, which identifies the C-terminal decapeptide of splice variants of $G_s\alpha$ [6], have previously been described.

Synthetic peptides corresponding to the C-terminal decapeptides of the α subunits of Gq/G11, G12, $G_L1$ (G14), G16, Gz, $G_i1/G_i2$, $G_i3$ and $G_o1$ (Table I) which were used to assess potential interactions of antiserum 13CB with these other G-proteins were obtained either commercially from Biomac (Glasgow, Scotland) or as gifts from Dr. C.G. Unson, Department of Biochemistry, Rockerfeller University, New York, USA or Dr. S.J. Arkinstall, Glaxo Institute for Molecular Biology, Geneva, Switzerland.

Table I

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

| | |
|---|---|
| G13 | HDNLKQLMLQ-COOH |
| Gq/G11 | QLNLKEYNLV-COOH |
| G12 | QENLKDIMLQ-COOH |
| Gz | QNNLKYIGLC-COOH |
| $G_L1$(G14) | QLNLREFNLV-COOH |
| G16 | ARYLDEINLL-COOH |
| $G_i1/G_i2$ | KNNLKDCGLF-COOH |
| $G_i3$ | KNNLKECGLY-COOH |
| $G_o$ | ANNLRGCGLY-COOH |

The above peptides with, in the cases of Gq/G11, G12, G13 and G16, an additional N-terminal cysteine were used in competitive ELISAs with antiserum 13CB (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 13CB.

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2.2. *Immunoblotting*

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 13CB against 100 ng 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 [7,8]. 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 and human platelets were prepared as in [9].

## 3. RESULTS AND DISCUSSION

Competitive ELISA of the interaction of antiserum 13CB with the peptide used for its generation (100 ng) indicated that half-maximal reactivity was observed at a 1:2,000 dilution of the crude antiserum (Fig. 1). No substantive cross-reactivity of this antiserum was noted with synthetic peptides which represent the equivalent C-terminal regions of the α subunits of the G-proteins, Gz, Gq + G11, G12, G14, G15 + G16, $G_i1$ + $G_i2$, $G_i3$

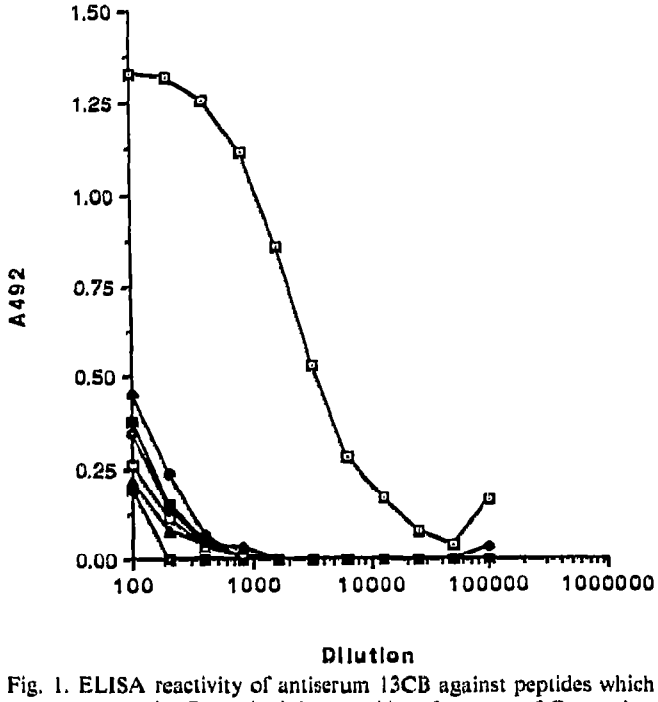


Fig. 1. ELISA reactivity of antiserum 13CB against peptides which correspond to the C-terminal decapeptides of a range of G-proteins. ELISA asays were performed using 100 ng of peptides corresponding to the C-terminal decapeptides of G13 (⊡), G12 (■), G11/Gq (▲), Gz (◆), $G_i1$ +$G_i2$ (□), $G_i3$ (◇) and $G_o1$ (◘). Further experiments with peptides equivalent to the C-terminal decapeptides of $G_L1$ (G14) and G15 + G16 revealed no cross-reactivity between these peptides and antiserum 13CB.

