Proteolipid protein (PLP), the major protein of central nervous system myelin, contains approximately 2 mol of covalently bound fatty acids. In this study, the in vivo turnover rate of the acyl chains bound to PLP was determined in 40-day-old rats after a single intracranial injection of [3H]palmitic acid. The apparent half-life of total fatty acids bound to PLP was approximately 7 days. After correction for acyl chain interconversion, the half-life of palmitate bound to PLP was only 3 days. This turnover rate is much more rapid than that of the protein moiety calculated under the same experimental conditions (t½ = 1 month). Additional evidence for the dynamic metabolism of acyl groups was provided by experiments in brain tissue slices which showed that acylation of PLP occurs in adult animals as well as during active myelination. Acylation of endogenous PLP in purified myelin and its subfractions was also studied during rat brain development using either [3H]palmitoyl-CoA or [3H]palmitic acid plus ATP and CoA. Labeling of endogenous PLP with [3H]palmitoyl-CoA was observed as early as 10 days postnatal and continued at the same rate throughout development. When [3H]palmitic acid was used as precursor in the presence of both ATP and CoA, acetylation of myelin PLP occurred rapidly in adult animals, indicating that both nonacylated PLP and acyl-CoA ligase are present in myelin. Finally, pulse-chase experiments in a cell-free system showed that PLP-bound fatty acids turn over with a half-life shorter than 10 min. These observations are consistent with the concept that acylation of myelin PLP is a dynamic process involved mainly in myelin maintenance and function.

Protein-fatty acylation is now recognized as a widespread post-translational modification which can be considered analogous to glycosylation or phosphorylation (for review, see Towler et al., 1988; Grand, 1989; Schmidt, 1989). A large number of proteins from eukaryotic cells have been shown to be acylated with long chain fatty acids (C14-C30) and, based on the nature of the fatty acid-protein linkage, two types of acylated proteins have been identified: those in which the major acyl groups of the cell (C16-C20) are attached in oxyster or thioester linkage to the side chain Ser, Thr, or Cys, and those in which myristic acid (C14:0) is bound to the amineterminal residue via an amide linkage. In contrast to myristoylation, which occurs during or immediately after polypeptide synthesis (Wilcox et al., 1987), palmitoylation appears to be a late post-translational event (Schmidt and Schlesinger, 1980; McIlhenny et al., 1985). Therefore, it is not surprising that, for most palmitoylated proteins, addition of palmitic acid continues for some time in the absence of protein synthesis. However, for the acylation of a large number of proteins, including p21RK protein (Mage et al., 1987), rhodopsin (St. Jules and O'Brien, 1966), ankyrin (Staufenbiel, 1987), and several erythrocyte membrane proteins (Staufenbiel, 1988), it has been found that the lack of sensitivity to inhibition of protein synthesis is due to a rapid turnover of the acyl chains rather than to the presence of a large pool of unacylated protein within the cell. These results suggest not only that palmitoylated proteins undergoing fatty acid turn over are not rare, but also that their acyl moieties could be playing dynamic roles.

