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Overproduction and Immuno-affinity Purification of Myelin Proteolipid Protein (PLP), an Inositol Hexakisphosphate-Binding Protein, in a Baculovirus Expression System

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Myelin proteolipid protein (PLP) is a major integral membrane protein of central nervous system myelin and is considered to play a significant role in myelination. PLP has a four-transmembrane structure, judging from the hydropathy profile. In addition, it has $InsP_6$ binding activity. Here, we have succeeded in producing PLP in large quantities of 3.9 pg/cell (6 mg/L) by using a baculovirus expression system and developing an efficient purification method, maintaining InsP6 binding activity. The recombinant PLP (rPLP) was purified by ion-exchange and immunoaffinity chromatography in a non-organic solvent. The final yield of purified rPLP was 36%. The K_d and B_{max} values for the $InsP_6$-PLP binding were 55 nM and 33 pmol/μg protein, respectively. The K_d value of purified rPLP is equal to that of mouse brain PLP. These results indicate that purified rPLP keeps its native conformation and binds $InsP_6$ in an almost one-to-one ratio.

Myelin proteolipid protein (PLP) is a putative four transmembrane protein, and is localized in the plasma membrane of oligodendrocyte of the central nervous system. The plasma membrane containing PLP forms multilamellar stacks surrounding individual axons called myelin. The physiological function of PLP has remained obscure, but recent mutant studies have indicated that PLP might form a stabilizing membrane junction in myelin sheaths (1). The amino acid sequences of PLP are remarkably well conserved among bovines, mice, rats, and humans, suggesting that PLP has indispensable functions (2,3). PLP is synthesized on the rough endoplasmic reticulum (ER) and passes through the Golgi apparatus to the cell surface. Recent reports indicated that a significant lag exists between biosynthesis of PLP on rough ER and its insertion into the expanding myelin membrane (4-6). This delay suggests that PLP may be involved in the integral membrane protein transport system.

Several inositol polyphosphates have been known to be biological messenger molecules. For example, Inositol 1,4,5-trisphosphate ($InsP_3$) regulates intracellular Ca^{2+} concentration after binding to the receptor protein for $InsP_3$ (7-10). Recently it was found that when cells are stimulated, the concentration of inositol high polyphosphate series (IHPS) including $InsP_4$, $InsP_5$, and $InsP_6$ is enhanced in the cell (11-13). In addition, $InsP_4$ may also play a role in regulation of Ca^{2+}-influx phase of the response and modulation of various intracellular Ca^{2+} pools (14-16). It was hypothesized that $InsP_6$ acts as an intracellular signal by stimulating specific populations of neurons (17), and was reported that $InsP_6$ enhanced Ca^{2+}-influx in cultured cerebellar neurons (18).

Several recent studies suggested that $InsP_6$-binding proteins such as a clathrin assembly protein, AP-2, have been involved in intracellular vesicular transport (19-21). We recently found that the C2B domain of synaptotagmin binds IHPS. C2B is considered to be important for fusion to the plasma membrane. IHPS,

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Abbreviations used: PLP, myelin proteolipid protein; $InsP_3$, inositol 1,4,5-trisphosphate; $InsP_4$, inositol 1,3,4,5-tetrakisphosphate; $InsP_6$, inositol hexakisphosphate; IHPS, inositol high polyphosphate series; CHAPS, 3-[(3-cholamidopropyl)dimetylammonio]-1-propanesulfate; Sf-9, Spodoptera frugiperda cell; AcNPV, Autographa californica nuclear polyhedrosis virus; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; NHS, *N*-hydroxysuccinimide; EPPS, *N*-(2-hydroxyethyl)piperazine-*N*′-3-propanesulfonic acid; PEG, polyethylene glycol; CBB, coomassie brilliant blue R-250; ER, endoplasmic reticulum; rPLP, recombinant PLP produced in insect cells; mPLP, PLP purified from mouse brain.

