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Immunochemical characterization of phosphatidylinositol 4-phosphate kinase from rat brain

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Affinity-purified antibodies were used to identify a protein of molecular mass 45 kDa (45 kDa protein) in rat brain cytosol as phosphatidylinositol 4-phosphate (PtdIns4*P*) kinase. Antibodies were raised in rabbits by immunization with the purified 45 kDa protein. Anti-(45 kDa protein) immunoglobulins were isolated by affinity chromatography of the antiserum on a solid immunosorbent, which was prepared by coupling a soluble rat brain fraction, the DEAE-cellulose pool containing 10–15% 45 kDa protein, to CNBr-activated Sepharose 4B. The purified IgGs were specific for the 45 kDa protein as judged by immunoblot and by immunoprecipitation. The purified anti-(45 kDa protein) IgGs inhibited the enzyme activity of partially purified PtdIns4*P* kinase, whereas preimmune IgGs were ineffective. Immunoprecipitation of the 45 kDa protein from the partially purified enzyme preparation with the purified IgGs resulted in a concomitant decrease in the amount of 45 kDa protein and in PtdIns4*P* kinase activity. The amount of 45 kDa protein remaining in the supernatant and the activity of PtdIns4*P* kinase correlated with a coefficient of $r = 0.87$. The evidence presented lends further support for the notion that the catalytic activity of PtdIns4*P* kinase in rat brain cytosol resides in a 45 kDa protein.

INTRODUCTION

Inositol phospholipids have been implicated in the regulation of membrane permeability and synaptic transmission in neurons (Abdel-Latif, 1983; Downes & Michell, 1982). An enhanced metabolism of these lipids was observed after receptor activation by transmitters, hormones and neuropeptides which utilize calcium ions as their second messenger (Abdel-Latif, 1983; Michell, 1979).

Recently we reported a procedure for the purification of PtdIns4*P* kinase from rat brain cytosol, using $(NH_4)_2SO_4$ precipitation, DEAE-cellulose column chromatography and preparative isoelectric focusing (Van Dongen *et al.*, 1984). The PtdIns4*P* kinase activity comigrated with a 45 kDa protein with pI 5.3 (Van Dongen *et al.*, 1984). In the present paper we report the production and purification of antibodies directed against the 45 kDa protein. The inhibition of the PtdIns4*P* kinase activity by these antibodies supports the proposal that the catalytic activity of brain PtdIns4*P* kinase resides in the 45 kDa protein.

MATERIALS AND METHODS

Purification of PtdIns4*P* kinase

Rats (150–180 g body wt.) of an inbred Wistar strain were used (TNO, Zeist, The Netherlands). The purification of PtdIns4*P* kinase was performed according to Van Dongen *et al.* (1984), with minor modifications. In short, a cytosolic fraction from whole brain (minus cerebellum) of 30 rats was prepared and fractionated by $(NH_4)_2SO_4$ precipitation (20–40% saturation). The precipitate was further purified by DEAE-cellulose column chromatography using a linear gradient of 150–250 mM-NaCl in buffer A (10 mM-Tris/HCl, pH 7.4/0.1 mM-dithiothreitol). After column chromatography the fractions were analysed by SDS/polyacrylamide-gel electrophoresis on 11% slab gels (see below). Fractions enriched in 45 kDa protein were pooled, dialysed against buffer A and concentrated 4–5-fold by an Amicon ultrafiltration cell equipped with a PM-10 filter membrane. This concentrated DEAE-cellulose pool (approx. 1 mg of protein/ml; 10–15% of the total protein was 45 kDa protein) was stored at −20°C.

Purification of 45 kDa protein

Concentrated DEAE-cellulose pool (4 mg of protein) was applied to 3 mm thick 11% SDS/polyacrylamide slab gels. After electrophoresis the proteins were stained with Coomassie Blue and the 45 kDa protein was excised from the gel. The protein was extracted from the gel strip with an Isco sample concentrator (model 1750; Isco, Lincoln, NE, U.S.A.) in 0.25 M-Tris/192.5 mM-glycine/0.1% SDS, pH 8.3. The thus-purified protein was used for immunization.

Production of antiserum

Three female New Zealand rabbits (weighing approx. 3 kg; nos. 8316, 8317 and 8318) were immunized with intradermal injections on multiple sites on the back. The immunogen consisted of 0.1–1.0 ml of solution of 45 kDa protein (100 μg) emulsified with an equal volume of Freund's adjuvant. Complete adjuvant was used for the first, third, fifth and seventh injection; incomplete Freund's adjuvant was used for the intervening injections (Oestreicher *et al.*, 1983). The rabbits received eight

Abbreviations used: PtdIns4*P*, phosphatidylinositol 4-phosphate; e.l.i.s.a., enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline.

immunizations at intervals of 3 weeks. Animals were bled from the ear vein 10 days after each injection. Preimmune serum was obtained from the animals prior to immunization. The serum samples were stored in small portions at −20 °C.