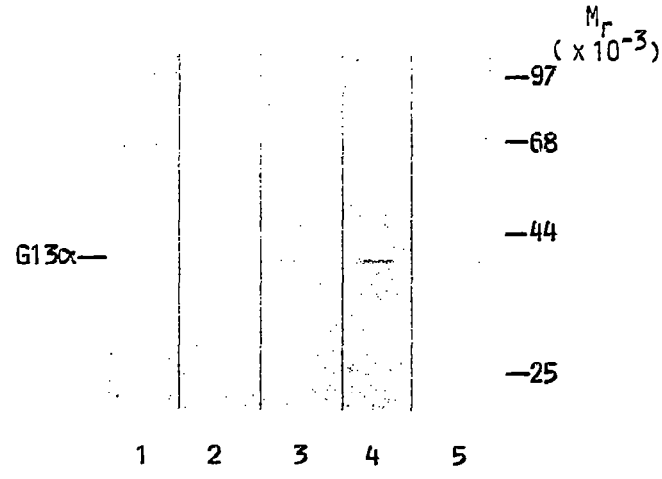


Fig. 2. Immunological detection of a 43-kDa polypeptide by antiserum 13CB is prevented by the G13α C-terminal decapeptide but not by the equivalent peptides of other pertussis toxin-insensitive G-proteins. Membranes (150 μg) of human platelets were separated by SDS-PAGE (10% acrylamide) and immunoblotted (lane 1) with antiserum 13CB (1:200 dilution) or with this dilution of antiserum 13CB in the presence of 1 μg/ml of the C-terminal decapeptides corresponding to (lane 2) G13α, (lane 3) G12α, (lane 4) G11α + Gqα or (lane 5) Gzα.

and $G_o1$ (Fig. 1 and results not shown). Such information indicates the likely specificity of antiserum 13CB for G13α.

Immunoblotting of membranes of human platelets with antiserum 13CB identified a single polypeptide of 43 kDa (Fig. 2). Pre-incubation of antiserum 13CB with 1 μg/ml of the peptide used to generate the antiserum abolished immunological detection of the 43-kDa polypeptide. In contrast, pre-incubation of antiserum 13CB with the equivalent decapeptides (1 μg/ml) of Gz, Gq/G11 and G12, failed to reduce the immunological detection of the 43-kDa polypeptide although these 4 other G-proteins, like G13, are predicted to be resistant to ADP-ribosylation catalysed by pertussis toxin, and share some 50–70% overall amino acid identity with G13α [2,3]. The polypeptide identified by antiserum 13CB was not a substrate for pertussis toxin-catalysed ADP-ribosylation. We have previously noted that $G_i2$ (and other pertussis toxin-sensitive G-proteins) migrate more slowly through SDS-PAGE following pertussis toxin-catalysed ADP-ribosylation (see [4] for example). Immunoblotting of membranes of untreated and pertussis toxin-treated C6 glioma cells with antiserum 13CB showed no difference in mobility of the identified 43-kDa polypeptide in the 2 samples. Parallel immunoblots of these membranes with antiserum SG1, which in these cells identifies only $G_i2α$, demonstrated the anticipated reduced mobility of the 40-kDa polypeptide in membranes from the toxin-treated cells (data not shown).

Cholera toxin treatment of cells has been shown to cause a considerable reduction in cellular levels of $G_sα$ (see [4] for example). Immunoblotting of membranes of untreated and cholera toxin-pretreated C6 glioma cells

with antiserum CS1, which identifies forms of $G_s\alpha$ [6], confirmed these observations, but parallel immunoblots of these membranes with antiserum 13CB indicated that no alteration in membrane level of G13α was produced by cholera toxin treatment (data not shown).

As PCR RNA analysis has suggested that brain has only very low levels of G13α transcripts relative to other tissues [3], we immunoblotted rat brain cortical membranes with antiserum 13CB in concert with membranes from human platelets (Fig. 3b). As anticipated, immunoreactivity in the rat brain membranes was below detectable levels. In a parallel experiment rat brain cortical membranes and human platelets were immunoblotted with antiserum CQ2 [4] to detect the presence of Gq/G11 (Fig. 3a). CQ2 identified a single polypeptide of 42 kDa in both brain and platelet membranes although immunoreactivity to this antiserum was markedly greater in platelet membranes than in brain membranes.

