The Journal of Experimental Biology 204, 487–493 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
JEB2818

INOSITOL-1,4,5-TRISPHOSPHATE-BINDING PROTEINS CONTROLLING THE PHOTOTRANSDUCTION CASCADE OF INVERTEBRATE VISUAL CELLS

AKIO KISHIGAMI[1,*], TOMIO OGASAWARA[2], YUTAKA WATANABE[2], MASATO HIRATA[3], TOMOKO MAEDA[4], FUMIO HAYASHI[4] AND YASUO TSUKAHARA[1]

[1]*Institution of Chemical and Physical Research (RIKEN), Sendai 980-0845, Japan,* [2]*Department of Resources Chemistry, Faculty of Engineering, Ehime University, Matsuyama 790-8577, Japan,* [3]*Department of Biochemistry, Faculty of Dentistry, Kyushu University, Fukuoka 812-8582, Japan* and [4]*Department of Biology, Faculty of Science, Kobe University, Kobe 657-0013, Japan*

*e-mail: squid@postman.riken.go.jp

Accepted 10 November 2000; published on WWW 15 January 2001

Summary

The main phototransduction cascade in invertebrate visual cells involves the turnover of phosphatidylinositol, an important biochemical mechanism common to many signal-transduction systems. Light-activated rhodopsin stimulates guanine nucleotide exchange on the Gq class of G-protein, which activates phospholipase C to hydrolyze phosphatidylinositol 4,5-bisphosphate to inositol-1,4,5-trisphosphate and diacylglycerol. Subsequently, inositol-1,4,5-trisphosphate-binding proteins continue the signal cascade. Here, we report on the first inositol-1,4,5-trisphosphate-binding proteins demonstrated in an invertebrate visual system with our investigation of the photosensitive rhabdoms of squid. We screened the ability of proteins to interact with inositol-1,4,5-trisphosphate by affinity column chromatography with an inositol-1,4,5-trisphosphate analogue. We detected an inositol-1,4,5-trisphosphate-binding affinity in phospholipase C, receptor kinase and five other proteins in the cytosolic fraction and, surprisingly, rhodopsin in the membrane fraction. A binding assay with ^{3}H-labelled inositol-1,4,5-trisphosphate demonstrated the inositol-1,4,5-trisphosphate affinity of each of the purified proteins. Since rhodopsin, receptor kinase and phospholipase C are involved upstream of phosphatidylinositol turnover in the signal cascade, our result suggests that phosphatidylinositol turnover is important in feedback pathways in the signalling system.

Key words: inositol-1,4,5-trisphosphate, phospholipase C, rhodopsin kinase, rhodopsin, phototransduction, vision, cephalopod, squid, *Todarodes pacificus.*

Introduction

In many cell types, stimulation by hormones, neurotransmitters and growth factors activates the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) catalyzed by phospholipase C (PLC) (Exton, 1996). Many studies have demonstrated light-dependent phosphatidylinositol (PI) turnover through rhodopsin and a Gq class G-protein in invertebrate visual cells (Yarfits and Hurley, 1994). Studies of the cellular expression of *trp* and *trpl* genes, which are essential for the light-induced cation conductance in *Drosophila melanogaster* visual cells (Niemeyer et al., 1996), have demonstrated that the proteins TRP and TRPL have Ca^{2+} channel activity and that the channel gating mechanism is located downstream of PI turnover (Friel, 1996; Montell, 1997; Chyb et al., 1999). Phosphatidylinositol turnover is very important for the activation process in invertebrate phototransduction. The *Drosophila inaC* gene encodes a photoreceptor-specific protein kinase C (PKC) regulated by Ca^{2+} and diacylglycerol (Smith et al., 1991). The INAC protein is necessary for deactivation and desensitization (Smith et al., 1991; Ranganathan et al., 1991). Because PI turnover generally activates PKC, it is also involved in deactivation and adaptation processes in invertebrate phototransduction.

No previous study has reported an interaction between inositol-1,4,5-trisphosphate (IP_3) and proteins in an invertebrate visual system, although IP_3 plays an important role as a second messenger continuing the signal cascade after PI turnover. Here, we demonstrate an interaction between proteins and IP_3 in a rhabdomeric fraction of squid visual cells that produces IP_3 in response to light (Vandenberg and Montal, 1984a; Vandenberg and Montal, 1984b; Szuts et al., 1986; Brown et al., 1987; Wood et al., 1989). We investigated IP_3-binding proteins using affinity chromatography with a column coupled to a synthetic IP_3 analogue. This IP_3 column has proved excellent for characterizing the specific IP_3-binding activity of proteins in rat brain (Hirata et al., 1990; Kanematsu et al., 1992). The large eyes of squid provide a useful preparation for biochemical techniques such as affinity chromatography, because it is easy to collect sufficient proteins from the visual

system for experimentation. We prepared two fractions from the rhabdomeric microvillar fraction purified from squid visual cells to load onto the IP_3 column. One was the buffer-soluble fraction prepared by washing rhabdomeric membranes. The other was the membrane protein fraction solubilized from washed rhabdomeric membranes using a detergent. Detection using specific antibodies indicated that elutions of the buffer-soluble fraction contained PLC and receptor kinase, while rhodopsin was present in the detergent-soluble fraction.

