The Cytoplasmic Domain of Myelin Glycoprotein P₀ Interacts with Negatively Charged Phospholipid Bilayers*

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The intracellular COOH-terminal domain of the glycoprotein, P₀, has been proposed to be involved in the formation of the major dense line of peripheral myelin. We have addressed this hypothesis by generating and subsequently isolating a peptide fragment that contains 65 of the 69 residues of the cytoplasmic region of rat P₀. This peptide, termed P₀ (intramembrane), bound to artificial phospholipid vesicles and caused their rapid aggregation. The peptide-induced aggregation of membrane bilayers appeared to result from ionic interactions, since P₀ (intramembrane) vesicle association was decreased by 1) reducing the phosphatidyserine content of the membranes, 2) increasing the NaCl concentration of the surrounding buffer, or 3) elevating the divalent cation concentration within the buffers. Cationic disc gel electrophoresis of P₀ (intramembrane) revealed at least four charge isoforms of the peptide. Treatment of sciatic nerve slices with phospholipase C prior to isolation of P₀ (intramembrane) increased the amount of the more negatively charged species, suggesting that at least some of the change heterogeneity of the peptide can be attributed to differing phosphorylation states. The ability of P₀ (intramembrane) to bind to phospholipid bilayers implies that the cytoplasmic domain of P₀ may be responsible for the formation and maintenance of the myelin major dense line.

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The rapidly conducting axons of vertebrates are surrounded by myelin, a multilayered, membranous sheath of insulation that serves to greatly increase the velocity and the efficiency of action potential propagation (Bray et al., 1981). In the peripheral nervous system (PNS), myelin is elaborated by Schwann cells as a result of interaction with axons (Aguayo et al., 1976a, 1976b; Weinberg and Spencer, 1975, 1976). The most abundant protein in this specialized membrane is P₀, a highly conserved integral membrane glycoprotein that accounts for over 50% of the protein of PNS myelin (Greenfield et al., 1973).

P₀ has been the subject of numerous studies owing in part to its proposed role as an organizer of peripheral myelin (i.e. see Lemke and Axel, 1985) and to the controlled regulation of its expression (LeBlanc et al., 1987; LeBlanc and Poduslo, 1990; Trapp et al., 1988). P₀ of rats has a single transmembrane domain that separates the NH₂-terminal 124 residue extracellular region from the COOH-terminal 69 amino acid intracellular domain (Lemke and Axel, 1985). The extracellular portion of this myelin glycoprotein contains a region that is homologous to an immunoglobulin variable region domain (Salzer and Colman, 1989), and P₀ is therefore recognized as a member of the immunoglobulin gene superfamily. Other members of this large family of proteins are known to mediate cell-cell interactions in both the nervous and immune systems, and transfection of non-Schwann cells with vectors containing P₀ cDNA results in the aggregation of the cells (D’Urso et al., 1990; Filbin et al., 1990). These works support the hypothesis that the extracellular domain of P₀ is capable of homophilic interaction that presumably plays a role in the formation and maintenance of the myelin intraperiod line.

Little is known of the function(s) of the COOH-terminal cytoplasmic domain of P₀, although it has been suggested that this highly positively charged region (Lemke and Axel, 1985) may play a role in the PNS that is analogous to that of myelin basic protein (MBP) in the central nervous system (CNS). The latter has been shown to interact with negatively charged phospholipids (Cheifetz et al., 1985; Cheifetz and Moscarello, 1985; Young et al., 1982), and it has been proposed that the association of MBP with CNS myelin lipids is responsible for the apposition of the intracellular membrane leaflets that comprise the major dense line of myelin.