In the central nervous system, proteolipid protein (PLP) comprises more than 50% of the total myelin protein (Lees and Brostoff, 1984). It is an intrinsic, highly hydrophobic membrane protein and contains approximately 2% by weight of ester-bound fatty acids, mainly palmitic, oleic, and stearic acids (Stoffyn and Polch, 1971; Bizzozero et al., 1985). The post-translational addition of fatty acids to the polypeptide backbone has been demonstrated in vivo and in several in vitro systems using radioactive palmitic (Agrawal et al., 1982; Townsend et al., 1982; Bizzozero et al., 1983, 1986, and 1987b), oleic (Bizzozero et al., 1986), and, more recently, linoleic and linolenic acids (Bürgisser and Matthieu, 1989). PLP acylation is a late post-translational event that takes place close to or within the myelin membrane and not at its site of synthesis (Townsend et al., 1982; Bizzozero et al., 1983). Indeed, incubation of purified myelin membranes with [3H]palmitoyl-CoA results in specific labeling of endogenous PLP (Bizzozero and Lees, 1986a; Bizzozero et al., 1987a), indicating that a pool of nonacylated PLP is present in this membrane. Metabolic experiments in brain tissue slices have also shown that palmitoylation of PLP is not affected by inhibition of its synthesis or transport for long periods of time (i.e. after the newly synthesized PLP has been depleted into myelin) (Bizzozero et al., 1983; Townsend and Benjamin, 1983a; Pasquin et al., 1987). Although these observations clearly suggest that PLP acylation is a dynamic event, pulse-chase experiments failed to demonstrate a rapid acyl chain turnover (Bizzozero et al., 1983).
1983; Townsend and Benjamins, 1983b). However, since chase experiments are technically difficult due to the extensive reutilization of radiolabeled fatty acids from the large lipid pool, the latter results should be taken cautiously. The present study was therefore undertaken to re-examine the possible independent turnover of the acyl chains modifying PLP. The results from in vivo long term, developmental, and cell-free studies indicate that PLP is rapidly acylated and deacylated in the myelin membrane. A preliminary report of these studies has been presented in abstract form (Lees and Bizzozero, 1988).

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals were of the highest purity available. Supplies for SDS-gel electrophoresis were from Bio-Rad. [9,10-3H]Palmitic acid (30 Ci/mmol), U-14C-labeled L-amino acid mixture, and L-[4,5-3H]leucine (60 Ci/mmol) were from Du Pont-New England Nuclear. \([\text{3H}]\)Palmitoyl-CoA (1 Ci/mmole) was chemically synthesized from [9,10-3H]palmitic acid as described previously (Bizzozero et al., 1987a).

In Vivo Labeling of Myelin PLP—Forty-day-old rats of either sex were lightly anesthetized with ether, and 5 μl of either \([\text{3H}]\)palmitic acid (500 μCi) dissolved in 0.9% NaCl containing 1% fatty acid-free bovine serum albumin or \([\text{3H}]\)leucine (100 μCi) in 0.9% NaCl were injected intracranially into both hemispheres by means of a 10-μl syringe equipped with a needle guard to stop the penetration at 2 mm below brain surface. At different intervals after injection, animals were killed by asphyxiation with CO₂, and the forebrain was rapidly removed and placed on ice. Myelin membranes were isolated by the method of Norton and Poduslo (1973). Light and heavy myelin were obtained by the procedure of Autilio et al. (1984). Total protein was assayed by the procedure of Lowry et al. (1951) using bovine serum albumin as the standard.

Labeling of Endogenous Myelin PLP—Rats of either sex were killed by decapitation, and the forebrain was removed and homogenized in 0.32 M sucrose. Purified myelin, myelin-like, and myelin subfractions (A-D) were prepared (Bizzozero et al., 1984) and stored in small aliquots at -20°C. Myelin membranes (50-120 μg of protein) were incubated at 37°C in 0.2 ml of 50 mM Tris-HCl, pH 7.5, containing 2 mM MgCl₂, 1 mM dithiothreitol, 1 mM NaF, and 0.5% Triton X-100 (Bizzozero et al., 1987a). The reaction was started by the addition of either \([\text{3H}]\)palmitoyl-CoA (1 Ci/mmole) or \([\text{3H}]\)palmitic acid (5 Ci/mmole) plus 1 mM ATP and 0.1 mM CoA. After incubation, the radioactive fatty acids were extracted with ethanobether (2:3), dried under nitrogen, and counted. In some experiments, aliquots of the total lipid extract were used to isolate the phospholipid fraction by silicic acid column chromatography (Vance and Sweeney, 1967). Phospholipids were methanolyzed with HC1/methanol (Kishimoto and Hoshi, 1972), and the released radioactive methyl esters were analyzed by reversed-phase TLC.

