Protein-lipid interactions and myelination

Beest, Martin Bernhard Adriaan ter

Summary and concluding remarks

The myelin sheath surrounds the axons, in this way providing an environment in which efficient pulse conduction can occur. The importance of myelin in the correct functioning of the nervous system is clear from the drastic effects of some neurodegenerative diseases involving myelin.

Despite its simple composition, myelin can be regarded as a complex structure, unique for the central and peripheral nervous system. Special requirements with respect to the architecture of myelin are evident from the presence of specific proteins such as MBP and PLP. Abundant information concerning the nature of both proteins and their interaction with (artificial) membranes, is available, as discussed in *chapter 1*. However, less is known about the precise function(s) of these proteins, the regulation of this/these function(s) and the role of protein heterogeneity in the structure of myelin.

The objective of this thesis was to study the aforementioned questions for both MBP and PLP. For this purpose both proteins were isolated and the interaction of isolated MBP or PLP with membranes was studied. The aggregation of these artificial membranes, induced by MBP or PLP, was used as a model for myelin compaction.

Myelin basic protein

Apparently the function of MBP is related to the stabilization of the major dense line, i.e., the compacted cytoplasmic space (*chapter 1*). This compaction is obtained via the interaction of MBP with the myelin membrane (figure 1).

MBP shows a complex behavior with respect to its interaction with artificial membranes as discussed in *chapter 2*. This interaction was monitored by measuring the aggregation of unilamellar vesicles by MBP, and this aggregation may be regarded as a method to measure MBP 'activity'. The addition of MBP to vesicles containing negatively charged lipids results in an immediate aggregation of the vesicles. Although some leakage is observed, no major destabilization of the membranes occurs, at least when PC is present in the vesicles. Incubation of the MBP-vesicle complex with trypsin results in a complete dissociation of vesicle aggregates, which is also an indication of the maintenance of membrane integrity. These data stress the importance of MBP in cross-linking membranes without disturbing the membrane integrity.

Yet, the interaction of MBP with membranes is more complex as one would expect from these data. This can be clearly inferred from studies of the interaction of MBP with vesicles lacking negatively charged lipid, but rather consisting of PC and cholesterol (*chapter 2*). Also these vesicles are aggregated upon addition of

MBP, which clearly demonstrates the ability of MBP to interact hydrophobically with membranes.

MBP-induced aggregation of vesicles is also dependent on the interaction of the protein with other MBP molecules. This MBP dimerization is highly pH-dependent and indeed, when PC/chol vesicles were aggregated by MBP as a function of pH, a decrease of aggregation was observed upon lowering the pH. This result stresses the importance of MBP-MBP interaction in vesicle aggregation and therefore also in myelin compaction.

The presence of calcium at moderate concentrations results in an extensive decrease of MBP-induced vesicle aggregation. Even preformed MBP-vesicle complexes can be dissociated in the presence of calcium. This calcium dependent MBP-membrane dissociation could regulate myelin compaction. Both, a competition effect and a direct influence of calcium on MBP structure are plausible in explaining these results. As it was shown that monovalent [1], divalent cations and pH mediate the MBP-induced vesicle aggregation, the interaction of MBP with the myelin membrane is prone to regulation via the presence of ion-channels. Subsequently these ion-channels may be under control of exogenous and/or endogenous signals. As pore formation by PLP/DM-20 has been suggested [2], it is tempting to speculate a role of PLP/DM-20 in myelin compaction via such a mechanism.

Recent studies showed that MBP is heterogeneous with respect to charge and molecular weight. Posttranslational modification of MBP, giving rise to the different charge isomers, is a well-characterized phenomenon but its precise function is still not known. It has been implied that it may influence the interaction of MBP with the myelin membrane, and as a result may influence myelin compaction. Yet, in *chapter 3* we show that, although aggregation kinetics are influenced by this modification of MBP, the extent of aggregation is only marginally influenced by posttranslational modification. Vesicles consisting of lipids mimicking the inner leaflet of the myelin membrane were aggregated to the same extent by the different charge isomers. Only with the C-8 charge isoform, which is extensively modified, a total absence of aggregation is observed. However, posttranslationally modified MBP showed an increased potency to self-associate and to interact with other proteins, such as tubulin. Thus, MBP modifications such as phosphorylation may be important in the initial myelination events, when they can regulate the affinity of MBP for membranes and proteins.

How are the interaction of MBP with the myelin membrane and as a consequence, the myelin compaction regulated? In *chapter 2* we already observed that changing the calcium concentrations can have extensive effects on MBP-membrane association and on the MBP-induced vesicle aggregation. In *chapter 4* we show that sphingosine, a metabolite of sphingolipids, influenced the interaction

of MBP with membranes. The presence of sphingosine (or psychosine) reduced the MBP-induced aggregation considerably. The mechanism appears to be related to the positive charge of the molecule, causing interference with the binding of MBP to the membrane. This interference can be a result of charge neutralization of the negatively charged PS or a direct interference of sphingosine with MBP-membrane interaction, involving the charge repulsion. The potency of MBP to interact hydrophobically and electrostatically with membranes reduces the effect of sphingosine on MBP-membrane interaction.