We report here studies describing the generation of a peptide fragment of P₀ containing nearly the entire intracellular domain of the glycoprotein. This peptide was produced by specific chemical cleavage of P₀ isolated from rat sciatic nerve. The COOH-terminal P₀ fragment (designated P₀ (intramembrane)) binds to artificial phospholipid vesicles containing negatively charged lipids and causes aggregation of these membranes. The properties of P₀ (intramembrane) described herein suggest the intracellular portion of the major PNS myelin protein may be involved in the close juxtaposition of cytoplasmic membrane surfaces, thus forming the major dense line.

MATERIALS AND METHODS

Protein Extraction from Rat Sciatic Nerve—Adult Sprague-Dawley rats were euthanized by sodium pentobarbital overdose, and the sciatic nerves, including the tibial, peroneal, and sural branches, were excised. The microdissection technique of Dyck et al. (1970) was used to separate the endoneurium from the perineurium and epineurium. In most studies, the isolated endoneurial segments were stored at –80°C until they were used for protein purification. In one experiment, endoneurial slices were incubated for 3 h at 37°C in 95% O₂, 5% CO₂ in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) containing 500 mg/ml PMA and/or 85 ng/ml okadaic acid. These endoneurial tissue were then frozen as above. The frozen endoneurial segments were homogenized on ice in pre-cooled 20 mM sodium phosphate, pH 7.4, 150 mM NaCl (0.5–1 M endoneurium), utilizing a battery-driven homogenizer (Omni International, Inc.). The homogenate was centrifuged (75,000 rpm, Sorvall

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‡ The abbreviations used are: PNS, peripheral nervous system; MBP, myelin basic protein; CNS, central nervous system; PAGE, polyacrylamide gel electrophoresis; Tricine, N-(2-hydroxy-1,1-bis(hydroxymethyl)-ethyl)glycine; PVDF, polyvinylidene difluoride; BiaTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-propane-1,3-diol; PS, phosphatidyserine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PC, phosphatidylcholine; FMA, phorbol 12-myristate 13-acetate.
Characterization of a COOH-Terminal P_0 Peptide

Instruments rotor model TPT 80.2) at 4 °C for 30 min. The supernatant was discarded, and the pellet was homogenized and centrifuged as above. The pellet from the second spin was homogenized in 0.5–1 ml of pre-cooled 3% (v/v) Triton-X 100 (Bio-Rad), 20 mM sodium phosphate, pH 7.4, 150 mM NaCl in an ice bath. The homogenate was kept on ice for 20 min and was then centrifuged as above. The supernatant was collected as crude P_0 extract. A second extraction of the final pellet with Triton-X 100 buffer resulted in significantly less P_0 than the first extraction, and some of the P_0 was partially degraded as revealed upon SDS-PAGE. Therefore, we routinely performed a single extraction with Triton-X 100 buffer.

Production of P_0 by Cation-exchange Chromatography—The crude P_0 extract was applied to a cellulose phosphate (Sigma; fine mesh) column (0.15 ml of resin/nerve) pre-equilibrated with 0.1% Triton-X 100, 20 mM sodium phosphate, pH 7.4, 150 mM NaCl. The column was washed with approximately 3 column volumes of 0.1% Triton-X 100, 20 mM sodium phosphate, pH 7.4, 150 mM NaCl to remove nonbound and loosely bound material. P_0 was then eluted with 3–4 column volumes of 0.1% Triton-X 100, 20 mM sodium phosphate, pH 7.4, containing 600 mM NaCl. Fractions were collected and assayed for protein content using a BCA assay (Pierce Chemical Co.). The purity of eluted P_0 was checked by SDS-PAGE as described above. Gels were stained in Coomassie Blue stain solution (Sigma). Lyophilized peptides were solubilized in trifluoroacetic acid. The peptide mixture was subsequently lyophilized for later use. Iso-PO fractions Non-P_0 fractions

