Lipid-metabolizing enzymes of myelin and their relation to the axon

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Abstract The old concept of myelin as a metabolically inert membrane has been considerably revised as a result of the discovery of numerous enzyme activities in the isolated membrane. The high degree of purification and low levels of contamination markers leave little doubt that the measured activities are intrinsic to myelin itself. Slightly more than half of the discovered activities involve lipid metabolism. One such enzyme, neutral cholesterol esterase, is myelin-specific, while the rest occur in other subcellular fractions as well as myelin. These include activities involved in synthesis of cerebrosides, phospholipids, and cholesteryl esters; only a few degradative enzymes are presently known. In vivo studies have shown that various substrates utilized by lipid-synthesizing enzymes of myelin can originate in the axon. Six such substrates have been characterized. The possibility exists that these enzymes may be wholly or partially dependent on the axon as the primary source of substrate, thereby suggesting a possible form of metabolic dependency of myelin on the axon.—**Ledeen, R. W.** Lipid-metabolizing enzymes of myelin and their relation to the axon. *J. Lipid Res.* 1984. **25:** 1548–1554.

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INTRODUCTION

The role of myelin in providing insulation for the axon and facilitation of saltatory conduction has made it a membrane of prime interest to neuroscientists. A fatty substance containing 70–75% lipid, it comprises the bulk of nerve tissue in the peripheral nervous system (PNS) and of white matter in the central nervous system (CNS). Electron microscopic studies have revealed a multilamellar structure formed by compaction of spiraling processes extending from the glial cell body. The myelin sheath is thus a specialized extension of the plasma membrane of the Schwann cell (PNS) or oligodendrocyte (CNS).

The earlier concept of myelin as a relatively inert membrane seemed consistent with its insulating role. This appeared to receive experimental support in the 1960's from the studies of Davison and coworkers (1–3) showing myelin to be the most metabolically stable of brain subcellular fractions. In addition, the initial search for enzymic activities in isolated myelin revealed almost no activity, the one exception being leucine aminopeptidase (4). However, the latter was to prove less and less of an exception as numerous enzyme activities came to be discovered over the next two decades. These findings have required rather drastic revision of the original concept, to the point where myelin is now viewed as possessing a rather surprising array of enzymes considering its plasma membrane origin. Also contributing to this newer viewpoint are more current metabolic studies (5) which, while confirming the relative metabolic stability of myelin, have revealed measurable turnover rates for several myelin constituents.

The systematic study of myelin enzymology began with the discovery of 2′,3′-cyclic nucleotide 3′-phosphohydrolase in 1967 (6), followed by several confirmatory reports (7–10, for reviews). Although the true substrate and functional significance of this highly active enzyme are not known, it has come to be regarded as the prototypic myelin-specific enzyme and is often used as a biochemical marker for this membrane. One other myelin-specific enzyme has been reported (11), which also happens to be the first lipid-metabolizing enzyme discovered in this membrane: neutral (pH 7.2) cholesterol ester hydrolase. These two enzymes showed high relative specific activity (ca. 5–10) in myelin relative to brain homogenate and high recovery (ca. 50–80%) of total brain activity in myelin. A distinction has been drawn between this category of enzyme, believed to be fairly specific to CNS myelin (but probably present in oligodendroglial membranes as well), and the much larger group of enzymes which are intrinsic to myelin but not

Abbreviations: PNS, peripheral nervous system; CNS, central nervous system; GM1 ganglioside II^{3}NeuAc-GgOse$_4$Cer; DAG, diacylglycerol.

specific to it (7–10). The latter group encompasses most lipid-metabolizing enzymes, which are summarized in **Table 1.** It also includes carbonic anhydrase (12–14) Na^+,K^+-ATPase (15, 16), and 5′-nucleotidase (17, 18), three enzymes involved in biological transport, and various protein kinases and phosphatases (7–10, for review).

The first part of this survey briefly recounts the findings to date on the lipid-metabolizing enzymes that have been found in myelin. The enzyme activities discussed (Table 1) are those for which reasonably firm evidence has been provided of their intrinsic association with myelin. For further details the reader is referred to recent reviews on this subject (7–10). The final section on functional significance examines evidence of axonal involvement, in particular the provision of substrates utilized by lipid-synthesizing enzymes of myelin.