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when injected inside the cell, inhibit the membrane fusion between the C2B domain of synaptotagmin and the plasma membrane, and thus inhibit exocytosis. When IHPS were injected in the synaptic terminal, not only exocytosis was blocked but also endocytosis was disturbed (22). Recently, we found that PLP is an $InsP_6$-binding protein, while the spliced isoform, DM-20, is not (23). These results suggest that $InsP_6$ may regulate the membrane dynamics including endo- or exocytosis or vesicular transport through the $InsP_6$-binding site. The transgenic mice overexpressed PLP shows abnormality in the formation of endomembrane such as Golgi and ER (24-26). PLP may be involved in membrane dynamics inside the cell.

In this study, in order to further characterize the property of PLP as an $InsP_6$ binding protein, we have set up a system to over-produce and purify recombinant PLP (rPLP) in insect cells and have confirmed the $InsP_6$-binding activity of rPLP as well as PLP purified from mouse brain (mPLP). In addition, we have purified native PLP by an immuno-affinity method, keeping PLP native. This expression system and immuno-affinity method can be used for the structural and functional analyses of PLP, such as the mechanism of $InsP_6$ binding, which may provide further insights into the physiological significance of this protein.

MATERIALS AND METHODS

Materials. Cell culture grade fetal bovine serum and cell culture medium were purchased from GIBCO BRL. [^{3}H] $InsP_6$ (444GBq/mmol) was purchased from Dupont-NEN. $InsP_6$ and CHAPS were purchased from Dojindo Laboratories. The anti-PLP monoclonal antibody (AA-3) was kindly supplied from Dr. Marjorie B. Lees (27). All of the other chemicals were of the highest purity commercially available.

Insect cell culture. *Spodoptera frugiperda* cells (Sf-9) were kindly provided by Dr. Y. Matsuura. They were grown in monolayer at 27°C in Grace's insect cell medium containing 10% (vol/vol) fetal bovine serum.

Vector construction. The PLP gene was obtained by polymerase chain reaction (PCR) using a plasmid containing mouse PLP cDNA (28) as a template. The DNA of 864bp, obtained from the PCR reaction mixture by electrophoresing on a 1.2% (wt/vol) agarose gel, was inserted in the BglII-BamHI site of the plasmid pVL1392 (InVitrogen) to construct the recombinant plasmid pVLPLP for recombinant virus production. The DNA sequence containing the PLP gene was confirmed with a DNA sequencer (373A, Applied Biosystems).

Vector virus production. Recombinant baculovirus was produced by cotransfecting Sf-9 cells in monolayer with 1 μg of the linearized *Autographa californica* nuclear polyhedrosis virus (AcNPV) DNA (PharMingen) and 5 μg of the transfer vector (pVLPLP) by the Lipofectin method. The recombinant virus AcNPV-PLP was isolated by single plaque assay and was amplified (29).

Production of rPLP. Sf-9 cells (3×10^7 cells on a 15cm plate) were infected with the recombinant baculovirus AcNPV-PLP at a multiplicity of infection of five, and the cell suspension was harvested three days after infection.

The cells were centrifuged at 1500 rpm for 5 min and washed twice with PBS (GIBCO BRL). The cells were resuspended in 0.32 M sucrose, 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μM leupeptin, 10 μM pepstatin A, 1 mM 2-mercaptoethanol, and 50 mM Tris-HCl, pH 8.0. The cell suspension was disrupted by fifty repetitions of sonicating at 40 W for 10 s and pausing for 50 s in an ice bath. The cell lysate was obtained by removing cell debris from the disrupted cell suspension with centrifugation at 1500 rpm for 5 min. The combined supernatant was centrifuged at 100,000 $\times$g for 60 min at 2°C to obtain the membrane protein fraction. This fraction was resuspended in 1% (vol/vol) Triton X-100, 1 mM EDTA, and 50 mM Tris-HCl, pH 8.0, and was then solubilized by fifty repetitions of sonicating at 40 W for 10 s and pausing for 50 s in an ice bath. The soluble fraction was obtained by removing the insoluble fraction with centrifugation at 26,000 $\times$g for 60 min at 2°C. The combined supernatant, named Triton extract, was applied to a column of DEAE-Sepharose equilibrated with 1% (vol/vol) Triton X-100, 10% (vol/vol) glycerol, 1 mM EDTA, and 50 mM Tris-HCl, pH 8.0 (Buffer 1). The DEAE-Sepharose flow-through fraction was collected with Buffer 1.