Materials and methods

Preparation of visual cell membranes

Live squid *Todarodes pacificus* were collected in the Japan Sea at night by the research ship *Choukai-maru* (Japan Sea National Fisheries Research Institute). They were frozen at −40 °C after dark-adaptation and stored in the dark prior to use. Under dim red light, frozen squid were partially thawed, and the frozen eyes were enucleated. The eye cups were prepared and agitated in buffer A [20 mmol l^{-1} Hepes, pH 7.5, 1 mmol l^{-1} dithiothreitol (DTT), 1 mmol l^{-1} $CaCl_2$, 1 mmol l^{-1} $MgCl_2$, 400 mmol l^{-1} NaCl, 0.1 mmol l^{-1} phenylmethylsulphonyl fluoride (PMSF), 5 μmol l^{-1} leupeptin, 5 μmol l^{-1} E64] to detach the outer segments from the visual cells. The outer segments were then collected by centrifugation at 30 000 ***g*** for 30 min at 4 °C and homogenized with 34 % sucrose in buffer A. After centrifugation to remove any remaining screening pigment, the floating membrane fraction consisting of detached rhabdomeric microvilli was diluted with an equal volume of buffer A and centrifuged at 100 000 ***g*** for 30 min at 4 °C. The resulting rhabdomal membrane pellet was then resuspended in buffer A (1 ml per retina) and stored in liquid nitrogen.

IP_3 affinity column chromatography

The IP_3 analogue 2-*O*-[4-(5-aminoethyl-2-hydroxy-phenylazo)benzoyl]-1,4,5-tri-*O*-phosphono-*myo*-inositol was synthesized (Hirata et al., 1990) for an IP_3 column (bed volume 1 ml), which was prepared and equilibrated with buffer B (20 mmol l^{-1} Tris-HCl, pH 7.5, 100 mmol l^{-1} NaCl, 1 mmol l^{-1} DTT, 1 mmol l^{-1} EDTA, 0.1 mmol l^{-1} PMSF). Rhabdomal membranes were washed by passage through a 21-gauge needle with buffer C [0.1 % (w/v) saponin, 20 mmol l^{-1} Hepes, pH 7.5, 1 mmol l^{-1} DTT, 400 mmol l^{-1} NaCl, 5 mmol l^{-1} $MgCl_2$, 1 mmol l^{-1} EGTA, 0.1 mmol l^{-1} PMSF]. After centrifugation at 100 000 ***g*** and 4 °C for 20 min, the supernatant was dialyzed against buffer B, and 1 mg of protein was loaded onto the IP_3 column. The protein concentration of the supernatant was determined using the method of Bradford (Bradford, 1976).

After washing with 40 ml of buffer B, attached proteins were eluted with 10 mmol l^{-1} phytic acid in buffer B, and 1 ml fractions were collected. Samples (10 μl) of each fraction were added to 200 μl of Bradford's solution on a 96-well microplate, and their absorption was measured at 590 nm using a microplate reader (NJ-2300; Intermed, Japan) to check the elution pattern. The washed and pelleted rhabdomeric membranes were further washed with saponin-free buffer C. After centrifugation, the pellet was resuspended in 2 % sucrose monolaurate (SML) in buffer B and incubated for 30 min on ice. After centrifugation at 100 000 ***g*** at 4 °C for 20 min, the supernatant was obtained. This extract, the membrane protein fraction, was loaded onto the IP_3 column, and column chromatography was performed in the same way as for the buffer-soluble fraction except that it was washed with 0.2 % SML in buffer B. The protein concentration of a sample, which included the dense detergent, was determined as described by Brown et al. (Brown et al., 1989). The absorption of 200 μl of each fraction was measured at 490 nm using a 96-well microplate and a microplate reader.

Sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis (PAGE) was performed (following Laemmli, 1970). Slab gels were stained with Coomassie Brilliant Blue (CBB). Western blotting was carried out (according to Lauriere, 1993), and proteins were transferred to a polyvinylidene difluoride (PVDF) membrane sheet to examine their immunoreactivity. Proteins bound to antibodies were detected using an ABC kit (Wako Pure Chemical Industries, Japan). Anti-β-adrenergic receptor kinase (βARK) antibody was purchased from Wako Pure Chemical Industries, Japan. Anti-squid PLC antibody was a gift from Dr T. Suzuki. Anti-squid rhodopsin antibody was a gift from Dr A. Terakita.

Purification of phospholipase C

Squid PLC was purified according to the method of Mitchell et al. (Mitchell et al., 1995) except that sulphate–Cellulofine (Seikagaku Kogyo Inc., Japan) was used instead of heparin sepharose. The soluble fraction of squid microvilli was loaded onto a sulphate column equilibrated with buffer D (20 mmol l^{-1} Hepes, pH 7.5, 1 mmol l^{-1} DTT, 1 mmol l^{-1} $MgCl_2$, 0.1 mmol l^{-1} PMSF) and eluted using a linear gradient of NaCl (0 mol l^{-1} to 1 mol l^{-1}). The fractions containing PLC were applied to a Bioscale-Q column (Bio-Rad) equilibrated with buffer D. The protein was eluted with a linear gradient of NaCl (0 mol l^{-1} to 1 mol l^{-1}).

Purification of receptor kinase

Squid receptor kinase was purified from the cytosolic extract of squid rhabdomeric membrane using a sulphate–Cellulofine column and a Q-column following the purification method described for octopus rhodopsin kinase (Kikkawa et al., 1998).

Purification of rhodopsin

The washed rhabdomeric membranes were dissolved in 2 % SML in buffer E (20 mmol l^{-1} Hepes, pH 7.5, 100 mmol l^{-1} NaCl, 1 mmol l^{-1} DTT, 0.1 mmol l^{-1} PMSF) and loaded onto a DEAE–cellulose column pre-equilibrated with 0.2 % SML in buffer E. The flow-through fraction was loaded onto a concanavalin A–Sepharose column (Amersham Pharmacia Biotech) pre-equilibrated with 0.2 % SML in buffer D. After washing with five bed volumes of 0.2 % SML in buffer E, rhodopsin was eluted with 100 mmol l^{-1} α-D-methylglycomanoside and 1 % SML in buffer E.