CRITERIA FOR MYELIN ASSOCIATION

Establishing an enzyme as intrinsic to myelin has usually required considerable evidence beyond the mere demonstration of activity in the isolated fraction. The procedure is relatively straightforward for those (few) enzymes that are specific to this membrane. In such cases the specific activity in purified myelin should exceed that of brain homogenate, and the recovered activity should be a sizeable fraction of that in whole brain. Decreased activity is generally expected in brains from myelin-deficient mutants while developmentally related increases should correspond to the period of active myelination in normal animals.

For the much larger group of myelin-associated enzymes which are not specific to this membrane, and which often have lower activity in myelin than other subcellular fractions, additional evidence is needed. One useful criterion has been constant specific activity after additional purification; it has been found, for example, that trace contaminants present in myelin isolated by the standard Norton-Poduslo procedure (15) can often be further reduced by an additional gradient in which the myelin is "floated up" following homogenization in 0.85 M sucrose (19). The ratio of the activity in question to markers for the suspected contaminating membrane(s) provides another indicator. Mixing experiments have been employed to advantage in some studies; addition of the suspected contaminant (e.g., microsomes) to the myelin undergoing isolation would be expected to produce elevated levels in the final preparation if that were the true source of the enzyme. Finally, it is sometimes possible to demonstrate different properties for the myelin-associated enzyme compared to its counterpart in other fractions. Such properties can include kinetic parameters, response to detergents and cofactors, and developmental patterns. One or more of these various strategies has been employed in establishing the myelin-intrinsic character of most of the enzymes listed in Table 1.

LIPID-SYNTHESIZING ENZYMES

Eucaryotic cells in general are believed to carry out de novo synthesis of complex membrane lipids in the endoplasmic reticulum, with additional participation by

TABLE 1. Lipid metabolizing enzymes of myelin

Enzymes	Reference
Synthetic enzymes	
UDP-Gal:ceramide galactosyltransferase	21–24
Cholesterol esterifying enzyme (pH 5.2)	32
Cholesterol esterifying enzyme (pH 7.4)	33
Phosphoinositide kinase	37
Diphosphoinositide kinase	37
CDP-ethanolamine:1,2-diacyl-*sn*-glycerol ethanolaminephosphotransferase	25
CDP-choline:1,2-diacyl-*sn*-glycerol cholinephosphotransferase	29
CTP:phosphoethanolamine cytidylyltransferase	26
CTP:phosphocholine cytidylyltransferase	30
Ethanolamine kinase	27, 51
Choline kinase	27, 51
Catabolic enzymes	
Cholesteryl ester hydrolase (pH 7.2)[a]	11
Polyphosphoinositide mono- and diphosphoesterases[b]	38
Neuraminidase	39, 41

[a] This enzyme is myelin-specific; the others are present in "microsomes" (i.e., endoplasmic reticulum) as well as myelin.

[b] The number of enzymes involved in these activities is not known.

the Golgi apparatus in some cases. Certain intermediates used by these enzymes are generated in the cytosol. This pattern appears to be as true for the myelin-forming units of the nervous system as for other cells. Hence, the majority of synthetic activities discussed in this section are localized in glial cell bodies as well as myelin, the former being considered the primary locus of myelinogenesis. Thus, radiolabeled membrane lipids have been shown to diffuse from the Schwann cell perikaryon into the myelin sheaths of sciatic nerve (20), and very likely the same process occurs in the CNS. The presence of these enzymes in the endoplasmic reticulum, and consequently in the heterogeneous "microsomal" fraction, has provided the rationale for comparison of the latter activity with that of myelin. It is interesting to note that the lipid-metabolizing enzymes now known to occur in myelin (Table 1) comprise slightly more than half of all enzyme activities discovered in that membrane to date (cf. refs. 7–10).

UDP-Galactose:ceramide galactosyltransferase, the enzyme completing the synthesis of the relatively myelin-specific lipid galactosylceramide, was shown to occur in rat CNS myelin with specific activity similar to that of whole brain (21, 22):

ceramide + UDP-galactose → galactosylceramide.