Table I

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<th>Protein extraction from endoneurium (383 ± 108 ng/nerve)</th>
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<th>70.0 ± 2.5%</th>
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<td>Triton-insoluble</td>
<td></td>
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<tr>
<td>Protein fraction</td>
<td>42.7 ± 4.8%</td>
<td>55.1 ± 4.6%</td>
</tr>
<tr>
<td>P_0 fractions</td>
<td>Non-P_0 fractions</td>
<td></td>
</tr>
<tr>
<td>Triton-soluble fraction applied to cellulose phosphate</td>
<td>52.3 ± 4.8%</td>
<td>23.5 ± 3.9%</td>
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Lipid Vesicle Preparation—Bovine brain phosphatidylserine (PS), bovine liver phosphatidylinositol (PI), synthetic phosphatidylglycerol (PG), and egg phosphatidycholine (PC) were obtained from Avanti Polar lipids. Various ratios of an acidic phospholipid and PC in chloroform were mixed to a total weight of 9 mg, and the lipid mixtures were dried by rotary evaporation and 30 min of lyophilization before being used for the preparation of lipid vesicles. The dried lipids were suspended in 2.3 ml of 20 mM BisTris, pH 7.4, and 0.1 mM NaCl, and vesicles were prepared by a freeze-thaw method (Davidson et al., 1984). Briefly, the lipid suspension was sonicated in a bath-type sonicator (Bransonics; Inc.; model B-2200R-1) for approximately 40 min until the suspension was translucent. The suspension was then frozen in a dry ice/ethanol bath, thawed, and sonicated until the suspension became opalescent.

Vesicle Aggregation Assay—Lyophilized P_0 was solubilized in distilled water containing 0.1 mM NaCl to an approximate concentration of 1 mg/ml. The aggregation of lipid vesicles by P_0 was monitored by examining the turbidity (absorbance) of the mixture of peptide and lipid vesicles at 450 nm (Wood and Moscarello, 1989), utilizing Costar half-area 96-well microplates. Lipid vesicles and P_0 (typically 10 nmol of total lipid and 3 nmol of peptide) were added to microplate wells and mixed for 10 min at room temperature in a final volume of 150 μl of buffer as indicated in the figure legends. The absorbance of each well was read at 450 nm with a microplate reader (MR 4000, Dynatech Laboratories). For studies of the effects of divalent cations on vesicle aggregation, MgCl_2 and CaCl_2 were added to the buffer solution surrounding the lipid vesicles. Likewise, buffers of differing pH were utilized to study the effect of pH in peptide-mediated aggregation. Poly-L-lysine (Sigma, M = 4,000 by viscosity measurement) was used as a positive control in the aggregation assay, and aprotinin from bovine lung (Sigma; 60 amino acids, 6 G or A, and 8 Arg and Lys, M_s approximate, 6,500) was employed as a negative control. In all studies, background absorbance of vesicles incubated in the absence of peptide was subtracted from data.

Vesicle Co-aggregation Assay—P_0 was subsequently lyophilized for later use. This procedure was repeated with different concentrations of P_0 and for each peptide. The results were expressed as a percentage of the signal obtained from control samples.

RESULTS

Isolation of P_0—Purified P_0 was obtained using a relatively simple isolation procedure that avoids the denaturing conditions that have been used in previous isolation procedures (Bisazzeri et al., 1989; Kitamura et al., 1976; Roomi et al., 1978; Mezey and Verpoorte, 1981; Brunden et al., 1987). In summary (see Table I), adult rat sciatic nerve endoneurium was homog-
Characterization of a COOH-Terminal \( P_0 \) Peptide

![Image of SDS-PAGE gel with molecular mass markers](image)

**Fig. 1. Purification of \( P_0 \).** \( P_0 \) was purified as described under "Materials and Methods," and the resulting preparation was analyzed by 15% SDS-PAGE (lane 1). If sciatic nerve endoneurial membranes were solubilized with Triton X-100 buffer containing increased NaCl (0.65 M), a faster migrating form of \( P_0 \) was obtained after purification (lane 2). The migrations of molecular mass markers are indicated on the figure.