IP$_3$-binding assay

The amount of [^{3}H]IP$_3$ binding to each purified protein was assayed as described by Hirata et al. (Hirata et al., 1990). The assay mixture (0.45 ml) contained 50 mmol l^{-1} Tris-HCl (pH 8.3), 1 mmol l^{-1} EDTA, 1 nmol l^{-1} [^{3}H]IP$_3$ (0.37 kBq) and the purified protein. After incubation on ice for 15 min, bound [^{3}H]IP$_3$ was separated from free [^{3}H]IP$_3$ by precipitation by the addition of 50 µl of a solution of bovine γ-globulin and 0.5 ml of polyethylene glycol 6000 (30 %, w/w). After centrifugation, the pellet was dissolved in 0.5 ml of 0.1 mol l^{-1} NaOH, and the level of radioactivity was determined by liquid scintillation counting.

Results

Fig. 1A is a representative elution profile of the buffer-soluble fraction of squid rhabdom, eluted in 10 fractions with buffer containing phytic acid (inositol hexakisphosphate). Elution with phytic acid produced at least eight proteins in SDS–PAGE (CBB-stained) (Fig. 1B). Each protein was named according to its molecular mass as P130, P95, P77, P50, P35, P32, P26 and P10.

Two proteins (P130 and P95) showed IP$_3$ affinity and reacted with anti-PLC antibody (Suzuki et al., 1995b) (Fig. 1C). The protein P130 is the squid visual PLC, which is classified as a β4-type, and P95 is a PLC degradation product (Mitchell et al., 1995; Suzuki et al., 1995a). An IP$_3$-binding assay for purified squid PLC (P130) confirmed its ability to bind IP$_3$ (Fig. 2). The IP$_3$-binding activity of squid PLC is shown in Fig. 2B, which also shows the effect of including 300 µmol l^{-1} unlabelled IP$_3$ in the assay. Fig. 2C shows that PLC has higher affinity for IP$_3$ than inositol tetrakisphosphate (IP$_4$) in an inhibition assay that examines [^{3}H]IP$_3$ binding in the presence of various inositol polyphosphates. The level of affinity does not correspond to the number of phosphate groups present, suggesting that PLC has a specific binding activity with inositol phosphate.

The protein P77 bound anti-βARK antibody (Fig. 1D), indicating that P77 is a squid receptor kinase allied to βARK. The protein P77 could be purified by the same method as that used to prepare octopus rhodopsin kinase (Kikkawa et al., 1998). An IP$_3$-binding assay for purified squid receptor kinase confirmed its IP$_3$-binding properties, as shown in Fig. 3. The squid receptor kinase showed [^{3}H]IP$_3$-binding activity, which was reduced in the presence of 300 µmol l^{-1} unlabelled IP$_3$ (Fig. 3B). An inhibition assay shows that the level of affinity does not correspond to the number of phosphate groups present (Fig. 3C). The binding affinity of the receptor kinase (Fig. 3C) is greater than that of PLC (Fig. 2C), which corresponds to the finding that P77 shows longer tailing than P130 in the elution profile of the column (Fig. 1B).

In Fig. 4, we show the result of IP$_3$ affinity chromatography for the membrane protein fraction. Our results show that squid rhodopsin has an affinity for IP$_3$, because the eluate had an orange colour, with absorption at 490 nm (Fig. 4A), and the