For the second step of purification, an immunoaffinity column was prepared by coupling purified anti-PLP monoclonal antibody (AA-3) to NHS-activated Sepharose (Pharmacia). Purified antibody was dialyzed at 4°C against 0.1 M $NaHCO_3$ containing 0.5 M NaCl, pH 8.3, and was concentrated to 7 mg/ml using an Amicon concentrator and a YM-50 membrane (Amicon). One ml of concentrated AA-3 was added to 1 ml of NHS-activated Sepharose that had been washed according to the manufacturer's instructions.

To the DEAE-Sepharose flow-through fraction, 5M NaCl was added to give a final concentration of 0.5 M. This solution was applied to the immunoaffinity column equilibrated with 10 mM CHAPS, 0.5 M NaCl, and 50 mM EPPS-NaOH, pH 8.0 and was circulated in the column for 1 h at 4°C using a tube pump. Recombinant PLP was eluted by the addition of 10m M CHAPS, 0.5 M NaCl, and 0.1 M glycine-HCl, pH 2.3 to the column. The pH of the eluate was immediately adjusted to 7 with one-tenth volume of 1 M Tris-HCl (pH9.0).

Measurement of [^{3}H] $InsP_6$ binding. $InsP_6$-binding was measured by the polyethylene glycol (PEG) precipitation method with minor modification (30). The assay mixture contained 2 mg/ml γ-globulin, 20 mM HEPES-KOH at pH 7.2 for the [^{3}H] $InsP_6$-binding assay.

SDS-PAGE and immunoblot analysis. Samples were incubated at room temperature for 20 min with sample buffer finally containing 2% (wt/vol) SDS, 20% (wt/vol) glycerol, 10 mM Tris-HCl, and 0.02% (wt/vol) coomassie brilliant blue R-250 (CBB), and were electrophoresed at 20 mA for 2 h using a 0.1% (wt/vol) SDS-15% (wt/vol) PAGE gel (PAGEL SPU-15S, ATTO). The proteins were stained with CBB.

The gel was equilibrated with 25 mM Tris-HCl/192 mM glycine buffer containing 20% (vol/vol) methanol, and the proteins were transferred to a nitrocellulose membrane (Clear Blot-P, ATTO) using a semidry system (ATTO). The membrane was soaked in skim milk (BlockAce, Dainippon Pharmaceutical) to block nonspecific binding, and was incubated in PBS containing 10% (vol/vol) BlockAce and AA-3 antibody at 37°C for 1 h. After washing with PBS containing 0.1% (vol/vol) Tween-20 (T-PBS), the membrane was incubated in PBS containing 10% (vol/vol) BlockAce, biotinylated goat anti-rat IgG antibody at 37°C for 1 h. After washing, immunoreactive bands were stained using Vectastain ABC kit (Vector Laboratories) according to the manufacturer's instructions with treatment of T-PBS containing 0.01% (wt/vol) diaminobenzidine, 0.1% (wt/vol) imidazol, 0.03% (wt/vol) hydrogen peroxide.

NH_2-terminal sequence analysis. The amino terminal sequence was determined by Edman degradation of purified samples using an automatic protein sequencer (477A, Applied Biosystems). The eluate of immunoaffinity chromatography or blotted membrane pieces were used for the purified samples containing rPLP. PAGEL running buffer (ATTO) containing 20% (vol/vol) methanol was used as a transfer buffer.