Lipid Analysis—Total lipids were extracted from myelin with chloroform:methanol (2:1), and the individual species isolated by TLC on Silica Gel G plates developed with chloroform:methanole:20 mM CaCl₂ (50:40:10). Spots were detected with iodine vapors, scraped, and counted. In some experiments, aliquots of the total lipid extract were used to isolate the phospholipid fraction by silicic acid column chromatography (Vance and Sweeney, 1967). Phospholipids were methanolyzed with HC1/methanol (Kishimoto and Hoshi, 1972), and the released radioactive methyl esters were analyzed by reversed-phase TLC.

**RESULTS**

In Vivo Turnover of PLP-bound Fatty Acids—To determine the in vivo turnover rate of PLP-bound fatty acids and to compare it with that of the polypeptide backbone, 40-day-old rats were injected intracranially with either \([\text{3H}]\)palmitic acid or \([\text{3H}]\)leucine of high specific radioactivity. At different intervals postinjection, animals were killed, myelin was isolated, and the radioactivity associated with PLP was determined after electrophoresis. Twenty-four h after an intracranial injection of \([\text{3H}]\)leucine, most myelin proteins were labeled (Fig. 1). In contrast, when palmitate was used as the radioactive precursor, only PLP and DM-20 among all myelin proteins were significantly labeled. Myelin basic proteins (MBP), Wolfgram, and other high molecular weight proteins were not labeled. This labeling pattern is similar to that described previously (Bizzozero et al., 1984, 1986).

The decay of \(^{3}H\) radioactivity associated with PLP as a function of time is shown in Fig. 2. To minimize the errors produced by the accumulation of myelin which occurs during

![FIG. 1. SDS-PAGE of myelin proteins labeled in vivo either with \([\text{3H}]\)palmitate or \([\text{3H}]\)leucine. Forty-day-old rats were injected intracranially with either 500 μCi of \([\text{3H}]\)palmitic acid (30 Ci/mmol) or 100 μCi of \([\text{3H}]\)leucine (60 Ci/mmol). After 24 h, animals were killed, myelin was isolated, and proteins were analyzed by SDS-PAGE as described under "Experimental Procedures." LBP and SBP, large and small basic protein, respectively; PLP, major proteolipid protein; WP, Wolfgram protein.](image-url)
the course of the experiment, rats beyond the peak period of maximal myelin synthesis (i.e. 40 days of age) were used. Nevertheless, to calculate the apparent half-lives, the label associated with PLP was expressed as radioactivity per forebrain, which circumvents that problem. Apparent half-lives for turnover were calculated from the slope of a plot of log (total radioactivity per forebrain) versus time after injection. Incorporation of [3H]leucine into PLP reached a maximum 24 h after injection, and it decayed thereafter with an apparent half-life of approximately 1 month (Fig. 2B). This value is similar to that reported by others (Fisher and Morell, 1974) and is consistent with the known stability of the myelin sheath. In marked contrast, after intracranial injection of [3H]palmitate, the radioactivity associated with PLP reached a maximum at 2 h, and it decayed after 2 days with an apparent half-life of approximately 7 days (Fig. 2B). This value is identical with the half-life of decay of total radioactivity (Norton and Cammer, 1984). As shown in Fig. 5B, the rates of decay of the palmitoyl chains bound to PLP were identical in both subfractions and indistinguishable from that in total myelin. These results suggest that PLP molecules from both compacted and uncompacted myelin undergo rapid turnover.

The turnover rate of palmitoyl chains in PLP derived from two different myelin subfractions (light and heavy myelin) was also determined. These subfractions are obtained on the basis of the heterogeneous buoyant density of myelin and apparently represent different domains of the membrane sheath with different degrees of compaction and/or perhaps different developmental stages in myelin synthesis (Norton and Cammer, 1984). As shown in Fig. 5B, the rates of decay of the palmitoyl chains bound to PLP were identical in both subfractions and indistinguishable from that in total myelin. These results suggest that PLP molecules from both compacted and uncompacted myelin undergo rapid turnover.