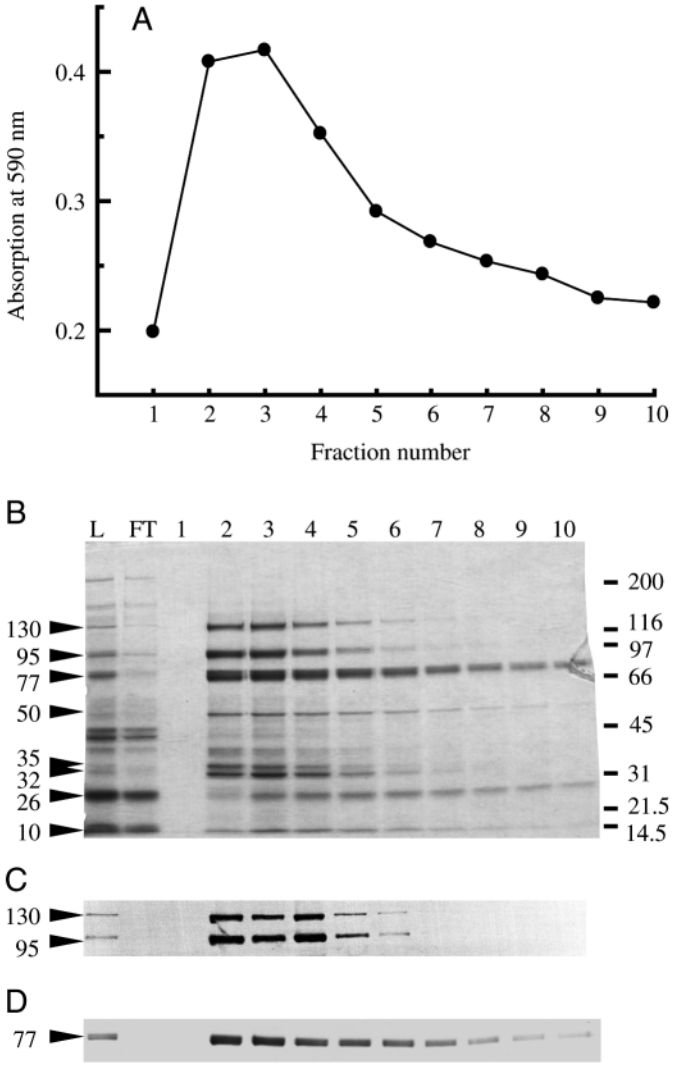

Fig. 1. Representative elution profile of inositol-1,4,5-trisphosphate (IP$_3$) column affinity chromatography for the buffer-soluble fraction of squid rhabdoms. (A) Elution profile. Filled circles, absorption at 590 nm. (B) Coomassie-Brilliant-Blue-stained gel of the fractions indicated in A. L, loaded sample; FT, flow-through fraction. The numbers above each lane refer to fraction numbers. The numbers on the right show molecular mass standards (kDa). The numbers on the left give the molecular mass (kDa) of proteins showing IP$_3$ affinity. (C) Anti-phospholipase C (PLC) immunoblot. (D) Anti-β-adrenergic receptor kinase (βARK) immunoblot.

45 kDa band in SDS–PAGE reacted with an anti-squid rhodopsin antibody (Fig. 4B,C). When purified rhodopsin was attached to the column, irradiation of the rhodopsin did not markedly affect its binding to the IP$_3$ column (data not shown). An IP$_3$-binding assay for purified rhodopsin confirmed its IP$_3$-binding properties (Fig. 5). Binding between squid rhodopsin and [^{3}H]IP$_3$ is shown in Fig. 5B. The effects of various inositol polyphosphates on binding to rhodopsin are shown in Fig. 5C. IP$_3$ and inositol pentakisphosphate (IP$_5$) show a higher binding affinity than IP$_4$ and inositol hexakisphosphate (IP$_6$).

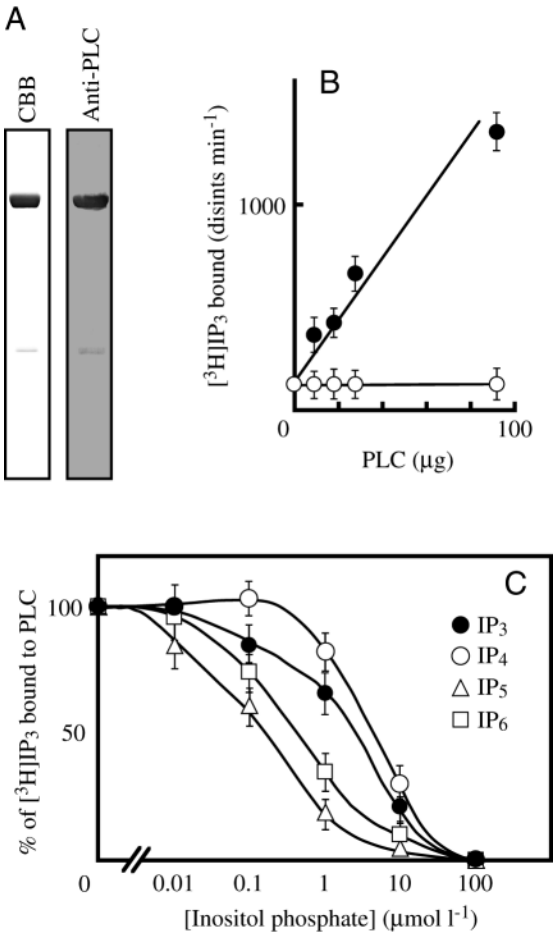

Fig. 2. Squid phospholipase C (PLC) binds inositol-1,4,5-trisphosphate (IP_3). (A) Purified PLC (130 kDa) was subjected to SDS–PAGE and stained with Coomassie Brilliant Blue (CBB). A western blot probed with anti-PLC shows the reactivity of a CBB-stained protein. (B) IP_3-binding activity of purified PLC. Filled circles show [^{3}H]IP_3 binding to varying amounts of PLC. Open circles show [^{3}H]IP_3 binding in the presence of 300 μmol l^{-1} IP_3. Values are means ± S.E.M. (*N*=6). (C) Competitive inhibition of [^{3}H]IP_3 binding to squid PLC by various inositol phosphates. Values are means ± S.E.M. (*N*=6). Filled circles, inositol-1,4,5-trisphosphate; open circles, inositol-1,4,5,6-tetrakisphosphate (IP_4); open triangles, inositol pentakisphosphate (IP_5); open squares, inositol hexakisphosphate (IP_6).