Purification of antibodies

IgGs against 45 kDa protein were purified with an immunosorbent column which was prepared according to the method described by Oestreicher *et al.* (1983). Briefly, the DEAE-cellulose pool (5 mg total protein) enriched in PtdIns4*P* kinase activity was concentrated to 2 ml and exhaustively dialysed against 0.1 mM-$NaHCO_3$/0.5 mM-NaCl (pH 9.0). This fraction was incubated for 16 h at room temperature with 1 g of swollen CNBr-activated Sepharose 4B (Pharmacia) in the same buffer. After 16 h, all protein had been conjugated to the column as revealed by SDS/polyacrylamide-gel electrophoresis. Remaining free CNBr groups were saturated with 1 M-glycine, pH 8.0, for 2 h at room temperature. Excess glycine was removed by repeated washing in 4 ml of 1 M-NaCl in 0.1 M-sodium acetate (pH 4.0) and in 0.1 M-sodium borate (pH 8.5), successively. Finally, the column was rinsed with 0.1 M-ammonium formate (pH 2.7) and neutralized with phosphate-buffered saline (PBS; 3.44 g of NaH_2PO_4, 33.3 g of Na_2HPO_4 and 82 g of NaCl per litre adjusted to pH 7.4). The column was stored at −20 °C. Affinity chromatography was performed by the procedure described by Oestreicher *et al.* (1983).

The eluates containing IgGs were lyophilized, dissolved in PBS (approx. 0.5 μg/μl), dialysed against PBS and stored at −20 °C. Control IgGs were isolated from preimmune serum or normal rabbit serum by affinity chromatography on a protein A–Sepharose CL-4B column (2 ml of swollen gel) by a procedure similar to that described by Oestreicher *et al.* (1983).

Detection of antibodies

The titre of anti-(45 kDa protein) antibodies was monitored by e.l.i.s.a. (Engvall, 1980), using the 45 kDa protein (25 ng/ml per well) as the antigen.

The specificity of the antibodies was examined by two methods: (1) the SDS-gel/immunoperoxidase method (Van Raamsdonk *et al.*, 1977) with the modifications described by Oestreicher *et al.* (1983) and (2) immunoblotting according to the method of Nielsen *et al.* (1982). For the last procedure, 100 μg of the DEAE-cellulose fraction was subjected to SDS/polyacrylamide-gel electrophoresis on 11% slab gels using a gel without slots. The separated proteins were electrophoretically transferred to nitrocellulose filters (0.45 μm pore size). A strip of the nitrocellulose was stained for protein with Amido Black (see also Schrama *et al.*, 1984) and the remainder of the blot was incubated overnight with 0.1% (w/v) gelatin in 50 mM-Tris/HCl (pH 7.4), containing 150 mM-NaCl and 0.5% (v/v) Triton X-100 (Tris/saline/Triton X-100). Thereafter, the blot was cut into strips of approx. 0.5 cm and incubated with dilutions of immune, preimmune serum or anti-(45 kDa protein) IgGs. After 2 h the strips were washed repeatedly in Tris/saline/Triton X-100. Then the strips were incubated with goat anti-(rabbit IgG) conjugated to horseradish peroxidase (1:2000 dilution). The peroxidase reaction was performed as described by Schrama *et al.* (1984).

Immunoprecipitation with protein A–Sepharose

Immunoprecipitation of 45 kDa protein with the purified antibodies was performed using protein A–Sepharose CL-4B (Pharmacia). The DEAE-cellulose pool (20 μg of total protein) was incubated with 20 μg of the anti-(45 kDa protein) specific IgGs in PBS containing 0.1 mM-dithiothreitol under gentle agitation at 0–4 °C. As controls, buffer only or 20 μg of control IgGs were incubated with the DEAE-cellulose pool. To prevent non-specific adsorption 160 μg of bovine serum albumin was added. After 6 h, protein A–Sepharose (10 μl of swollen material) was added and the incubation was continued for 16 h. The final volume was 160 μl. Bound and free proteins were separated by centrifugation for 5 min at 10000 ***g***. The supernatant was removed and the pellet was washed three times with 150 μl of PBS containing 0.1 mM-dithiothreitol. The pellet was suspended in 60 μl of this buffer.