Synthesis and Acylation of PLP during Development—The independent turnover of fatty acids was also demonstrated by comparing the rates of synthesis and acylation of PLP during development using rat brain tissue slices. The incorporation of radioactive precursors (i.e. amino acids and fatty acids) into myelin PLP using this in vitro labeling system has been atted palmitic acid, nonacylated proteins such as myelin basic proteins were still unlabeled (data not shown). However, the palmitic acid used for labeling underwent elongation and desaturation. Twenty-four h after injection, approximately 20% of the radioactivity associated with both PLP and phospholipids was present in fatty acids other than palmitic acid, mainly stearic and oleic acids (Fig. 4A). As a result of fatty acid interconversion, the percentage of radioactive palmitic acid (i.e. the major radiolabeled species) could also have been incorporated into PLP. Thus, because of the possible reutilization of the radioactive donor, the actual turnover rate cannot be accurately determined and may differ by orders of magnitude from that experimentally calculated. Nevertheless, it is clear that palmitoyl groups covalently bound to PLP turn over much more rapidly than the protein backbone.

FIG. 3. Removal of PLP-bound radioactivity by hydroxylamine. Myelin proteins labeled with [3H]palmitic acid were analyzed by SDS-PAGE after different time periods. After electrophoresis, gels were soaked in 0.5 M hydroxylamine, pH 7.5, for 12 h as described under “Experimental Procedures.” Radioactivity associated with PLP was determined by liquid scintillation counting. Data are expressed as relative to those from untreated gels. Each point represents the average of duplicate determinations.

FIG. 2. Decay of 3H radioactivity in myelin PLP with time after the intracranial injection of either [3H]leucine (A) or [3H]palmitic acid (B) into 40-day-old rats. Rats were injected intracranially with either 100 µCi of [3H]leucine (60 Ci/mmol) or 500 µCi of [3H]palmitic acid (30 Ci/mmol). At different intervals, animals were killed, myelin was isolated, and proteins were analyzed by SDS-PAGE. Radioactivity associated with PLP was determined by liquid scintillation counting. Each point represents the average of two to three independent determinations.
previously characterized (Bizzozero et al., 1983, 1986). Both the relative and total (not shown) incorporation of [14C]-amino acids into PLP was maximal at 18 days of age and decreased thereafter (Fig. 6), following the developmental pattern of the rate of myelin synthesis (Norton and Cammer, 1984). In contrast, incorporation of palmitic acid into PLP was proportional to the amount of protein present in this membrane but not to the rate of myelin or protein synthesis, suggesting the independent turnover of PLP-acyl chains. In control experiments, radioactive palmitic acid was added to the unlabeled brain homogenates, and myelin was prepared. Analysis of the radioactivity associated with PLP revealed that it was not significant (<6%), suggesting that PLP acylation does not occur after the incubation period (i.e. during myelin isolation).

Acylation of Endogenous Myelin PLP—Since different intracellular fatty acid pools may exist at different times during development, the incorporation of the radioactive precursor may not be proportional to the actual acylation rate. To circumvent this problem, we measured the incorporation of radioactive palmitate into endogenous myelin PLP in a cell-free system. Acylation of myelin PLP in this cell-free system has been previously characterized, and it was found to be of physiological importance (Bizzozero et al., 1987a). Incubation of myelin with [14C]palmitoyl-CoA showed that, at all ages examined, PLP and the minor DM-20 protein were the only proteins labeled (Fig. 7A). Incorporation of palmitate into these proteins was detected as early as 10 days after birth and increased with age. However, as found in tissue slices, labeling was always proportional to the amount of endogenous PLP in the fraction, and, therefore, the specific radioactivity remained essentially constant. To determine whether the non-acylated pool was located in a particular domain of the myelin sheath, acylation of endogenous PLP was measured in myelin subfractions isolated from 25-day-old rats (Fig. 7B). Again, no difference in PLP specific radioactivity was found among the subfractions. Comparable results were obtained at 17 and 34 days (data not shown). These results, along with those obtained in vivo, clearly suggest an identical metabolic behavior for the PLP-bound fatty acids from subfractions representing compacted and uncompacted myelin.