enzed in PBS, and a membrane fraction was obtained following centrifugation. This membrane pellet was resuspended in buffer containing 3% Triton X-100, a nonionic detergent that is relatively nondenaturing (Dean and Suarez, 1984). \( P_0 \) can be readily purified from the detergent mixture by separation on a cellulose phosphate column, with proteins other than the myelin glycoprotein eluting when several column volumes of PBS are passed through the resin. \( P_0 \) eluted as a broad peak with phosphate buffer containing >0.6 M NaCl. Utilizing such an isolation scheme, we routinely obtained 50–100 \( \mu \)g of purified 30-kDa \( P_0 \)/sciatic nerve (see Fig. 1, lane 1, and Table I). The apparent heterogeneous binding of \( P_0 \) to the matrix is consistent with the fact that the glycoprotein has multiple posttranslational modifications that affect its charge, including sulfation (Matthieu et al., 1975), sialylation (Roomi et al., 1978), and phosphorylation (Brunden and Poduslo, 1987; Singh and Spritz, 1976; Wiggins and Morell, 1980).

During the optimization of the \( P_0 \) isolation protocol, we noticed that increasing the concentration of NaCl in the Triton X-100 membrane solubilization buffer increased the efficiency of protein extraction. However, subsequent cellulose phosphate chromatography of high salt Triton extract resulted in the purification of a \( P_0 \) species with an apparent molecular mass of 24 kDa instead of the usual 30 kDa (Fig. 1, lane 2). This lower molecular mass \( P_0 \) is similar in size to that described by Agrawal et al. (1990), who found a proteolytically cleaved truncated form of the glycoprotein during incubation of sciatic nerve slices in vitro. As noted by this group, we found that the conversion of 30-kDa \( P_0 \) to 24-kDa degradation product was not inhibited by conventional protease inhibitors such as phenylmethylsulfonyl fluoride, L-1-tosylamide-2-phenylethyl chloromethyl ketone, and leupeptin (data not shown). Because of the increasing degradation of \( P_0 \) when elevated salt concentrations were used in the Triton mixtures, 150 mM NaCl was routinely used in the extractions.

**Generation and Isolation of \( P_{\text{intra}} \).**—The isolated \( P_0 \) was treated with iodosobenzoic acid (Mahoney et al., 1981) to obtain proteolytic fragments resulting from specific cleavage at tryptophan residues. \( P_0 \) contains 5 tryptophan residues in its NH\(_2\)-terminal extracellular domain, but only a single tryptophan at amino acid 154 within the COOH-terminal intracellular region comprised of residues 151–219 (Lemke and Axel, 1985). The resulting cleavage products were analyzed by SDS-PAGE, resolving at least five distinct molecular species (Fig. 2). To determine which of the peptides corresponded to the intracellular fragment beginning at amino acid 155 (\( P_{\text{intra}}^{\text{am}} \)), digests were separated electrophoretically and transferred to PVDF membranes. After staining of the transferred peptides, individual bands were excised and subjected to amino acid sequencing. The species with a molecular mass of approximately 11 kDa was found to have the predicted NH\(_2\)-terminal sequence of \( P_{\text{intra}}^{\text{am}} \). The apparent molecular mass of this peptide is greater than would be predicted from its sequence (amino acids 155–219, 7.4 kDa; see Lemke and Axel, 1985). This discrepancy presumably results from the abundance of lysyl and arginyl residues in \( P_{\text{intra}}^{\text{am}} \), causing aberrant migration during SDS-PAGE.