PtdIns4*P* kinase activity

To study the direct effect of specific IgGs on the activity of PtdIns4*P* kinase, 10 μg of IgGs was incubated for 16 h at 0–4 °C with the DEAE-cellulose fraction (2–3 μg of total protein) in PBS containing 0.1 mM-dithiothreitol in a final volume of 25 μl.

PtdIns4*P* kinase activity was studied according to the method described by Van Dongen *et al.* (1984). In brief, the incubations were performed as follows. The total reaction volume contained 15 μl of enzyme fraction; 7.5 μM-ATP containing 2 μCi of [γ-^{32}P]ATP (approx. sp. radioactivity 3000 Ci/mmol; NEN), 48 μM-PtdIns4*P* (Sigma), 15 μg of bovine serum albumin, 10 mM-sodium acetate, 10 mM-magnesium acetate and 0.1 mM-calcium acetate at pH 6.5 in a final volume of 25 μl. The samples were preincubated for 5 min at 30 °C and the reaction was started by the addition of ATP. The reaction was terminated 5 min later with 2 ml of ice-cold chloroform/methanol/12 M-HCl (200:100:0.75, by vol.). The extraction and further analysis of radioactive phosphate incorporated into phosphatidylinositol 4,5-bisphosphate was performed as described by Jolles *et al.* (1981).

Other analytical procedures

The method of Lowry *et al.* (1951) was used to determine protein, with bovine serum albumin as standard. One-dimensional SDS/polyacrylamide-gel electrophoresis was performed on 11% slab gels (thickness 1.3 mm) according to Zwiers *et al.* (1976), unless indicated otherwise. Samples containing IgGs were boiled in the presence of the denaturing solution (Zwiers *et al.*, 1976) for 2–4 min before application to the gel. Routinely, the proteins were stained with Fast Green. The amount of 45 kDa protein was estimated by densitometric scanning of the Fast Green stained protein pattern using known amounts of bovine serum albumin on the same gel as standard. Scanning was performed at 650 nm with a Zeiss PM-QII spectrophotometer with KM3 chromatography attachment.

RESULTS

Purification of anti-(45 kDa protein) antibodies

For the production of antibodies against 45 kDa protein, three rabbits were injected eight times with pure (SDS/polyacrylamide-gel electrophoresis-excised)