Immunologically detected G13α was expressed widely. We were able to detect a single polypeptide which co-migrated with the polypeptide expressed in human platelets in each of neuroblastoma × glioma hybrid, NG108-15, rat glioma C6 cells, human monocytic U937 and human kidney EMBK cells (Fig. 4). On a per protein basis, of these cell lines glioma C6 cell membranes expressed the highest levels of G13α (Fig. 4). The widespread distribution of the G13α polypeptide was to be expected given the widespread detection of mRNA encoding this polypeptide. The function of this protein is currently unknown but it must presumably act to transmit information which is not disrupted by pertussis toxin treatment of cells. Antisera directed against the C-terminal regions of a variety of other G-proteins have been invaluable in defining interactions between these G-proteins and particular classes of receptors [9–14]. It is hoped that the antibodies described in this report may similarly prove useful in defining the function of G13.

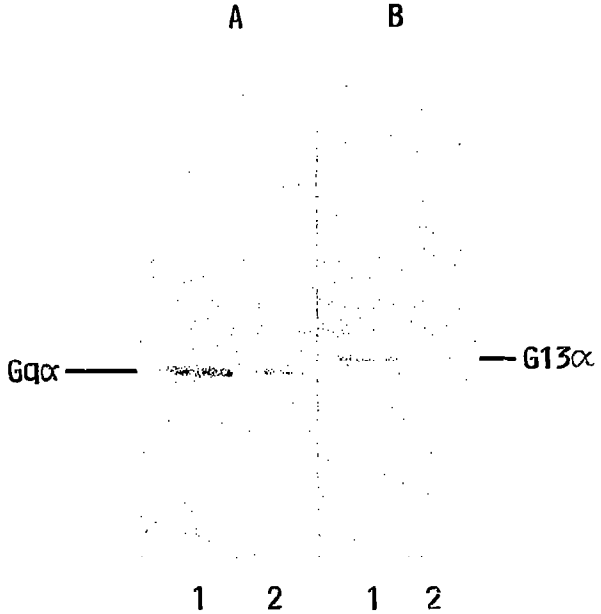


Fig. 3. Immunological detection of G13α in platelet but not brain membranes. Membranes (20 μg, Fig. 3a; 150 μg, Fig. 3b) from human platelets (lanes 1) and rat brain (lanes 2) were resolved as described in the legend to Fig. 2 and immunoblotted with either antiserum CQ2 (Fig. 3a) or antiserum 13CB (Fig. 3b). Immunoreactivity corresponding to G13α was detected in platelet but not brain whilst immunoreactivity corresponding to Gqα/G11α was detected in both tissues.

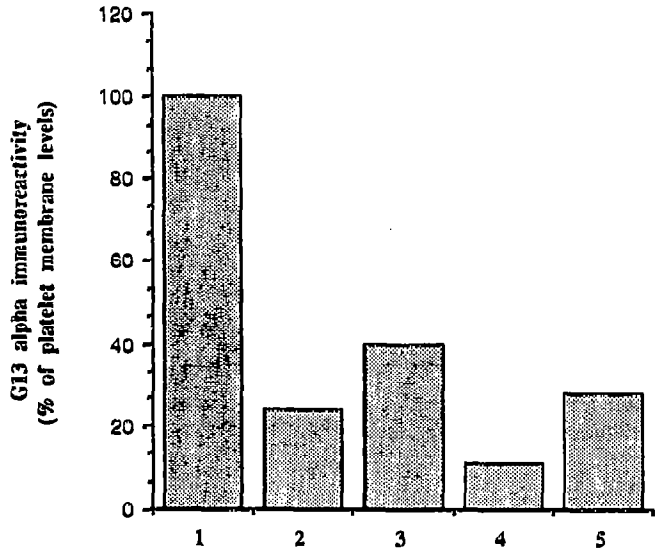


Fig. 4. Widespread immunological detection of G13α. Membranes (200 μg) from (1) human platelets, (2) neuroblastoma × glioma NG108-15 hybrid cells, (3) rat glioma C6BU1 cells, (4) CHO cells, and (5) EMBK cells were resolved as in the legend to Fig. 2 and immunoblotted with antiserum 13CB (1:200 dilution) and the signal corresponding to G13α quantitated by densitometric scanning. Data are presented for the level of G13α relative to that of platelets which have the highest level of this polypeptide that we have yet detected.

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