Immunogold labeling and electron microscopy. Transfected Sf-9 cells were fixed in 100 mM phosphate buffer containing 4% paraformaldehyde. Ultrathin sections of dehydrated samples were blocked

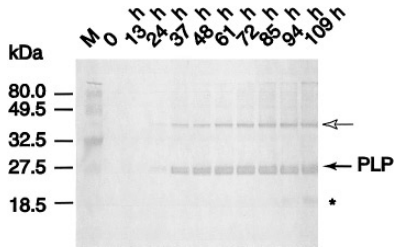

FIG. 1. The time course of PLP production in insect cells detected with immunoblotting. Sf-9 cells (4×10^6 cells on a 6cm plate) were infected with recombinant baculovirus AcNPV-PLP. Each sample contained the lysate of sonicated cells (2.25×10^4) harvested after a certain period of infection. The blots were detected by peroxidase-coupled antibody as described under Materials and Methods. The time after infection is shown at the top. Lane M, prestained proteins (BioRad) were used as molecular weight markers. Sizes of markers are shown at the left. The band indicated by the open arrow is a 43k PLP which is also shown in Fig. 5. The band indicated by the asterisk seems to be a degradation product of PLP.

with 4% BSA, incubated with AA-3 monoclonal antibody, washed with PBS, and incubated with gold-coupled anti-rat IgG polyclonal antibody (Amersham). The sections were refixed in 2% glutaraldehyde, postfixed in 2.5% uranyl acetate, and examined in a Hitachi H-7100 electron microscope at 100 keV.

Purification of mouse brain PLP (mPLP). Myelin was purified from the brain of adult mouse according to the procedure of Norton et al. (31). The purification of PLP from myelin was performed by four steps of chromatography after solubilization in a non-organic solvent (23).

RESULTS

Expression of the PLP gene. The coding sequence for the mouse PLP gene was inserted into the multiple cloning site of the baculovirus transfer vector pVL1392. The obtained pVLPLP plasmid was used to construct the recombinant virus AcNPV-PLP, as described in Materials and Methods. Sf-9 cells were infected with AcNPV-PLP and the amount of rPLP produced was determined by immunoblotting. Figure 1 shows the time course of rPLP production. At 72 h, the rPLP production reached a maximum, and two major protein bands with similar density were observed at the molecular weights of 26k and 24.5k. The total amount of rPLP produced was estimated at 12 mg/L by densitometry of the immunoblots. Therefore the productivity level of 26k PLP, which proved to have native conformation (described below), was 3.9 pg/cell (approximately 6mg/L).

Cellular localization of rPLP products in Sf-9 cells were visualized by indirect immunogold labeling and transmission electron microscopy (Fig. 2). The rPLP products were located not only on the cell surface but also in the cytoplasm. Unlike the authentic PLP in myelin, therefore, a considerable amount of rPLP was shown to remain in the organelles or cytosol.

Purification of rPLP. PLP produced by insect cells (3.8×10^8 cells) infected with AcNPV-PLP was purified as follows. Three days after infection, Sf-9 cells were harvested by centrifugation and sonicated. The cell lysate was obtained by removing cell debris from the disrupted cell suspension with centrifugation. The membrane protein fraction obtained by ultracentrifugation was solubilized with 1% (vol/vol) Triton X-100, 1 mM EDTA, and 50 mM Tris-HCl, pH 8.0, using a sonicator. The insoluble fraction was removed from the solubilized sample by ultracentrifugation. The combined Triton extract was resolved by immunoblotting as shown in Fig. 3. About 80% of the 26k PLP products was recovered in Triton extract, and their electrophoretic mobility was the same as that of mPLP purified from mouse brain. On the other hand, most of the 24.5k PLP products were insoluble with Triton X-100 solution and their mobility was higher than that of mPLP. These results indicated that the 24.5k PLP had a non-native structure.