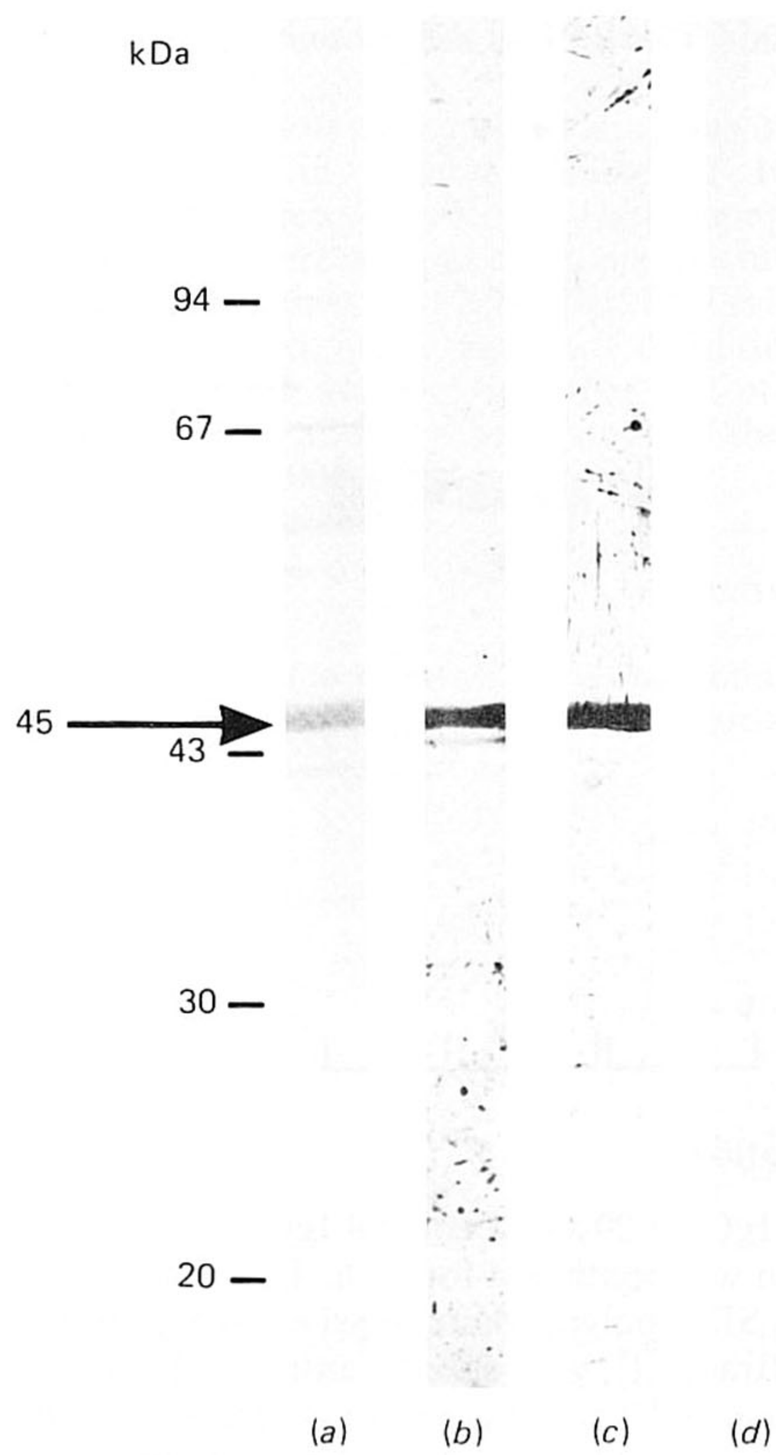

Fig. 1. Analysis of anti-(45 kDa protein) serum by using nitrocellulose blots

The DEAE-cellulose fraction (100 μg of total protein) was separated on a 11% (w/v) slab gel using a comb without slots and transferred electrophoretically to nitrocellulose as described in the Materials and methods section. Track (*a*), protein staining pattern of a nitrocellulose strip with Amido Black; tracks (*b*–*d*), immunostaining pattern after incubation with anti-(45 kDa protein) serum (track *b*), with anti-(45 kDa protein) IgGs (track *c*) and with control IgGs (track *d*). The protein blots of the nitrocellulose strips were incubated with 1:2000 dilutions of the antibodies. After washing of the strips the second antibody was added for detection (see the Materials and methods section).

45 kDa protein which was rerun on SDS gel electrophoresis to estimate the amount of 45 kDa protein and the purity of the preparation. After four injections the titres of the collected antisera ranged from 2560 to 5120 as determined by the e.l.i.s.a. The titres of the antisera of the three rabbits were similar and did not alter after subsequent immunizations.

The specificity of the antisera to the 45 kDa protein was examined using the immunoblot technique. Fig. 1(*a*) shows the protein pattern after Amido Black staining. Next, the immunostaining pattern with antiserum of rabbit 8318 (Fig. 1, track *b*) is compared with the staining pattern observed after incubation with preimmune serum (Fig. 1, track *d*). The immune serum stained two protein bands with apparent molecular masses 45 kDa and 43 kDa, whereas preimmune serum did not stain any of the proteins in the DEAE-cellulose pool. We found the same results for the antisera of the rabbits 8316 and 8317. The 43 kDa protein is a minor component of the DEAE-cellulose pool, which could not be detected in the protein staining pattern either of the blot (Fig. 1, track *a*) or the original gel.

The IgG fraction from the antiserum to 45 kDa protein was purified by affinity chromatography on an immunosorbent column to which the proteins of the DEAE-cellulose pool had been bound. The conservation of immunoreactivity of the purified antibodies was analysed by immunoblotting. The purified antibodies only reacted with the 45 kDa protein of the DEAE-cellulose pool (Fig. 1, track *c*; cf. track *b*).

The specificity of the anti-(45 kDa protein) IgG could be confirmed by another immunochemical procedure, i.e. the SDS-gel/immunoperoxidase method (Oestreicher *et al.*, 1983; see also Van Raamsdonck *et al.*, 1979) (results not shown). The recovery of immunoreactivity for the 45 kDa protein was about 45% as estimated by the e.l.i.s.a.

Effect of the anti-(45 kDa protein) IgGs on PtdIns4*P* kinase activity

In order to avoid the interference of other antibodies and serum proteins with PtdIns4*P* kinase, we have purified the IgGs by affinity chromatography to study their effect on the PtdIns4*P* kinase activity.

Incubation of the DEAE-cellulose pool with the anti-(45 kDa protein) IgGs for 16 h reduced the activity of the PtdIns4*P* kinase (Table 1); 10 μg of the anti-(45 kDa protein) IgGs inhibited PtdIns4*P* kinase activity by about 50%. This effect was specific, since control IgGs were ineffective in inhibiting the enzyme activity (Table 1).