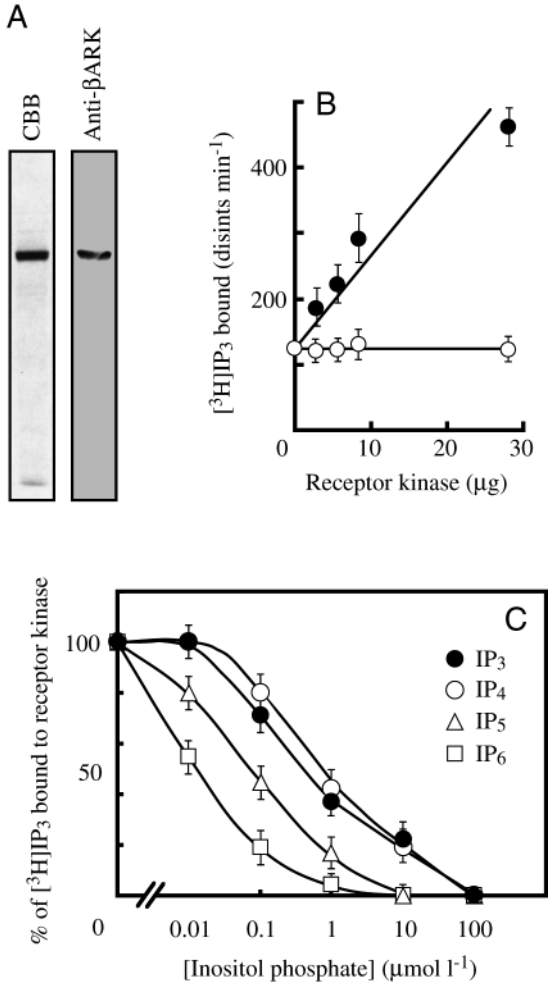

Fig. 3. Inositol-1,4,5-trisphosphate (IP_3)-binding properties of squid receptor kinase. (A) Purified receptor kinase was subjected to SDS–PAGE and stained with Coomassie Brilliant Blue (CBB) or transferred to PVDF membrane, where it is shown to bind anti-β-adrenergic receptor kinase (βARK). (B) IP_3-binding activity of purified receptor kinase. Filled circles, [^{3}H]IP_3 binding to increasing amounts of receptor kinase; open circles, [^{3}H]IP_3 binding in the presence of 300 μmol l^{-1} IP_3. Values are means ± S.E.M. (*N*=6). (C) Competitive inhibition of [^{3}H]IP_3 binding to squid receptor kinase by various inositol phosphates. Values are means ± S.E.M. (*N*=6). Filled circles, inositol-1,4,5-trisphosphate; open circles, inositol-1,4,5,6-tetrakisphosphate (IP_4); open triangles, inositol pentakisphosphate (IP_5); open squares, inositol hexakisphosphate (IP_6).

Discussion

It has been reported (Szuts et al., 1986) that, in intact squid retinas, the amount of IP_3 present was light-dependent, but no IP_4, IP_5 or IP_6 could be detected. The maximal light-induced concentration of IP_3 was calculated to be 1.2 mmol l^{-1} within the cytoplasm of the distal segment of squid photoreceptor cells, estimated from the PIP_2 content in the rhabdomere (Szuts, 1993). Inositol trisphosphate is rapidly reduced by hydrolysis (Wood et al., 1989; Wood et al., 1990). These results indicate that IP_3 is the main inositol phosphate present in squid photoreceptor cells and that the higher inositol polyphosphates are absent. Therefore, we believe that squid PLC, receptor kinase and rhodopsin are IP_3-binding proteins, although they may also bind other inositol phosphates (Figs 2C, 3C, 5C).

Squid PLC may have a specific binding site for inositol phosphate. It has been shown that PLC-δ in rat brain binds to this IP_3-analogue column at its pleckstrin homology (PH) domain rather than at its substrate-binding site (Kanematsu et al., 1992; Yagisawa et al., 1994); however, the primary structure of PLC in the squid visual system has no PH motif (Carne et al., 1995). The G-protein G βγ, which regulates PLCs *via* the PH domain, does not activate the squid PLC (Suzuki et

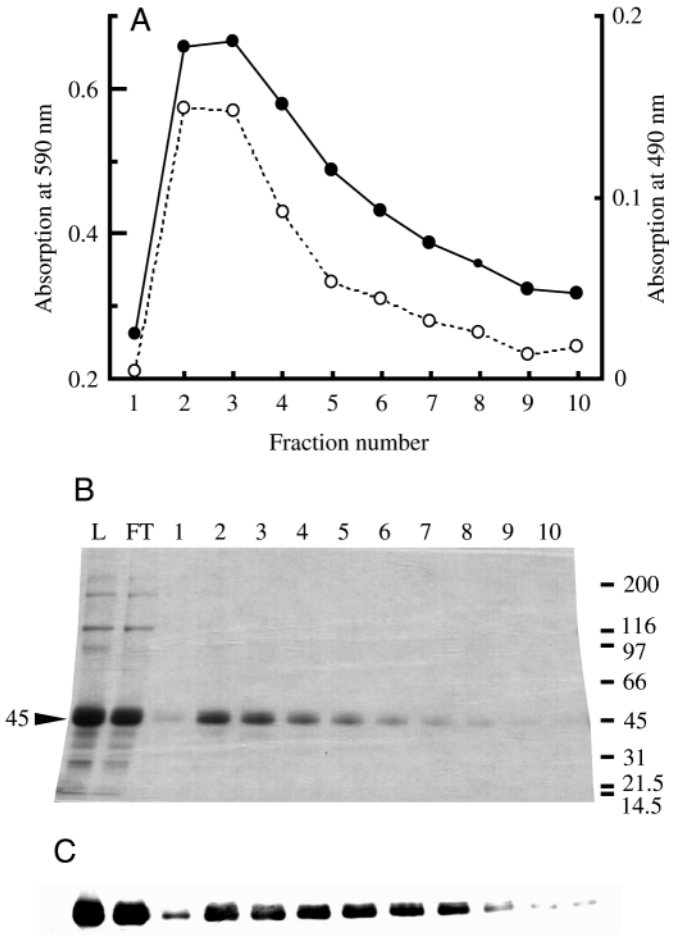

Fig. 4. Preparative elution profile of inositol-1,4,5-trisphosphate (IP_3) column affinity chromatography for the membrane protein fraction of squid rhabdoms. (A) Elution profile. Filled circles, absorption at 590 nm after staining; open circles, absorption at 490 nm with no staining. (B) Coomassie-Brilliant-Blue-stained gel of the fractions indicated in A. L, loaded sample; FT, flow-through fraction. The numbers above each lane refer to fraction numbers. The numbers on the right of the gel show molecular mass standards (kDa). The arrowhead on the left shows the molecular mass (kDa) of a protein showing IP_3 affinity. (C) Anti-rhodopsin immunoblot.