After desalting of the iodosobenzoic acid digest, the \( P_0 \) peptides could be separated by cellulose phosphate chromatography. The intracellular domain of \( P_0 \) is highly positively charged, and \( P_{\text{intra}}^{\text{am}} \) should thus bind tightly to the negative charges of the column matrix. Application of the digested \( P_0 \) to cellulose phosphate and subsequent washing of the resin with buffer containing 0.2 M NaCl revealed that most of the peptides were not strongly retained on the column. Gradient elution of bound peptides (0.3–0.7 M NaCl) resulted in the 11-kDa peptide corresponding to \( P_{\text{intra}}^{\text{am}} \), eluting as a broad peak starting at approximately 0.5 M NaCl. This late eluting material appeared to be primarily of a single molecular mass, as judged by SDS-PAGE (Fig. 3A, lane 2). The fractions from the dispersed \( P_{\text{intra}}^{\text{am}} \) peak were pooled, desalted, and lyophilized for subsequent studies. Using this procedure, approximately 100–200 \( \mu \)g of the intracellular fragment can be isolated per mg of digested \( P_0 \). In most of the \( P_{\text{intra}}^{\text{am}} \) preparations, there was a small amount (5–10%) of slightly lower molecular mass (7 kDa) material that co-eluted with the 11-kDa fragment (Fig. 3A, lane 2). This faster migrating species contained the same NH\(_2\)-terminal sequence as \( P_{\text{intra}}^{\text{am}} \), and appeared to result from chemical cleavage of the aforementioned 24-kDa truncated \( P_0 \). In fact, iodosobenzoic acid treatment of isolated 24-kDa \( P_0 \) resulted in a 7-kDa species eluting from cellulose phosphate with concentrations of NaCl >0.5 M (Fig. 3A, lane 3).

The 11-kDa \( P_{\text{intra}}^{\text{am}} \) appears to be composed of at least four charge isomers that were resolved by cathodic disc gel electrophoresis (Fig. 3B, lane 3). At least some of these isomers appear to represent different phosphorylated forms of \( P_0 \) that are affected by protein kinase C activation with phorbol esters (Ash-
Characterization of a COOH-Terminal P₀ Peptide

A

kD

17.0
14.5
10.6
8.2
6.2

1 2 3

Fig. 3. Purification and characterization of P₀ intra. A, P₀ intra was isolated as described under "Materials and Methods" and analyzed by Tris-Tricine SDS-PAGE (lane 2). P₀ intra, that has a truncated COOH terminus was isolated when 24-kDa P₀ was digested with iodosobenzoic acid (lane 3). Molecular mass markers are shown in lane 1. B, P₀ intra preparations from untreated sciatic nerve slices (lane 2), sciatic nerve slices treated for 3 h with okadaic acid (lane 2), or sciatic nerve slices treated for 3 h with okadaic acid and PMA (lane 3) were analyzed by cationic disc gel electrophoresis. More positively charged species migrate faster in this system.

B

Fig. 4. Dose-dependent vesicle aggregation caused by P₀ intra. Vesicle aggregation assays were performed as described under "Materials and Methods." Various amounts of P₀ intra and RPS (mole/mole) vesicles were mixed in 20 mM BisTris, pH 7.0, containing 0.1 M NaCl, and light scattering was monitored by determining the absorbance at 450 nm. Aprotinin was employed as a negative control, whereas poly-l-lysine (inset) was used as a positive inducer of vesicle aggregation.

resulted in aggregation of the membranes as monitored by light scattering at 450 nm. The extent of vesicle association was dependent on P₀ intra concentration (Fig. 4). As P₀ intra exceeded 60 μg in the assay, the amount of turbidity decreased. This may have reflected peptide-mediated disruption of the vesicle structure, resulting in decreased aggregation. The peptide-dependent vesicle association caused by P₀ intra appeared to be specific, as a peptide of similar size but reduced positive charge density (aprotinin; molecular weight of approximately 6,500) did not induce a change in the turbidity of the vesicle preparations (Fig. 4). In contrast, the positively charged polymer, poly-l-lysine, caused vesicle aggregation at concentrations lower than those needed with P₀ intra (Fig. 4, inset).