In a subsequent experiment, the influence of removal of the 45 kDa protein from the DEAE-cellulose pool was investigated by immunoprecipitation of the antigen–antibody complex with protein A–Sepharose. After incubation with control (non-specific) IgGs the amounts of all proteins in the supernatant were unchanged as was demonstrated by SDS/polyacrylamide-gel electrophoresis (Fig. 2*a*; compare tracks 3 and tracks 1). However, the amount of the 45 kDa protein was reduced to approx. 40% after immunoprecipitation with anti-(45 kDa protein) IgGs (Fig. 2*a*, tracks 2).

In the supernatant fractions in which the content of the 45 kDa protein was lowered as result of the specific immunoreaction, the PtdIns4*P* kinase activity was found

Table 1. Direct effect of antibody against 45 kDa protein on PtdIns4*P* kinase activity

DEAE-cellulose fraction (2 μg of total protein) was incubated in the absence or presence of 10 μg of IgG for 16 h at 0–4 °C. PtdIns4*P* kinase assay was performed as described in the Materials and methods section. The results are expressed as means ± S.E.M. ($n = 3$). *$2P < 0.001$ (Student's *t*-test). A second, independent experiment ($n = 3$) gave similar results.

Addition	Activity (fmol/min per μg of protein)
None	620 ± 56
Anti-(45 kDa protein) IgG	314 ± 32*
Control IgG	633 ± 40