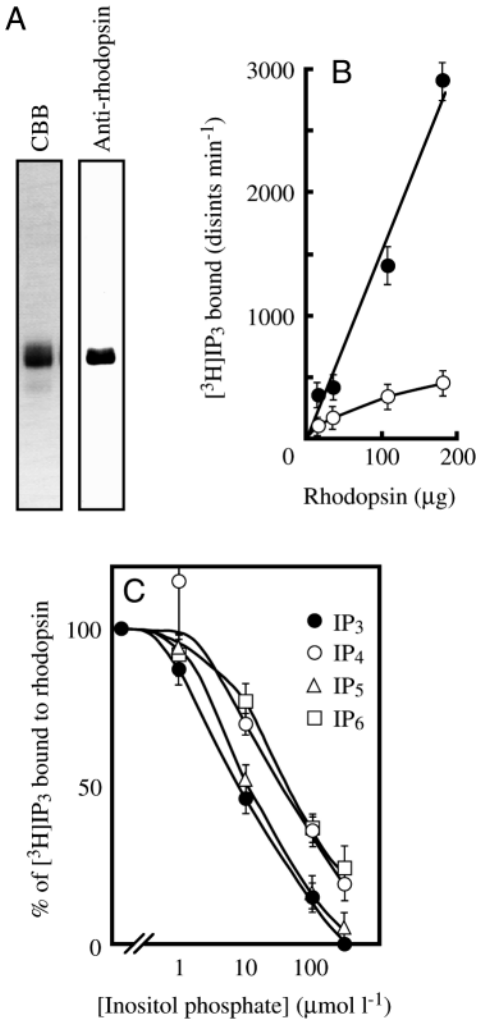

Fig. 5. The ability of purified squid rhodopsin to bind inositol-1,4,5-trisphosphate (IP_3). (A) Purified rhodopsin was subjected to SDS–PAGE and stained with Coomassie Brilliant Blue (CBB) or blotted and probed with an antibody to rhodopsin. (B) IP_3-binding activity of purified rhodopsin. Filled circles, [^{3}H]IP_3 binding to increasing amounts of rhodopsin; open circles, [^{3}H]IP_3 binding in the presence of 300 μmol l^{-1} IP_3. Values are means ± S.E.M. (*N*=6). (C) Competitive inhibition of [^{3}H]IP_3 binding to squid rhodopsin by various inositol phosphates. Values are means ± S.E.M. (*N*=6). Filled circles, inositol-1,4,5-trisphosphate; open circles, inositol-1,4,5,6-tetrakisphosphate (IP_4); open triangles, inositol pentakisphosphate (IP_5); open squares, inositol hexakisphosphate (IP_6).

al., 1995a). In the visual system, PLC has IP_3-binding activity but lacks a PH domain; however, like other PLCs, the squid visual PLC has a C2 domain (Carne et al., 1995). Using a BLAST database search (Altschul et al., 1990) to look for homologues with the peptide sequence of the C2 domain of the squid visual PLC, a sequence from 684R to 704N was shown to be similar to the sequence from 322R to 342S in the C2B domain of synaptotagmin I and II. This conserved sequence of synaptotagmin II has been shown to be an inositol-1,3,4,5-tetrakisphosphate-binding site (Fukuda et al., 1995). This finding suggests that inositol phosphate may modulate the enzymatic activity of squid PLC through this conserved region by means of feedback regulation.

In this report, we have shown that squid receptor kinase (P77) has IP_3-binding activity. It has recently been reported (Kikkawa et al., 1998) that an octopus rhodopsin kinase is similar to βARK in its structure and properties and also has a PH motif. It is therefore considered that P77 is squid rhodopsin kinase and that it has IP_3 affinity *via* its PH motif. Light-dependent phosphorylation of cephalopod opsin has been reported (Kikkawa et al., 1998; Paulsen and Hoppe, 1978; Vandenberg and Montal, 1984b; Tsuda et al., 1989). In the vertebrate system, work with transgenic mice has demonstrated that phosphorylation of rhodopsin is necessary to switch off the photoresponse (Makino et al., 1995). In the cephalopod system, rhodopsin phosphorylation by rhodopsin kinase may

be involved in the deactivation of rhodopsin for a similar quenching of the photoresponse. The function of cephalopod rhodopsin kinase must be dependent on phosphatidylinositol *via* a PH motif, as for βARK (Pitcher et al., 1996; DebBurman et al., 1996; Touhara, 1997). Visual PKC, which is activated by diacylglycerol and Ca^{2+}, is required for deactivation (Smith et al., 1991; Ranganathan et al., 1991). Regulation by the visual PKC in the invertebrate system includes phosphorylation of rhodopsin kinase because the PH domain is a common substrate of PKC (Mayer et al., 1993; Haslam et al., 1993). The metabolism of PIP_2 intervenes in the deactivation process through regulation of the βARK-like rhodopsin kinase.

Our identification of P130, P95 and P77 shows that this IP_3 column system is useful for identifying IP_3-binding proteins in visual cells, suggesting that other attached proteins have IP_3-binding properties and important roles in the visual system. Not all the elution profiles of attached proteins showed an identical pattern (Fig. 1B), suggesting that each protein has an individual affinity for IP_3 that would enable it to have a physiological function. Studies of unusual photoresponse mutants of *Drosophila melanogaster* have shown that abnormal enzymes in the inositol phospholipid metabolic cascade affect the visual system (Pak, 1995; Hardie and Minke, 1995; Zuker, 1996). In the squid retina, light-induced IP_3 is degraded to inositol bisphosphate by IP_3 phosphatase (Wood et al., 1989; Wood et al., 1990). The IP_3-binding proteins in the soluble protein fraction include signalling proteins and enzymes of PI metabolism such as IP_3 phosphatase.