The Triton extract was applied to an anion-exchange column, and rPLP was shown to be included in the flow-through fraction. NaCl was added to the pooled flow-through fraction to a final concentration of 0.5M to reduce possible non-specific interactions against resin of the immunoaffinity column. The conditioned flow-through fraction was applied to an immunoaffinity column coupled with anti-PLP monoclonal antibody (AA-3). A detergent-containing alkaline buffer is generally used as an eluent in the immunoaffinity chromatography of membrane proteins and receptors (32,33). In our first attempt, therefore, rPLP was eluted by the addition of 10mM CHAPS, 0.5M NaCl, and 0.1M diethylamine, pH 11.5, to the column. However, the peak of PLP was broad and the eluate lost $InsP_6$-binding activity (data not shown). When PLP was eluted by an acidic buffer (pH2.3), on the other hand, the peak of PLP was symmetrical and the PLP eluted by the acidic buffer proved to be $InsP_6$-binding active (described below).

Each fraction at various stages of purification was characterized by SDS-PAGE as shown in Fig. 4. Immunopurified rPLP yielded several bands on SDS-PAGE with molecular weights of 26k, 43k, 60k, and 70k. The N-terminal amino acid sequence of rPLP in the eluate pool was determined without any disturbance of contaminating proteins and was identical to that deduced from the PLP gene. Furthermore, the N-terminal amino acid sequence of the bands of 26k and 43k shown in Fig. 4 was identical to that of mPLP. Figure 5 shows the band pattern of immunopurified rPLP and mPLP. Both rPLP and mPLP yielded bands of 26k, 43k, 60k, and 70k as the result of immunoblotting. These results indicate that immunopurified PLP was separated to several bands under the mild denature condition on SDS-PAGE, and that bands of 26k, 43k, 60k, and 70k are identified as the mPLP protein. Its purity was estimated at more than 90% (the yield of 26k PLP was