FIG. 4. Distribution of radioactivity in PLP and phospholipids after an intracranial injection of [14C]palmitic acid. Rats were injected intracranially with 500 µCi of [14C]palmitic acid. At different intervals, animals were killed, myelin was isolated, and proteins were analyzed by SDS-PAGE. Radioactive fatty acids associated with PLP were released with NaOH, converted to methyl esters, and analyzed by reversed-phase TLC as described under “Experimental Procedures.” Phospholipids were isolated by silicic acid column chromatography and methanolyzed with HCl:methanol, and the released methyl esters were analyzed as before. A, distribution of radioactivity in PLP and phospholipids 24 h after the intracranial injection of [14C]palmitic acid. B, percentage of total acyl radioactivity in PLP and phospholipids identified as palmitic acid as a function of time after injection.

FIG. 5. In vivo turnover rate of palmitoyl chains bound to PLP in total myelin (A) and myelin subfractions (B). A, the rate of decay of the radioactive palmitoyl chains in myelin PLP was calculated by multiplying the data in Fig. 2B by the corresponding proportions of radioactive palmitate bound to PLP (Fig. 4). B, aliquots of total homogenate from animals injected with [14C]palmitic acid were used to isolate heavy and light myelin as described under “Experimental Procedures.” The rate of decay of the radioactive palmitoyl chains in PLP from those subfractions was calculated by multiplying the amount of radioactivity associated with PLP by the corresponding proportion of radioactive palmitate bound to the protein (Fig. 4). Values are expressed as the percent of maximal incorporation and represent the average of two separate determinations.
of the myelin-associated acyl-CoA synthetase, since activated (i.e. palmitoyl-CoA) and not free fatty acid is the immediate acyl chain donor of this reaction (Bizzozero and Lees, 1986a). As shown in Fig. 8A, PLP acylation occurred at all ages studied between 10 and 90 days, indicating that not only acylatable PLP but also acyl-CoA synthetase must be present throughout development. The extent of labeling was slightly higher at 25 days of age, perhaps reflecting developmental changes of the ligase. When myelin subfractions from a 25-day-old rat were tested for their ability to acylate endogenous PLP from free fatty acids plus ATP and CoA, endogenous PLP was labeled in all subfractions including uncompacted myelin (i.e. myelin-like) (Fig. 8B).

**Pulse-Chase Experiments**—The above experiments indicate that acyl groups attached to PLP turn over very rapidly. Paradoxically, pulse-chase experiments carried out in brain tissue slices (Bizzozero et al., 1983; Townsend and Benjamins, 1983b) have shown that the label associated with PLP remains essentially constant after addition of the unlabeled substrate, suggesting that the acylation reaction is a net addition of fatty acids. However, chase experiments involving fatty acids are particularly difficult because the precursor pool for protein acylation cannot be adequately diluted (Staufnhiel, 1988). To directly demonstrate a turnover of PLP-bound fatty acids, pulse-chase experiments were carried out in a cell-free system. Isolated myelin membranes were labeled with [3H]palmitic acid in the presence of ATP and CoA for 30 min, after which an excess of unlabeled palmitoyl-CoA and desulfocoenzyme A was added, and the incubation continued for another 90 min. Addition of the cold substrate resulted in a dramatic and immediate decrease of the label associated with PLP (Fig. 9), indicating a rapid exchange of fatty acids. The half-life of the radioactive palmitoyl chains attached to PLP was only 10 min, as determined from the decay values between 5 and 30 min. The rapid turnover of fatty acids was also suggested from the time course of incorporation of [3H]palmitic acid into PLP, which was linear for only 90 min. After this period, the label associated with PLP rapidly decreased, probably due to the consumption of the small amounts of [3H]...
by action of the endogenous acyl-CoA synthetase is very small.