We found that squid rhodopsin has a specific affinity for IP_3. The possibility that the IP_3-binding property is involved in a physiological regulatory process through rhodopsin is complicated because the IP_3-binding site occasionally interacts with PIP_2 since PIP_2 and IP_3 share an inositol structure with similar charge distribution. In addition, rhodopsin has three interaction proteins, G-protein, rhodopsin kinase and arrestin. For an accurate study of the interactions among the proteins, it would be necessary to carry out experiments with purified proteins to determine whether rhodopsin binds to PIP_2, to IP_3 or to both of them under physiological conditions.

In this paper, we have demonstrated the ability of PLC, receptor kinase and rhodopsin from squid visual cells to interact with IP_3. All three are abundant in the cell and relate to the upstream process of PI turnover. The results indicate that light-dependent PI turnover has feedback effects on upstream steps in the signalling system. PI turnover also operates the gating of light-dependent channels in the invertebrate visual system since it has been shown that inositol lipid signalling controls Ca^{2+} channel activity in cells expressing *trp* and *trpl* (Friel, 1996; Montell, 1997; Chyb et al., 1999). The turnover of PI organizes the division of signalling into activation, deactivation and adaptation steps, forming a complete signal-transduction system in the invertebrate visual cell.

We thank Drs S. Hasegawa, H. Kidokoro and I. Yosho (Japan Sea National Fisheries Research Institute) for their help with fresh squid collection. We thank Dr A. Terakita for his gift of anti-squid rhodopsin antibody and Dr T. Suzuki for his gift of anti-squid PLC antibody. We specially thank Dr H. Kosugi (Tohoku University) and Mr K. Magome (Sendai Wako Chemicals Co.) for introducing A.K. to Y.W. We thank Dr I. Gleadall for comments on the manuscript.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990). Basic local alignment search tool. *Mol. Biol.* **215**, 403–410.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analyt. Biochem.* **72**, 248–254.

Brown, J. E., Watkins, D. C. and Malbon, C. C. (1987). Light-induced changes in the content of inositol phosphates in squid (*Loligo pealei*) retina. *Biochem. J.* **247**, 293–297.

Brown, R. E., Jarvis, K. L. and Hyland, K. J. (1989). Protein measurement using bicinchoninic acid: elimination of interfering substances. *Analyt. Biochem.* **180**, 136–139.

Carne, A., McGregor, R. A., Bhatia, J., Sivaprasadarao, A., Keen, J. N., Davies, A. and Findlay, J. B. (1995). A β-subclass phosphatidylinositol-specific phospholipase C from squid (*Loligo forbesi*) photoreceptors exhibiting a truncated C-terminus. *FEBS Lett.* **372**, 243–248.

Chyb, S., Raghu, P. and Hardie, R. C. (1999). Polyunsaturated fatty acids activate the *Drosophila* light-sensitive channels TRP and TRPL. *Nature* **397**, 255–259.

DebBurman, S. K., Ptasienski, J., Benovic, J. L. and Hosey, M. M. (1996). G protein-coupled receptor kinase GRK2 is a phospholipid-dependent enzyme that can be conditionally activated by G protein βγ subunits. *J. Biol. Chem.* **271**, 22552–22562.

Exton, J. H. (1996). Regulation of phosphoinositide phospholipases by hormones, neurotransmitters and other agonists linked to G proteins. *Annu. Rev. Pharmac. Toxicol.* **36**, 481–509.

Friel, D. D. (1996). TRP: its role in phototransduction and store-operated Ca^{2+} entry. *Cell* **85**, 617–619.

Fukuda, M., Kojima, T., Aruga, J., Niinobe, M. and Mikkoshiba, K. (1995). Functional diversity of C2 domains of synaptotagmin family. Mutational analysis of inositol high polyphosphate binding domain. *J. Biol. Chem.* **270**, 26523–26527.

Hardie, R. C. and Minke, B. (1995). Phosphoinositide-mediated phototransduction in *Drosophila* photoreceptors: the role of Ca^{2+} and trp. *Cell Calcium* **18**, 256–274.

Haslam, R. J., Koide, H. B. and Hemmings, B. A. (1993). Pleckstrin domain homology. *Nature* **363**, 309–310.

Hirata, M., Watanabe, Y., Ishimatu, T., Yanaga, F., Koga, T. and Ozaki, S. (1990). Inositol 1,4,5-trisphosphate affinity chromatography. *Biochem. Biophys. Res. Commun.* **168**, 379–386.

Kanematsu, T., Takeya, H., Watanabe, Y., Ozaki, S., Yoshida, M., Koga, T., Iwanaga, S. and Hirata, M. (1992). Putative inositol 1,4,5-trisphosphate binding proteins in rat brain cytosol. *J. Biol. Chem.* **267**, 6518–6525.

Kikkawa, S., Yoshida, N., Nakagawa, M., Iwasa, T. and Tsuda, M. (1998). A novel rhodopsin kinase in octopus photoreceptor possesses a pleckstrin homology domain and is activated by G protein βγ-subunits. *J. Biol. Chem.* **273**, 7441–7447.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

Lauriere, M. A. (1993). A semidry electroblotting system efficiently

transfers both high- and low-molecular-weight proteins separated by SDS–PAGE. *Analyt. Biochem.* **212**, 206–211.