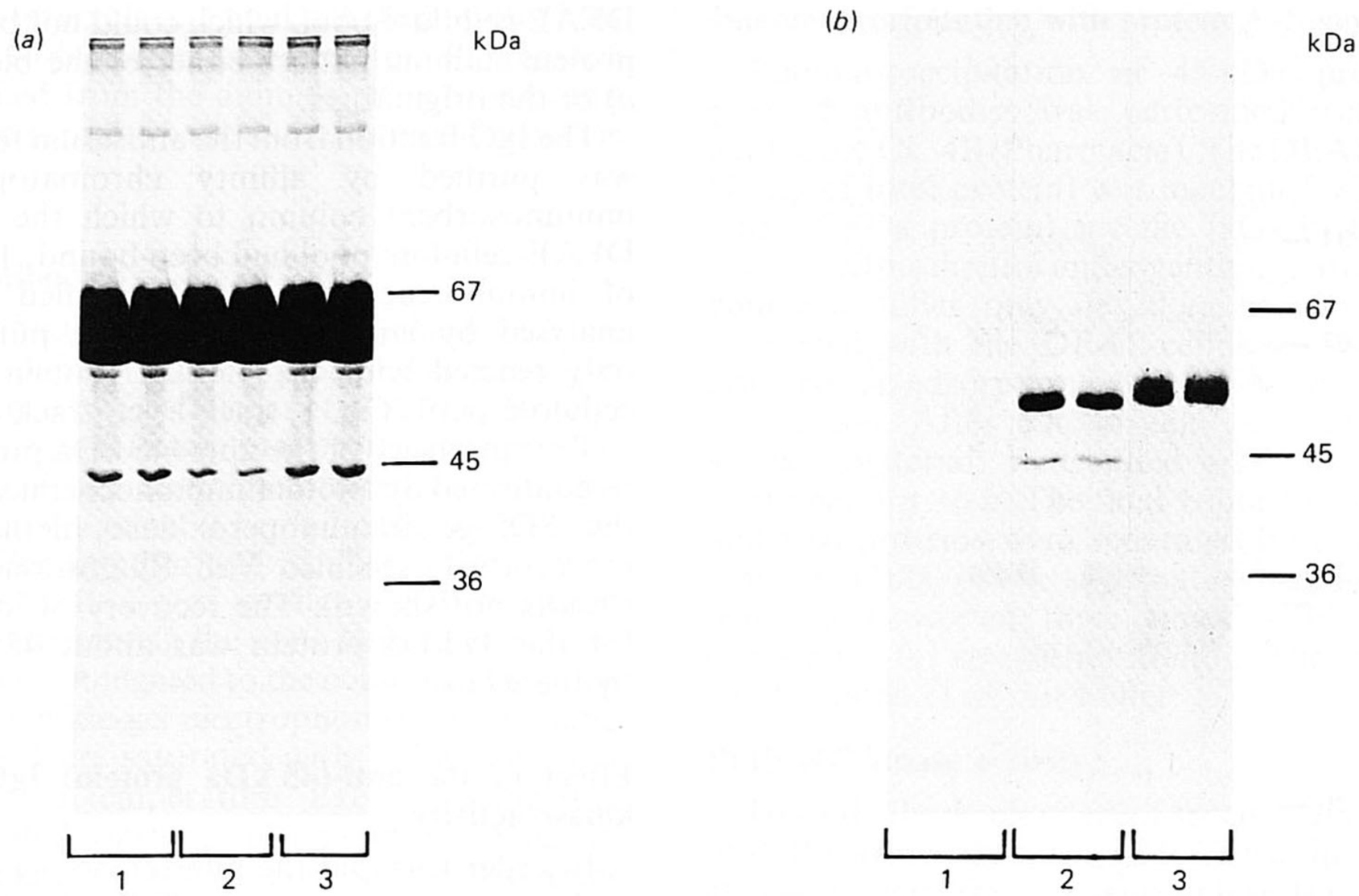

Fig. 2. Protein pattern of the supernatant (*a*) and pellet (*b*) after immunoprecipitation

The DEAE-cellulose pool was incubated in the presence of 20 μg of specific IgG or 20 μg of control IgG or in the absence of IgG. After 6 h incubation protein A–Sepharose was added and the incubation was continued for 16 h. The immunoprecipitate was removed by centrifugation and 20 μl of the supernatant was separated on SDS/polyacrylamide-gel electrophoresis. (*a*) The protein profiles of the supernatants are shown after incubation without IgG (tracks 1), with specific anti-(45 kDa protein) IgG (tracks 2), or with control IgG (tracks 3). (*b*) After the immunoprecipitation the pellet was washed three times with PBS buffer containing 0.1 mM-dithiothreitol. Finally the pellet was resuspended in 60 μl of this buffer and 20 μl was applied to a gel for SDS/polyacrylamide gel electrophoresis. The numbers of the tracks shown in (*b*) correspond with incubation conditions described in (*a*).

Table 2. PtdIns4*P* kinase activity remaining in the supernatant (*a*) and in the immunoprecipitated pellet (*b*) after immunoprecipitation

DEAE-cellulose fraction (2 μg of protein) was incubated with or without 20 μg of IgG as described in the legend to Fig. 2. An immunoprecipitate was performed by addition of protein A–Sepharose. PtdIns4*P* kinase activity was tested under standard conditions in 15 μl of the supernatant (*a*) and in 15 μl of the suspension obtained from the immunoprecipitated pellet (*b*), resuspended in 60 μl of PBS, containing 0.1 mM-dithiothreitol. Results (means ± S.E.M.; $n = 3$) are from one representative experiment from a total of three; * $2P < 0.05$ (Student's *t*-test).

Addition	Activity (fmol/min per μg of protein)
(*a*) None	620 ± 70
Anti-(45 kDa protein) IgG	400 ± 36*
Control IgG	620 ± 60
(*b*) None	60 ± 3
Anti-(45 kDa protein) IgG	87 ± 6
Control IgG	72 ± 4

to be decreased by 36%, whereas incubation with control IgGs did not alter PtdIns4*P* kinase activity (Table 2*a*).

The immunoprecipitated pellets were analysed for PtdIns4*P* kinase activity (Table 2*b*). As was expected, the heavy (H) chains of the IgGs were only recovered in the pellets of samples treated with anti-(45 kDa protein) IgGs or control IgGs (Fig. 2*b*). The enzyme activity recovered in the precipitates was very low. In fact, the activity was similar after using either anti-(45 kDa protein) IgG or control IgGs. Analysis of the immunoprecipitated pellets by SDS/polyacrylamide-gel electrophoresis showed that the pellets formed with anti-(45 kDa protein) IgGs contained exclusively the 45 kDa protein, whereas the pellets formed with control IgG did not (see protein staining pattern, Fig. 2*b*; compare tracks 2 and tracks 3). It appears that once the 45 kDa protein is immunoprecipitated as a pellet, the enzyme activity is inhibited. Attempts were made to examine if the immunocomplex could be dissociated and the enzyme activity recovered. As shown in Table 3, various agents known to dissociate immunocomplexes (MacSween & Eastwood, 1981) were tested for their effect on the PtdIns4*P* kinase activity of the DEAE-cellulose pool. All the reagents reduced the enzyme activity and, therefore, were not useful to dissociate active PtdIns4*P* kinase from the immunocomplex.

The immunoprecipitation of antibody–45 kDa protein was repeated several times with different amounts of anti-(45 kDa protein) IgGs and control IgGs. IgGs isolated from preimmune serum had no effect on either the activity of PtdIns4*P* kinase nor the amount of 45 kDa protein remaining in the supernatant. Addition of varying amounts of anti-(45 kDa protein) IgGs caused a simultaneous decrease of PtdIns4*P* kinase activity and 45 kDa protein content. When the amount of 45 kDa protein remaining after immunoprecipitation in the

Table 3. Influence of various agents on PtdIns4*P* kinase activity

DEAE-cellulose fraction (20 μg of protein) was incubated with several agents in a final volume of 160 μl of PBS/0.1 mM-dithiothreitol buffer at room temperature. After 15 min the mixture was put into a dialysis tubing and dialysed overnight against the PBS buffer. PtdIns4*P* kinase activity was measured under standard conditions. The results are expressed as fmol/min per μg of protein (mean ± S.E.M., $n = 3$) and as a percentage of the control incubation; * $2P < 0.005$ (Student's *t*-test).