The induction of vesicle aggregation by P₀ intra implied that the cationic peptide was binding avidly to the phospholipid bilayers. This was directly demonstrated by subjecting a mixture of P₀ intra and vesicles to centrifugation, with resulting co-sedimentation of the lipids and peptide (Fig. 5). The peptide sedimentation was due to specific binding of P₀ intra to phospholipids, since centrifugation of the peptide alone did not result in its deposition. Moreover, centrifugation of an aprotinin/vesicle mixture did not result in aprotinin localization with the membrane pellet (Fig. 5).

To examine whether negatively charged lipids were necessary for the binding and changes in turbidity induced by P₀ intra, the peptide was examined in vesicle preparations containing varying amounts of PS. As seen in Fig. 6A, P₀ intra did not induce appreciable aggregation of vesicles made of 6% PS, 94% PC. However, the peptide caused a similar increase of turbidity in vesicle preparations containing 9 or 12% PS. The dependence on PS concentration for P₀ intra binding and aggregation of vesicles appears to reflect a general requirement for negatively charged lipid instead of a specific interaction with PS. P₀ intra also caused aggregation of phospholipid vesicles containing 12% PI or PG (Fig. 6B), whereas aprotinin did not elicit a change in turbidity (data not shown).

The effect of salt concentration was examined in the turbidity assays to further test the concept that the interaction of
et al. have examined the properties of the cytoplasmic region of P₀, by generating, through iodosobenzoic acid digestion, a proteolytic fragment of P₀ (P₀(intra)) that contains 65 of the 69 amino acids comprising the intracellular domain.

Addition of P₀(intra) to artificial phospholipid vesicles containing negatively charged lipids results in rapid aggregation of the vesicles as judged by light scattering at 450 nm. The interaction of the peptide with the bilayer structures appears to be largely ionic in nature, since 1) lowering the amount of PS in the lipid preparations to 6% effectively eliminates vesicle aggregation.

**FIG. 5. Binding of P₀(intra) to phospholipid vesicles.** Aprotinin or P₀(intra) were suspended in buffer containing 5% PS (mole/mole) vesicles. Following a 20-min incubation, the samples were subjected to centrifugation, and the amount of nonsedimented peptide was determined by analyzing aliquots of the resulting supernatants by SDS-PAGE followed by densitometric quantitation. The results are expressed as a percentage of the values obtained from control peptide samples that were treated as above in the absence of added phospholipid vesicles. Results are from triplicate assays, with standard deviations indicated by error bars.

% of Control

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<th>Aprotinin + vesicles</th>
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**FIG. 6. Dependence of P₀(intra)-mediated vesicle aggregation on acidic phospholipids.** A, P₀(intra) and lipid vesicles containing differing amounts of PS were mixed in 20 mM BisTris, pH 7.0, 0.1 M NaCl, and aggregation was monitored as described under "Materials and Methods." Results are from triplicate assays, with standard deviations indicated by error bars. B, P₀(intra) and lipid vesicles containing either 5% PS, PG, or PI were mixed in 20 mM BisTris, pH 7.0, 0.1 M NaCl, and aggregation was monitored as above. Results are from triplicate assays, with standard deviations indicated by error bars.

**A.**

**B.**

% of Control

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**DISCUSSION**

Although the extracellular domain of P₀ is likely to define certain aspects of myelin structure (D’Urso et al., 1990; Filbin et al., 1990), little is known about the role of the COOH-terminal cytoplasmic region of the protein. The high content of basic residues in this domain suggests that it may have properties similar to MBP, which is capable of interaction with phospholipid bilayers (Cheifetz et al., 1985; Cheifetz and Moscarello, 1985; Young et al., 1982). It is generally believed that MBP maintains the close apposition of adjacent cytoplasmic membrane leaflets that result in the major dense line of CNS myelin. The relatively low MBP content in the PNS (5-10% of total protein versus 35% in the CNS) suggests that P₀ may either serve to augment or substitute for MBP action in the PNS. This premise is supported by examination of the mouse mutants *shiverer* and *shiverer*mut, which have severely reduced levels of MBP and disrupted CNS myelin with relatively unaffected PNS myelin (Roach et al., 1983, 1985; Popko et al., 1988). We have examined the properties of the cytoplasmic region of P₀ by...
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10769