Makino, C. J., Peachey, N. S., Baylor, D. A. and Simon, M. I. (1995). Mechanisms of rhodopsin inactivation *in vivo* as revealed by a COOH-terminal truncation mutant. *Science* **267**, 374–377.

Mayer, B. J., Ren, R., Clark, K. L. and Baltimore, D. (1993). A putative modular domain present in diverse signaling proteins. *Cell* **73**, 629–630.

Mitchell, J., Gutierrez, J. and Northup, J. K. (1995). Purification, characterization and partial amino acid sequence of a G protein-activated phospholipase C from squid photoreceptors. *J. Biol. Chem.* **270**, 854–859.

Montell, C. (1997). New light on TRP and TRPL. *Mol. Pharmac.* **52**, 755–763.

Niemeyer, B. A., Suzuki, E., Scott, K., Jalink, K. and Zuker, C. S. (1996). The *Drosophila* light-activated conductance is composed of the two channels TRP and TRPL. *Cell* **85**, 651–659.

Pak, W. L. (1995). *Drosophila* in vision research. *Invest. Ophthalmol. Vis. Sci.* **36**, 2340–2357.

Paulsen, R. and Hoppe, I. (1978). Light-activated phosphorylation of cephalopod rhodopsin. *FEBS Lett.* **96**, 55–58.

Pitcher, J. A., Touhara, K., Payne, E. S. and Lefkowitz, R. J. (1996). Phosphatidylinositol 4,5-bisphosphate (PIP_2)-enhanced G protein-coupled receptor kinase (GRK) activity. Location, structure and regulation of the PIP_2 binding site distinguishes the GRK subfamilies. *J. Biol. Chem.* **270**, 11707–11710.

Ranganathan, R., Harris, G. L., Stevens, C. F. and Zuker, C. S. (1991). A *Drosophila* mutant defective in extracellular calcium-dependent photoreceptor deactivation and rapid desensitization. *Nature* **354**, 230–232.

Smith, D. P., Ranganathan, R., Handy, R. W., Marx, J., Tsuchida, T. and Zuker, C. S. (1991). Photoreceptor deactivation and retinal degeneration mediated by a photoreceptor-specific protein kinase C. *Science* **254**, 1178–1181.

Suzuki, T., Narita, K., Yoshihara, K., Nagai, K. and Kito, Y. (1995a). Phosphatidyl inositol–phospholipase C in squid photoreceptor membrane is activated by stable metarhodopsin *via* GTP-binding protein, Gq. *Vision Res.* **35**, 1011–1017.

Suzuki, T., Terakita, A., Narita, K., Nagai, K., Tsukahara, Y. and Kito, Y. (1995b). Squid photoreceptor phospholipase C is stimulated by membrane Gqα but not by soluble Gqα. *FEBS Lett.* **377**, 333–337.

Szuts, E. Z. (1993). Concentrations of phosphatidylinositol 4,5-bisphosphate and inositol 1,4,5-trisphosphate within the distal segment of squid photoreceptors. *Vis. Neurosci.* **10**, 921–929.

Szuts, E. Z., Wood, S. F., Reid, M. S. and Fein, A. (1986). Light stimulates the rapid formation of inositol trisphosphate in squid retinas. *Biochem. J.* **240**, 929–932.

Touhara, K. (1997). Binding of multiple ligands to pleckstrin homology domain regulates membrane translocation and enzyme activity of β-adrenergic receptor kinase. *FEBS Lett.* **417**, 243–248.

Tsuda, M., Tsuda, T. and Hirata, H. (1989). Cyclic nucleotides and GTP analogues stimulate light-induced phosphorylation of octopus rhodopsin. *FEBS Lett.* **257**, 38–40.

Vandenberg, C. A. and Montal, M. (1984a). Light-regulated biochemical events in invertebrate photoreceptors. I. Light-activated guanosinetriphosphatase, guanine nucleotide binding and cholera toxin catalyzed labeling of squid photoreceptor membranes. *Biochemistry* **23**, 2339–2347.

Vandenberg, C. A. and Montal, M. (1984b). Light-regulated biochemical events in invertebrate photoreceptors. 2. Light-regulated phosphorylation of rhodopsin and phosphoinositides in squid photoreceptor membranes. *Biochemistry* **23**, 2347–2352.

Wood, S. F., Szuts, E. Z. and Fein, A. (1989). Inositol trisphosphate production in squid photoreceptors. Activation by light, aluminum fluoride and guanine nucleotides. *J. Biol. Chem.* **264**, 12970–12976.

Wood, S. F., Szuts, E. Z. and Fein, A. (1990). Metabolism of inositol 1,4,5-trisphosphate in squid photoreceptors. *J. Comp. Physiol.* B **160**, 293–298.

Yagisawa, H., Hiratra, M., Kanematsu, T., Watanabe, Y., Ozaki, S., Sakuma, K., Tanaka, H., Yabutaa, N., Kamata, H., Hirata, H. and Nojima, H. (1994). Expression and characterization of an inositol 1,4,5-trisphosphate binding domain of phosphatidylinositol-specific phospholipase C-δ 1. *J. Biol. Chem.* **269**, 20179–20188.

Yarfits, S. and Hurley, J. B. (1994). Transduction mechanisms of vertebrate and invertebrate photoreceptors. *J. Biol. Chem.* **269**, 14329–14332.

Zuker, C. S. (1996). The biology of vision of *Drosophila*. *Proc. Natl. Acad. Sci. USA* **93**, 571–576.