Agent	Concentration (M)	Activity (fmol/min per μg of protein)	(%)
None	—	620 ± 30	100
$MgCl_2$	1.75	31 ± 10*	5
$MgCl_2$	3.00	23 ± 12*	4
NaSCN	3	56 ± 23*	9
NaCl	2	155 ± 20*	25
Urea	3	186 ± 20*	30
Urea	6	62 ± 12*	10
Urea	9	18 ± 9*	3
Formic acid (pH 2.7)	0.1	10 ± 9*	2

supernatant is plotted against the enzymic activity of the supernatant, a straight line with a correlation coefficient of 0.87 is obtained (Fig. 3).

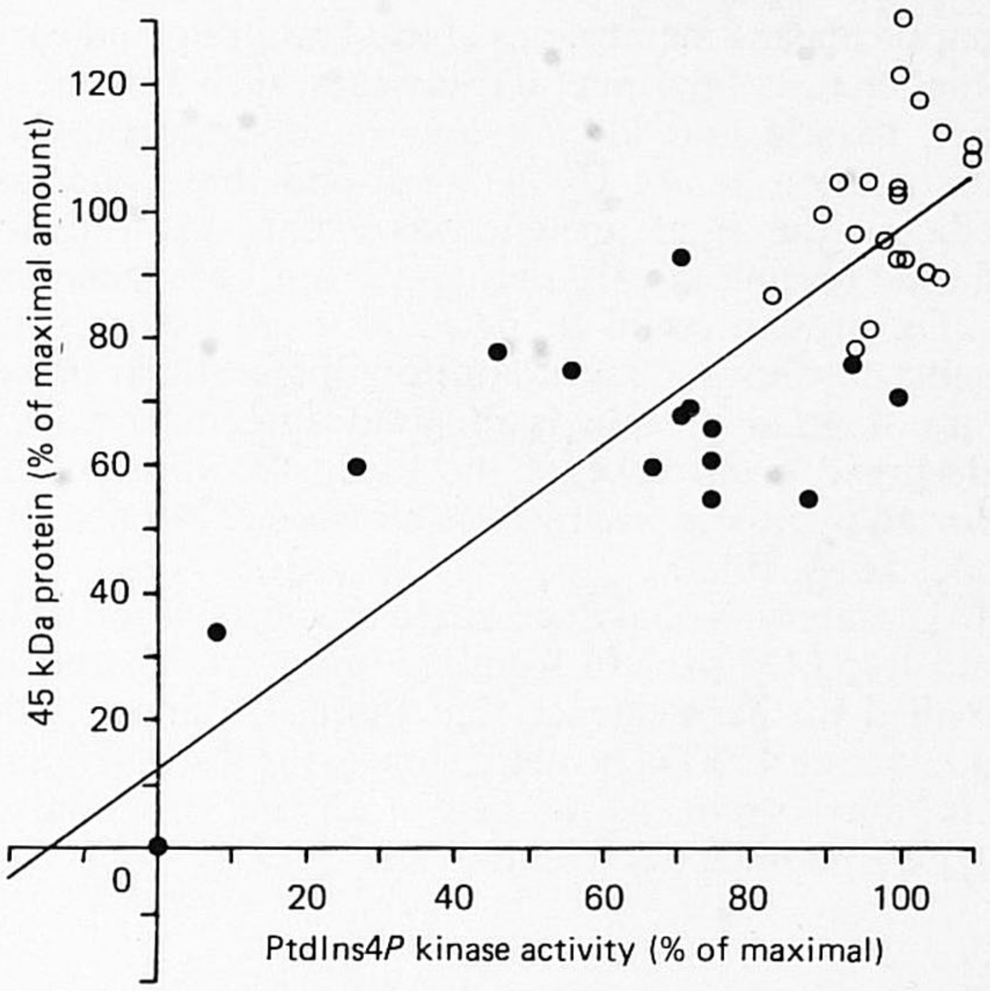

Fig. 3. Relation between the amount of 45 kDa protein and PtdIns4*P* kinase activity

DEAE-cellulose fraction was incubated with 0–25 μg of control IgG or anti-(45 kDa protein) IgG. An immunoprecipitate was formed as described in the legend to Fig. 2. The activity of PtdIns4*P* kinase and the amount of 45 kDa protein in 15 μl of the supernatant were tested. Each point represents one observation derived from five independent experiments. The data were correlated by the method of least mean squares from which a correlation coefficient of 0.87 was calculated. ○, Control IgG; ●, IgG against 45 kDa protein.