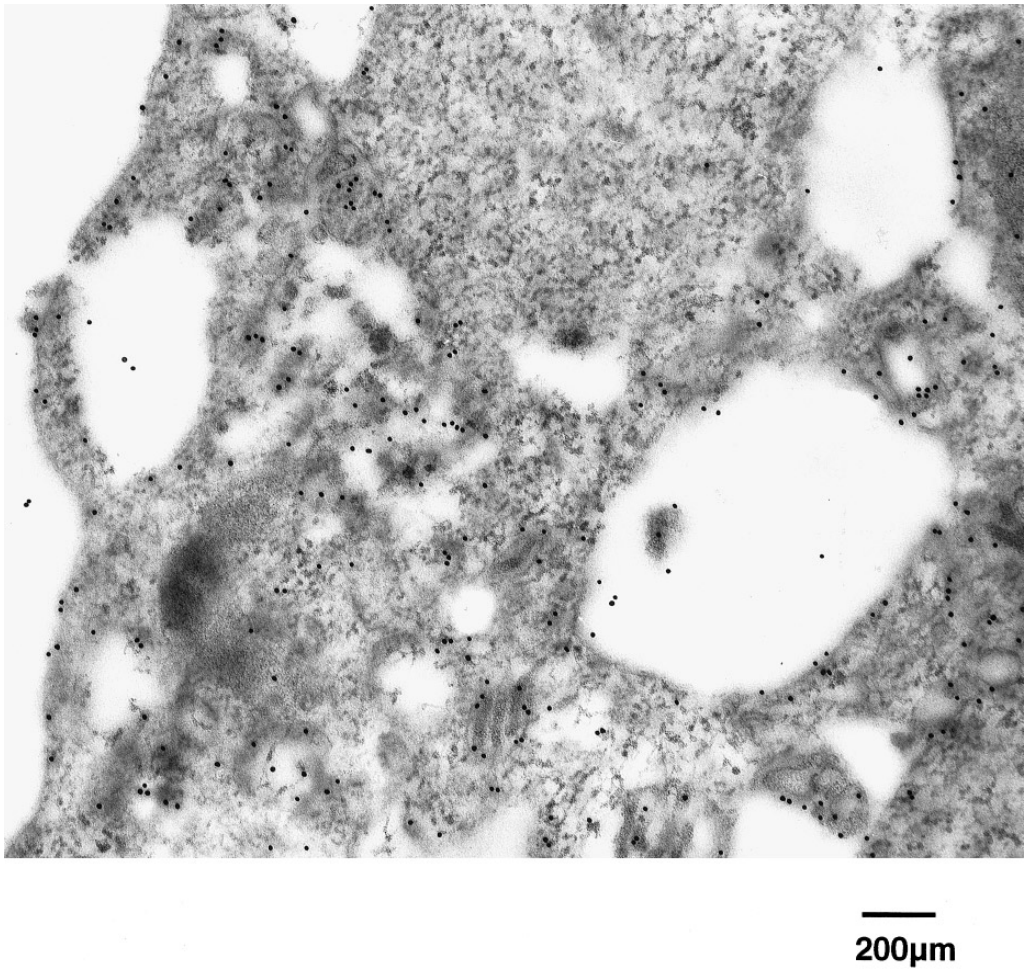

FIG. 2. Transmission electron micrograph of Sf-9 cells expressing PLP. Sf-9 cells, transfected with recombinant baculovirus AcNV-PLP, were harvested, fixed, and labeled with monoclonal antibody AA-3 followed by gold-coupled anti-rat IgG.

60%) from the intensity of bands on the SDS-PAGE gel stained with CBB. Eventually, 540 μg of purified rPLP were obtained from 3.8×10^8 Sf-9 cells.

$InsP_6$-binding assay. To determine whether purified rPLP has the same $InsP_6$-binding activity as mPLP, we analyzed the K_d and B_{max} of purified rPLP for [^{3}H] $InsP_6$. Recovery of PLP in the PEG precipitation method was 80%. Scatchard analysis of $InsP_6$ binding to immunopurified rPLP showed the K_d and B_{max} values of 55 nM and 33 pmol/μg, respectively (Fig. 6). The corresponding K_d value for mPLP is 52 nM (23). These results indicate that the $InsP_6$-binding activity of immunopurified rPLP is almost identical to that of mPLP. On the other hand, B_{max} of the immunopurified rPLP is larger than that of mPLP, 6.5 pmol/μg (23). The value 33 pmol/μg corresponds to the fact that 1 mol of PLP binds 0.87 mol of $InsP_6$, suggesting that the binding of $InsP_6$ to PLP occurs in a one-to-one ratio.

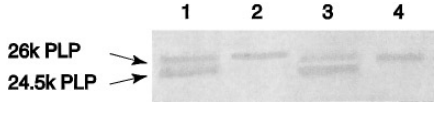

FIG. 3. Immunoblot analysis of PLP solubilized with Triton-X 100 buffer. The membrane protein fraction was obtained by centrifugation of the disrupted cell suspension with sonication. The membrane protein fraction was resuspended in 1 % (vol/vol) Triton X-100, 1mM EDTA, and 50mM Tris-HCl, pH 8.0. The soluble fraction was obtained by removing insoluble fraction with centrifugation at 26,000 ×g for 60 min. Lane 1, the membrane protein fraction resuspended in 1% (vol/vol) Triton X-100 buffer; lane 2, the soluble fraction; lane3, the insoluble fraction; lane4, mPLP purified from myelin described under Materials and Methods. Molecular weights of PLP are shown at the left as described in the legend to Fig. 1.

DISCUSSION

There have been several reports describing the expression of the PLP gene using mammalian systems. PLP has been expressed in human epithelial cells, human glioblastoma cells, and African green monkey kid-