The major conclusions to the amount of protein rather than the level of protein palmitoylation does not change during development and is proportional to the polypeptide backbone, 2) the rate of palmitoyl chains turn over in vivo much more rapidly than and independently of the polypeptide backbone, 3) the palmitic acid molecules bound to PLP have a half-life of approximately 10 min, as determined by pulse-chase experiments in a cell-free system. The major differences between this cell-free experiment and those carried out previously (Bizzozero et al., 1986; Bizzozero et al., 1987a) are that labeling was performed with small amounts of $[\text{H}]$palmitic acid of high specific radioactivity, and that a higher concentration of unlabeled palmitoyl-CoA (0.5 mM) was used for the chase. In addition, desulfo-coenzyme A, a competitive inhibitor of CoA, was included in the chase to block the further activation of radiolabeled fatty acids. Under these conditions, it is likely that the amount of radiolabeled precursor generated by action of the endogenous acyl-CoA synthetase is very small and is, therefore, effectively diluted with the large amounts of unlabeled palmitoyl-CoA added. In previous experiments, labeling was carried out with $[\text{H}]$palmitoyl-CoA (10 μM) and chase with nonradioactive palmitoyl-CoA (100 μM) (Fig. 4 in Bizzozero et al., 1987a). At this low, nonsaturating concentration of $[\text{H}]$palmitoyl-CoA, addition of a 10-fold excess of unlabeled palmitoyl-CoA not only diluted the radiolabeled precursor but also increased the velocity of the reaction by at least 5-fold. Thus, although $[\text{H}]$palmitoyl-CoA was efficiently diluted, the number of fatty acid molecules incorporated into PLP also increased, making the chase ineffective. Assuming that the efficiency of labeling decreased 2-fold, the turnover rate of palmitoyl chains in those experiments would be similar to that reported here.

The rapid metabolism of the fatty acids bound to PLP was found to occur throughout development and in mature, multilamellar myelin as well as in uncompacted myelin. These findings suggest that the enzymes responsible for fatty acylation and deacylation are closely associated with PLP, and that the acylation substrate (i.e. acyl-CoA) is readily accessible to the acylated amino acid (i.e. Cys<sup>188</sup>) (Bizzozero et al., 1990). In contrast to the acylation step which occurs in the absence of a separate enzyme (i.e. autoacylation), deacylation of PLP is not a consequence of the chemical instability of the acyl group, since they are stable in the isolated protein (Bizzozero et al., 1987b; Bizzozero and Good, 1990). We have recently obtained evidence for the presence of a myelin-associated fatty acylesterase activity which is capable of removing fatty acids from $[\text{H}]$palmitoyl-PLP. Since acylation of PLP is not mediated by a separate enzyme, it is likely that the acylesterase activity, as well as the local concentration of acyl chain donor (i.e. acyl-CoA), regulates the acylation state of the protein. Although acyl-CoA has not been found in myelin, Vaswani and Ledeen (1987) have found an acyl-CoA ligase in highly purified myelin, indicating that activation of fatty acids can occur in this membrane. Moreover, our data show that activation of fatty acids, like acylation of PLP, continues to occur beyond the peak period of myelin synthesis and in all myelin subfractions.

The rapid metabolism of the fatty acids bound to PLP explains why the incorporation of radioactive palmitate into myelin PLP is not affected by inhibition of protein synthesis or transport even for extended periods of time (Townsend et al., 1982; Bizzozero et al., 1983; Townsend and Benjamins, 1983a). Using time-staggered intracranial injections of $^3$H- and $^3$H-labeled palmitic acid into 20-day-old rats, we have previously shown that the entry of fatty acid into PLP of the various myelin subfractions was simultaneous (Bizzozero et al., 1984). That finding can now be explained by the fact that all myelin subfractions contain a pool of PLP available for acylation and that the turnover rate of the PLP-acyl chains in compacted and uncompacted myelin is identical.