DISCUSSION

This paper describes the production and purification of antibodies to 45 kDa protein and the ability of these antibodies to inhibit PtdIns4*P* kinase activity.

The antibodies were purified by affinity chromatography on a column to which the proteins of the DEAE-cellulose pool (enriched in PtdIns4*P* kinase activity) were coupled. Although the amount of 45 kDa protein in the DEAE-cellulose pool was 10–15% of the total protein, the use of this affinity column allowed the purification of IgGs reacting with the 45 kDa protein (Fig. 1). The yield of the IgGs was in the same order as reported by Oestreicher *et al.* (1983) for the isolation of anti-B-50 IgGs.

After incubation of the specific antibodies with the DEAE-cellulose pool enriched in PtdIns4*P* kinase for 16 h, the enzymic activity was reduced by 50% (Table 1). This suggests that the anti-(45 kDa protein) IgGs interacted with the PtdIns4*P* kinase activity. Since the enzyme activity assay was performed in the presence of the antigen–antibody complex, this complex might have decreased the enzyme activity by hindrance of the reaction between PtdIns4*P* kinase and its substrate. To exclude this possibility we then showed that, by removing the antigen–antibody complex and the free IgGs prior to the addition of substrates to the remaining supernatant, PtdIns4*P* kinase activity was reduced as well (Fig. 2; Table 2*a*). The amount of enzyme activity remaining in the supernatant was correlated with the amount of 45 kDa protein left in this supernatant (Fig. 3). These results indicate that the 45 kDa protein is either PtdIns4*P* kinase or that it contains the catalytic subunit. Relatively high amounts of IgGs were required to inhibit the PtdIns4*P* kinase activity, suggesting that probably part of the isolated IgGs were isolated as immunoinactive (Oestreicher *et al.*, 1983). This was also reflected as a variation in the effectiveness of the 45 kDa IgGs to precipitate the 45 kDa protein concomitant with a decrease in enzyme activity. Despite this drawback a correlation coefficient of 0.87 was found (Fig. 3).

In agreement with Table 1, in which was shown that the anti-(45 kDa protein) IgGs inhibited the enzyme activity, little or no activity could be expected in the immunoprecipitated pellet. This pellet contained the 45 kDa protein (Fig. 2*b*, tracks 2), but the enzyme was virtually inactivated (Table 2*b*). It was not possible to dissociate an active 45 kDa protein from the complex by means of the reagents tested in Table 3. These control experiments indicate that considerable loss of activity occurred due to conditions necessary for the disruption of the immune complex. If mild conditions for dissociation can be found, then the anti-(45 kDa protein) IgGs may be used to purify the active 45 kDa protein.

In previous studies (Oestreicher *et al.*, 1983; Gispen *et al.*, 1985) we reported that, in synaptic plasma membranes isolated from rat brain, the extent of the phosphorylation of the brain-specific 48 kDa B-50 protein modulates the activity of PtdIns4*P* kinase (see also Van Dongen *et al.*, 1985). In a series of immunochemical experiments, using the SDS-gel/immunoperoxidase method (Van Raamsdonk *et al.*, 1977) and employing specific IgGs to B-50 (Oestreicher *et al.*, 1983) and to the 45 kDa protein, we have compared the presence of B-50 and the 45 kDa protein, in solubilized protein fractions derived from a homogenate and

synaptic plasma membranes of total rat brain and various homogenates of peripheral rat tissues, such as liver, lung, heart, muscle and kidney. As we reported previously (Kristjansson *et al.*, 1982), we found that, whereas as predicted the B-50 protein was exclusively present in synaptic plasma membranes and brain homogenate, the 45 kDa protein could be detected in all tissues studied (results not shown). The ubiquitous intracellular presence of the 45 kDa protein is in good agreement with the widespread occurrence of the PtdIns4*P* kinase activity reported by others (see reviews Downes & Michell, 1982; Abdel-Latif, 1983).

In summary, we have presented the purification of IgGs against 45 kDa protein from rat brain cytosol and have provided further evidence that PtsIns4*P* kinase activity resides in the 45 kDa protein. Hence, the PtdIns4*P* kinase IgGs may serve as a new tool in the study of polyphosphoinositide metabolism.

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