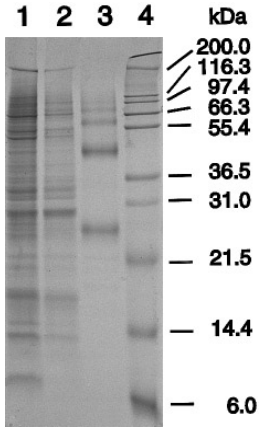

FIG. 4. SDS-PAGE analysis of PLP at various stages of purification. Triton extract (the soluble fraction, see Materials and Methods) was obtained by centrifuging the membrane protein fraction solubilized in Triton-X buffer. The DEAE-Sepharose flow-through fraction was obtained by applying the soluble fraction to a column of DEAE-Sepharose. The PLP was purified from the DEAE-Sepharose flow-through fraction with immunoaffinity column, prepared by coupling purified monoclonal antibody (AA-3) to NHS-activated Sepharose (Pharmacia). PLP was eluted by the addition of 10mM CHAPS, 0.5M NaCl, and 0.1M glycine-HCl, pH 2.3, to the column. Lane 1, Triton extract (2.25×10^4 cells); lane 2, DEAE-Sepharose flow-through fraction; lane 3, PLP purified by immunoaffinity chromatography; lane 4, Pre-stained proteins (NOVEX) were used as molecular weight markers. Sizes of markers are shown at the right.

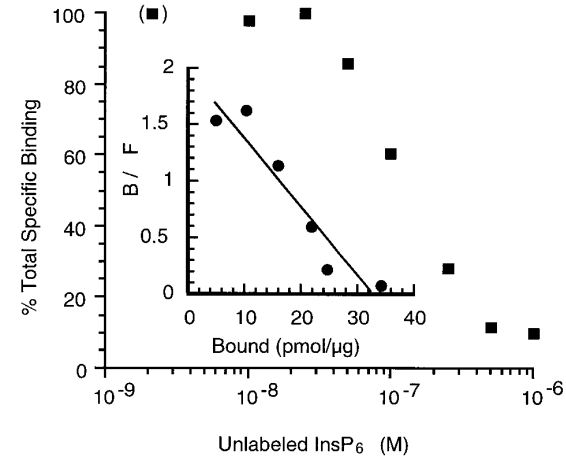

FIG. 6. Scatchard analysis of $InsP_6$ binding to PLP. Binding assay mixtures contained 0.25 μg of immunopurified PLP, 2.4 nM [^{3}H]$InsP_6$, various concentrations of unlabeled $InsP_6$, and 40 μg of γ-globulin in 20mM HEPES-KOH, pH 7.2 (100 μl). Samples were incubated for 10 min at 0 °C and binding activity was measured by polyethylene glycol precipitation method described under Materials and Methods. The inset shows the result of Scatchard analysis. B/F, bound/free. The estimated values were: Kd = 55nM, Bmax =33 pmol/μg protein.

ney cells (34). But the productivity of PLP in these cells was low and was not discussed. There are no reports of a baculovirus system for PLP production. Thus one of our objectives is the overproduction of biologically active PLP using the baculovirus system. In this study, we show the high-level expression (7.8 pg/cell) of PLP in insect cells. We obtained two separated peaks of rPLP (26k and 24.5k, Fig. 1) on SDS-PAGE. It was reported that two acylated types of PLP were separated in cation-exchange chromatography under organic conditions (35), which might be related to our result that the rPLP had at least two different structures. Under non-organic conditions, 26k PLP, but not 24.5k PLP, was soluble with Triton buffer. The electrophoretic mobility of 26k PLP was the same as that of mPLP (Fig. 3). These results strongly support the authenticity of 26k PLP. The quantity of 26k PLP was half of the total rPLP, suggesting that the production of native rPLP is 3.9 pg/cell (approximately 6 mg/L).

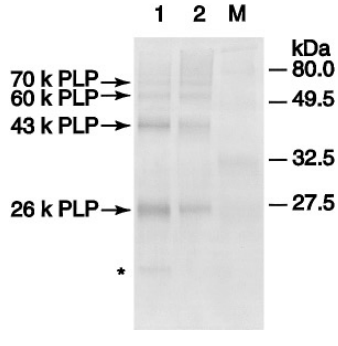

FIG. 5. Immunoblot analysis of PLP purified by immunoaffinity chromatography. Lane 1, rPLP purified from insect cells, the same sample as the lane 3 of Fig. 3; lane 2, mPLP purified from myelin as described under Materials and Methods. The purified mPLP was obtained by immunoaffinity chromatography in the same purification procedure as rPLP. Molecular weights of PLP were shown at the right as described in the legend to Fig. 1. The band indicated by the asterisk seems to be a degradation product of PLP.