A.

\[ \text{OD (450 nm)} \]

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B.

\[ \text{Mg}^{++}, \text{Ca}^{++} \]

\[ \% \text{ of Control} \]

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<tr>
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**FIG. 7.** Inhibition of \( P_{\text{intracellular}} \)-mediated vesicle aggregation by NaCl and divalent cations. \( P_{\text{intracellular}} \) was mixed with 9% PS (mole/mole) vesicles in 20 mM BisTris, pH 7.0, containing various amounts of NaCl, and vesicle aggregation was analyzed. Results are from triplicate assays, with standard deviations indicated by error bars.

**FIG. 8.** Effect of pH on \( P_{\text{intracellular}} \)-mediated vesicle aggregation. \( P_{\text{intracellular}} \) and lipid vesicles containing 9% (mole/mole) PS were mixed in 0.1 M NaCl and 10 mM L-histidine with pH values as indicated. Results are from triplicate assays, with standard deviations indicated by error bars.

gregation; 2) increasing the ionic strength of the vesicle solutions minimizes the ability of \( P_{\text{intracellular}} \) to mediate membrane aggregation; and 3) increasing the concentrations of the divalent cations, \( \text{Mg}^{++} \) and \( \text{Ca}^{++} \), decreases light scattering of vesicle/\( P_{\text{intracellular}} \) mixtures. The ability of the intracellular domain of \( P_0 \) to bind and aggregate vesicles is not the result of nonspecific peptide effects, as addition of aprotinin to the vesicle suspensions did not cause a change in turbidity, nor did this peptide co-sediment with phospholipids. In contrast, the highly charged polymer, poly-\( \text{L}-\text{lysine} \), resulted in rapid aggregation of the membrane preparations. This is consistent with what has been observed in studies with MBP, where poly-\( \text{L}-\text{lysine} \) was shown to be more effective than MBP in inducing vesicle aggregation (Young et al., 1982).

The ability of the cytoplasmic region of \( P_0 \) to bind negatively charged phospholipids lends credence to the proposal that such interactions in vivo result in the formation of the major dense line. Posttranslational phosphorylation of the intracellular domain of \( P_0 \) may regulate its affinity for phospholipid. This has been demonstrated for MBP, where more anionic isoforms had a reduced ability to aggregate PS-containing vesicles (Cheifetz and Moscarello, 1985). \( P_0 \) is known to be phosphorylated in peripheral nerve by protein kinase C on one or more serine residues (Brunden and Poduslo, 1987), and the glycoprotein can be modified with phosphates on three distinct serines in vitro (Suzuki et al., 1990). Cationic disc gel electrophoresis of the isolated intracellular fragment reveals at least four species. If one of these isoforms is nonphosphorylated, there may be three naturally occurring phosphorylated forms of the protein in vivo. In future studies, we hope to separate charged isomers of \( P_{\text{intracellular}} \) and determine whether they, like MBP, show differing abilities to bind and aggregate phospholipid vesicles.

Very recent studies (Hayasaka et al., 1993a, 1993b; Kulkens et al., 1993) have demonstrated that certain cases of Charcot-Marie-Tooth neuropathy type 1B appear to be attributable to mutations within \( P_0 \). All of these mutations are found within the extracytoplasmic Ig-like domain of \( P_0 \), suggesting that the demyelinating neuropathies in these individuals result from aberrant homophilic binding of the glycoprotein on adjacent myelin membranes surfaces. The ability of the cytoplasmic domain of \( P_0 \) to bind to membrane bilayers, and perhaps thus maintain the major dense line, suggests that certain cases of Charcot-Marie-Tooth neuropathy type 1B may be the consequence of mutations within the intracellular region of this myelin glycoprotein.

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