The developmental pattern of this enzyme in rat brain myelin differed considerably from that in rat brain microsomes (23); early in development myelin was found to have higher activity, but after several days it dropped below that of microsomes. This activity persisted in carefully purified myelin, whereas that of UDP-glucose:ceramide glucosyltransferase, another enzyme enriched in microsomes, was eliminated (22). Subfractionation studies (23, 24) revealed, as for many myelin-associated enzymes, enriched activity in the heavy myelin subfraction. The highest activity was found in an axolemma-enriched fraction from young animals (23), a finding that is both surprising and difficult to interpret in view of the heterogeneous nature of this fraction.

Phospholipid-synthesizing activity was first detected in purified myelin by observation of CDP-ethanolamine:1,2-diacyl-*sn*-glycerol ethanolaminephosphotransferase, the enzyme completing the synthesis of phosphatidylethanolamine (25). The relative specific activity (0.7–0.9) and percent recovery (11%) in myelin were similar to those of the galactosyl-transferase described above. The fact that the apparent K_m of CDP-ethanolamine was significantly lower for myelin than for microsomes was suggestive of isozymes. The same study demonstrated the virtual absence of three other lipid-synthesizing enzymes in myelin: serine phospholipid exchange enzyme, lactosylceramide sialyltransferase, and cerebroside sulfotransferase. Selectivity is thus an important feature of this aspect of myelin enzymology.

Further investigation revealed the presence of two additional enzymes in myelin required for the synthesis of phosphatidylethanolamine: CTP:phosphoethanolamine cytidylyltransferase (26) and ethanolamine kinase (27). Both of these are bimodally distributed in brain between soluble and membrane fractions, and although myelin had low specific activities for both enzymes relative to the cytosolic fraction, these activities were appreciable (20–30%) relative to microsomes. This fact is of some interest in light of the recent claim (28) that the active form of this enzyme (synthesizing CDP-choline) is the portion associated with microsomes, whereas that in the cytosol acts as a reserve. The absence of significant contamination was indicated by the very low activities detected for the marker enzymes NADPH-cytochrome C reductase (microsomes) and lactate dehydrogenase (cytosol). Thus, the evidence supports the presence in myelin of three enzymes needed to convert diacylglycerol (DAG) to phosphatidyl ethanolamine (**Fig. 1**). Additional work has pointed to the presence of the three parallel enzymes that convert DAG to choline glycerophospholipids (27, 29, 30); except for choline kinase, these latter studies are less detailed than the ones discussed above.

The kinases which complete the synthesis of diphosphoinositide and triphosphoinositide also occur in myelin

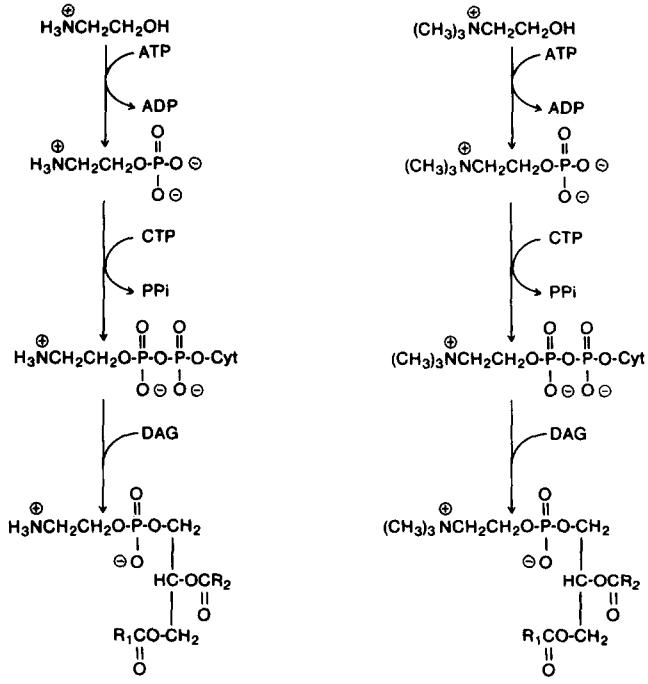

Fig. 1. Summary of pathways for conversion of diacylglycerol (DAG) to phosphatidylethanolamine and phosphatidylcholine. The six enzymes depicted have been detected in purified myelin.

(31). The latter was especially active, and that fact plus the high concentration of the triphosphoinositide product in myelin suggested this membrane to be the major locus of the kinase. However, despite high relative specific activity and recovery, this does not appear to be a myelin-specific enzyme. The fact that both kinases showed highest activity in the "heavy myelin" subfraction suggested to the authors that they are localized in the myelin-appurtenant regions. The presence of phosphatases that degrade polyphosphoinositides (see below) reinforces the concept of phosphoinositide metabolism in myelin.