It has been proposed that, in other proteins, acylation may be involved in a variety of functions, such as anchoring of proteins to membranes, triggering of membrane fusion, regulation of protein-protein interactions, prevention of protein oxidation and dimerization, and vectorial movement of proteins through the endoplasmic reticulum (Schmidt, 1989). In the case of PLP, the possibility that fatty acids can act as a signal for targeting the protein to myelin can be excluded since the addition of the fatty acid occurs in that membrane. However, the acyl chains could contribute to regulation of lipid-protein or protein-protein interactions within myelin and/or to maintenance of the protein in a specific conformation. We have previously demonstrated that removal of fatty acids produces a large conformational change (Bizzozero and Lees, 1986b), and this could ultimately be reflected in the biological activity of the protein.

The idea that insulation is the sole function of myelin has
been brought into question in recent years, since this membrane contains enzymes involved in dynamic post-translational modifications, reutilization of myelin components, and signal transduction (Norton and Cammer, 1984; Larroca et al., 1987; Kahn and Morell, 1988). Our finding of the rapid turnover of PLP-bound fatty acids is in agreement with such a concept. One of the rapid processes occurring in myelin is the phosphorylation and dephosphorylation of myelin basic protein (MBP) (Des Jardins and Morell, 1983), and acylation of PLP has several features in common with MBP phosphorylation: (a) both phosphate and fatty acyl groups turn over rapidly, (b) the turnover rates of these moieties are similar in young (i.e. rapidly myelinating) and older animals, and (c) both reactions occur within the myelin membrane and simultaneously in all subfractions. Therefore, it is conceivable that the two processes might be related. This suggestion is supported by the observation that phosphorib esters, known to stimulate protein kinase C and increase the phosphorylation state of MBP, reduce the incorporation of palmitic acid into PLP-bound fatty acids (Konat et al., 1987). Furthermore, we have recently found that stimulation of protein kinase C-dependent phosphorylation inhibits the activity of myelin-associated PLP fatty acylesterase (Bizzozero, 1991).

The integral membrane protein rhodopsin, like PLP, contains covalently bound fatty acids (O’Brien and Zatz, 1984). Acylation of these two proteins has many features in common: (a) the addition of the fatty acid occurs late after translation and is independent of protein synthesis (O’Brien and Zatz, 1984; Bizzozero et al., 1983), (b) there is fatty acid specificity (O’Brien et al., 1987; Bizzozero et al., 1986), (c) the attachment of the fatty acid is not mediated by an enzyme (i.e. autophosphorylation) (O’Brien et al., 1987; Bizzozero et al., 1987b), and (d) the amino acid sequence surrounding the palmitoylated cysteine in PLP (Thr-Thr-Leu-Cys-Gly-Lys) (Bizzozero et al., 1990) is almost identical with that found in rhodopsin (Thr-Thr-Leu-Cys-Cys-Gly-Lys) (Ovchinnikov et al., 1988). Furthermore, the fatty acyl chains in PLP, like in rhodopsin, exhibit a fast turnover rate (St. Jules and O’Brien, 1986). Despite these similarities, whether the fatty acids bound to PLP are involved in regulatory mechanisms as in rhodopsin remains unknown. In any case, a direct involvement of PLP acylation in myelin synthesis and assembly seems unlikely, since the rate of palmitoylation is as rapid in slowly myelinating animals (90 days) as it is in rapidly myelinating animals (15–25 days).

Our results demonstrate that PLP acylation is a dynamic metabolic process which explains previous findings from our laboratory and from other laboratories. They also raise a number of new questions, such as how the acylation state of PLP is regulated and the functional significance for neural function of this dynamic post-translational modification.

Acknowledgment—We thank Dr. Marjorie B. Lees for helpful suggestions and critical reading of the manuscript.

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