Immunocytochemistry of rPLP-expressing Sf-9 cells reveals that rPLP proteins are localized in both plasma membranes and cytoplasm (Fig. 2), indicating that some rPLP products synthesized on the rough ER are partially transported to the plasma membrane. This phenomenon practically corresponds to the results of other expression systems in mammalian cells (34). Overexpression of PLP may have caused some abnormality in trafficking of the protein through the $InsP_6$ binding site, or have caused full-occupancy of the transport system of PLP. The heterogeniety of rPLP localization in Sf-9 cells might correspond to the heterogeniety of electrophoretic mobility (26k and 24.5k) of PLP species and their solubility with Triton X-100.

Recently, PLP have been purified from adult male ddY mouse cerebella by four steps of chromatography after solubilization in a non-organic solvent (23). We have investigated an easier purification protocol of rPLP using immunoaffinity chromatography, and succeeded in purifying rPLP by two steps of chromatography.

In previous studies, alkaline eluents containing a de-

tergent have often been used in immunoaffinity chromatography of membrane proteins and receptors. For example, in immunoaffinity chromatography of M6, which showed high homology with PLP, 0.1 M diethylamine HCl, pH 11.5, was used as the eluent (33). In the case of PLP, however, the use of alkaline eluent resulted in the denaturation of PLP. By switching to the acidic buffer, we have found that PLP was readily eluted without loss of $InsP_6$-binding activity.

On SDS-PAGE and CBB staining analysis, the immunopurified rPLP was separated into several bands (Fig. 4). We ascribe this separation to an incompletely denatured PLP sample due to the following reasons : (a) PLP purified from mouse brain had the same band pattern (Fig. 5), (b) the N-terminal amino acid sequences of both 26k and 43k rPLP were identical to that deduced from the PLP gene, (c) these bands were immunoreactive (Fig. 5), (d) the same band pattern was reported for PLP purified from bovine brain (35), and (e) the denaturing condition was rather mild for hydrophobic membrane proteins such as PLP. Consequently, the several bands shown in Figs. 4 and 5 should be completely due to the PLP protein and PLP is expected to form a homomultimer or an aggregation in a non-organic solvent.

From the densitometry analysis of CBB-stained gel, the purity of rPLP in the final rPLP-containing fraction was estimated at more than 90%. Consequently, starting from $3.8x10^8$ insect cells containing 1480 μg rPLP, we finally obtained 540 μg of purified rPLP so that the final yield of rPLP was 36%.

We measured the $InsP_6$-binding activity of immunopurified rPLP. The Scatchard analysis showed K_d and B_{max} values of 55 nM and 33 pmol/μg protein, respectively (Fig. 6). This K_d value is nearly equal to the reported value, 52 nM, for mPLP (23), indicating that, as far as the $InsP_6$-binding is concerned, rPLP is indistinguishable from mPLP.

The estimated binding stoichiometry obtained from B_{max} value is $[InsP_6]/[rPLP] = 0.87$. This result indicates that most of the Triton-solubilized and purified rPLP retains its $InsP_6$-binding activity and that one PLP molecule specifically binds one $InsP_6$ molecule.

The role of $InsP_6$-binding in the physiological function of PLP is still unclear, but the "one-to-one" binding characteristics between PLP and $InsP_6$ might suggest the possibility of a positive contribution of $InsP_6$ to regulation of the structural change of PLP in a novel signaling reaction.

In summary, we have succeeded in expressing PLP gene in baculovirus-infected insect cells and obtaining large quantities of the recombinant PLP, which is indistinguishable from the native PLP in mouse brain, concerning the $InsP_6$-binding activity and the electrophoretic mobility. We have developed an easier procedure for the purification of PLP to near-homogeneity using immunoaffinity chromatography. This established PLP-expression and immunoaffinity purification system will be used for the structural analysis of PLP in the presence or absence of $InsP_6$, and in the systematic functional studies of PLP using site-directed mutagenesis.

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