Cholesterol-esterifying enzymes of two types, distinguishable by their pH optima and cofactor requirements, have been found in myelin as well as microsomes. The one with a pH optimum of 5.2 did not require ATP or CoA for esterification (32) whereas that with pH optimum at 7.4 required both cofactors (33). They showed enrichments over homogenate of 2.6- and 1.5-fold, respectively, when assayed in rat CNS myelin at 16 days of age. The activity of both declined thereafter. Purified myelin contained about 7–10% of total activity of both enzymes.

LIPID-CATABOLIZING ENZYMES

The list of known catabolizing enzymes in myelin is considerably shorter than that pertaining to lipid-synthesis, possibly owing to a less diligent search for this type. The myelin-specific cholesteryl ester hydrolase was discussed above. This was shown to be very hydrophobic and strongly influenced by exogenously added lipids (34, 35). Some progress has been reported in its isolation (36). The developmental pattern of the myelin cholesterol esterase, which correlated well with the accumulation of myelin, differed significantly from that of the microsomal enzyme which was already present at 50% of adult levels at birth (37).

The only catabolic activities reported to date for phospholipids[1] are the mono- and diphosphoesterases reactive toward the polyphosphoinositides (38). The activities in myelin, especially toward triphosphoinositide, were comparable to those in whole brain homogenate. Substantial activity was present in the three myelin subfractions, indicating that the hydrolases operative in the fast turnover of the inositide phosphate group(s) are probably distributed over the entire myelin structure. The number of enzymes responsible for these phosphohydrolase activities is not known.

A recently discovered catabolic activity of some interest is neuraminidase (39), an enzyme which converts the oligosialo gangliosides of brain to the monosialo ganglioside GM1. This behavior may be correlated with the fact that CNS myelin of most mammalian species contains GM1 as the major ganglioside (40). Neuraminidase activity of myelin isolated from rat brain cerebral hemispheres was higher than that of microsomes obtained from the same source (41). A rostral-caudal gradient was noted, myelin isolated from brain stem and spinal cord showing progressively less neuraminidase than that from cerebral hemispheres; relatively little activity was detected in PNS myelin.

FUNCTIONAL ROLE AND INVOLVEMENT OF THE AXON

The physiological purpose(s) served by the lipid-synthesizing and metabolizing enzymes of myelin is still a matter of conjecture. As mentioned previously, it seems unlikely that they are responsible for synthesis of the bulk constituents of compacted myelin during initial myelinogenesis. Present evidence supports the view that myelin components are synthesized within the glial cell perikaryon and enter the extended plasma membrane prior to compaction of the mesaxon (5, 7). Maintenance and remodeling of previously formed myelin seems a more likely function, particularly in view of the relatively long time required for arrival of new lipid synthesized in the glial cell (20). This might be expected to apply particularly to the specialized adaxonal structures (e.g., paranodal loops), and indeed the higher activities observed in the "heavy myelin" subfraction, believed to be enriched in these myelin appurtenant structures, would tend to support enzyme concentration in those regions. However, since substantial activity was always observed in all three myelin subfractions when such studies were carried out, the enzymes appear to have broad distribution throughout the compacted and specialized zones irrespective of the higher concentrations that may exist in the latter.

Related to functional considerations is the question of substrate source in vivo for the lipid-synthesizing (and other) enzymes of myelin. The myelin-forming glial cell itself is one possible source, although this would require that rather long distances be traversed between cell body and adaxonal regions of the membrane. The axon, which is relatively close to these regions, must also be considered and this possibility has received some support in recent studies. In both the CNS (42–44) and PNS (45–47) a portion of radioactive material undergoing axonal transport was observed to pass into the adjacent

[1] The report of a myelin-associated sphingomyelinase has been withdrawn (54).

myelin sheath with resultant labeling of specific lipids. This was attributed to two simultaneous processes: *a*) synthesis of new lipid in the glial cell body and/or myelin lamellae and *b*) transfer of intact lipid. The former process would involve utilization of labeled substrate coming from the axon, and the evidence supported synthesis within myelin itself (rather than the glial perikaryon).

In the initial studies referred to above, the precursors used to label axonally transported substances in the cell body were serine (43) and choline (45). Additional findings on choline, detailing reutilization by this transcellular process, were subsequently presented by Droz and coworkers (46, 48). More recently Toews and Morell (44) and Alberghina, Viola, and Giuffrida (42) suggested glial/myelin reutilization of acyl chains in similar fashion. Our studies (49) have shown that inorganic phosphate behaves as another transaxonal substrate but that glycerol is not utilized in this manner; rather it enters myelin through transfer of intact lipid.

Ethanolamine is another substrate that may be added to the growing list (**Table 2**) of putative substrates suggested to transfer from axon to myelin for utilization by lipid-synthesizing enzymes. Brunetti et al. (50) used this precursor to label ethanolamine glycerophospholipids undergoing axonal transport in peripheral nerve, and observed molecular specificity in regard to destination: the diacyl form stayed within the neuron, migrating primarily to the axolemma and membranous elements of nerve endings, while the alkenylacyl (plasmalogen) form transferred to myelin. This transfer could have involved intact phospholipid or ethanolamine, the latter then being utilized by the myelin-associated enzymes.

Recent work in our laboratory (51) has shown that following intraocular injection of [^{14}C]ethanolamine in rats, radiolabeled phosphoethanolamine and CDP-ethanolamine could be detected in extracts of optic nerve and tract. This would be consistent with liberation of ethanolamine in the axon through catabolism of ethanolamine glycerophospholipids, known to undergo fast axonal transport in the optic system (52). Such enzymic activity has been reported in the axon (53). The liberated ethanolamine could then presumably diffuse from the axon into adjacent myelin to be converted to the two detected intermediates (and ultimately phospholipid). Other mechanisms can of course be envisioned to explain their presence in the optic axons.

Such behavior of ethanolamine, if verified, would explain an apparent contradiction that arose in an earlier study of ours (43) in which radiolabeled ethanolamine glycerophospholipids—but not serine glycerophospholipids—were detected in optic tract myelin following intraocular injection of [^{14}C]serine. If serine were the only metabolite transferred from axon to myelin, this result would be difficult to explain, not only because of the precursor-product relationship of these two lipids but also because myelin appears to lack the serine phospholipid exchange enzyme (see above). However, since radiolabeled serine is known to label ethanolamine glycerophospholipids in the retina prior to axonal transport, this lipid could conceivably serve as a source of intraaxonal ethanolamine. Additional work is needed to determine the full range of substances provided by the axon as substrates for lipid-synthesizing enzymes of myelin.

Despite the rather extensive list of myelin-associated enzymes already compiled, it seems likely the list will continue to grow as work progresses in the field. In time we should also have a clearer picture of lipid-metabolizing enzymes which are not present in myelin, selectivity being a hallmark of this phenomenon. Thus, it may be possible to test preliminary indications (25) that myelin is somewhat deficient in enzymes that catalyze synthesis of acidic lipids. The large majority of studies to date have dealt with CNS myelin, leaving the subject of PNS myelin relatively unexplored. Developmental studies and regional variation are also potentially fruitful topics of investigation, perhaps leading eventually to localization of the various enzymes within the heterogeneous structures of myelin.

Finally, work is needed to test current hypotheses on the physiological role of myelin-associated enzymes. The prospect that these lipid-metabolizing enzymes may be wholly or partially dependent on the axon as a primary source of substrate opens intriguing new possibilities in the area of axon-myelin metabolic interaction, with possible ramifications in such phenomena as secondary demyelination. This needs further investigation. Thus, in assessing the importance of the axon as a general source of substrates for this group of myelin-localized enzymes, the consequences of this metabolic dependency will also require elucidation.

TABLE 2. Axon-myelin transfer of lipid precursors

Labeled Substances Administered to Neurons	Presumed Substrate Incorporated into Myelin Lipids	Reference
Serine	serine, ethanolamine	43
Choline	choline	45, 46, 48
Acetate	acyl chains	44
Palmitate	palmitate	42
Phosphate	phosphate	49
Ethanolamine	ethanolamine	50

The identity of substances listed in column 2 was inferred in most cases from the nature of the labeled lipids detected in myelin. Evidence suggests these (or related species) were transferred from the axon and enzymatically incorporated into myelin components.

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