The involvement of MBP heterogeneity in multiple sclerosis is discussed in *chapter 8*. The increased presence of the C-8 charge isomer in MS patients could be demonstrated indirectly (*chapter 8*).

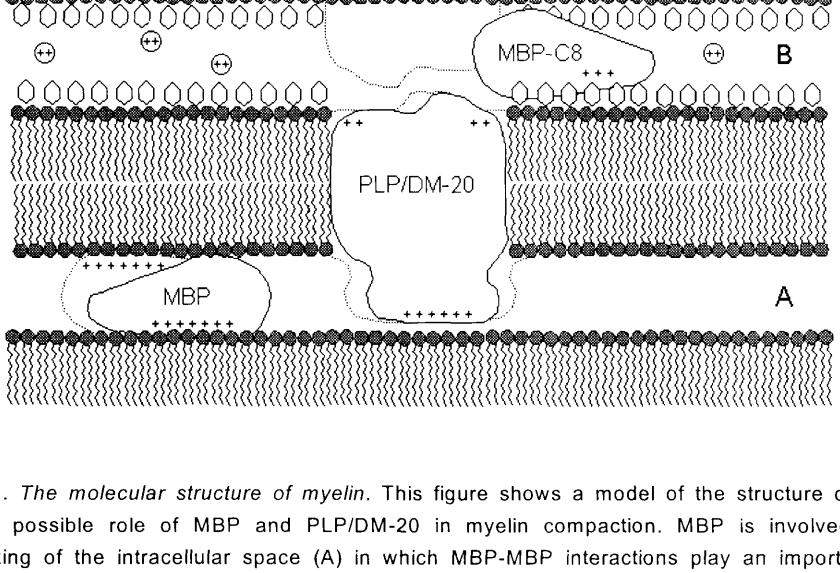

Figure 1. *The molecular structure of myelin.* This figure shows a model of the structure of myelin and the possible role of MBP and PLP/DM-20 in myelin compaction. MBP is involved in the crosslinking of the intracellular space (A) in which MBP-MBP interactions play an important role. This compaction may be regulated by divalent or monovalent cations or by modulation of the lipid composition. The involvement of PLP in myelin compaction at the intracellular space cannot be excluded (see text).

Interaction of PLP/DM-20 with membranes or the oligomerization of PLP/DM-20 are important in the compaction of the extracellular space (B). In addition this compaction may also be facilitated by the presence of calcium ions. Finally, the observation of MBP's presence at the extracellular site [4] may also imply a role for this protein in adherence of the membranes at the extracellular site.

The unchanged ratio of different isoforms of MBP when MS-patients were compared to healthy controls indicates that the observed increase of C-8 may not be directly related to remyelination.

Proteolipid protein

PLP is an integral membrane protein and is regarded as one of the most hydrophobic proteins. Its function in myelin is still a matter of debate but its relative abundance in myelin suggests a structural function. PLP was isolated, delipidated and transformed to a water soluble complex. In this complex most of the hydrophobic stretches are buried, and the hydrophilic stretches are exposed to the aqueous environment. With this complex we observed a pH dependent self-association (*chapter 5*). Interaction of this form with vesicles was highly dependent on the presence of negatively charged lipids. Yet, binding and penetration into zwitterionic vesicles was also observed. The presence of negatively charged lipids seemed to be a prerequisite to obtain PLP-induced aggregation of vesicles.

However, it is important to determine whether PLP can interact with membranes, when it is located in membranes. Thus the question arises whether PLP can form interlamellar attachments, when intercalated into membranes, i.e., its natural environment. Therefore a procedure was developed to reconstitute PLP in artificial membranes. This was most successfully accomplished at mild acid conditions (*chapter 6*). These vesicles still showed the tendency to form aggregates, indicating that self-association of PLP may be one of the mechanisms via which this protein can form interlamellar bridges. However, when vesicles containing PLP were incubated with vesicles containing the negatively charged lipid PS, aggregation occurs. This aggregation is accompanied by leakage and lipid dilution. PLP reconstituted in vesicles was able to aggregate vesicles containing sulfatide but to a lesser extent than that obtained with PS.

The natural occurring isoform of PLP, called DM-20, was used to investigate the role of a charged sequence (116-150) in PLP in the aggregation of vesicles. DM-20 showed a reduced aggregation and lipid mixing compared to PLP and its affinity for fluorescent probes was reduced. These observations show that the particular sequence is can modulate crosslinking of membranes. But also DM-20 has retained the ability to bind to charged membranes (*chapter 7*).

Interestingly it is known from the literature that PLP interacts preferentially with one of the charge isomers of MBP, namely C-8. [3] This charge isomer was also found to be predominantly present at the extracellular space [4] and therefore it cannot be excluded that C-8 together with PLP/DM-20 may be involved in myelin compaction at the extracellular space, as depicted in figure 1.

Suggestions for further research

Several important issues need further research. The function and properties of the different protein isoforms and isomers are still far from being elucidated. This

thesis presents evidence that localization and properties of the different MBP species imply differences in function. Possible interaction of MBP with other proteins or the cytoskeletal network in oligodendrocytes is evident from our results. Yet, these interactions have to be shown *in vivo*, and the regulation of these interactions will needs further characterization. Also, the effect of sphingolipid bases on MBP-membrane interaction *in vivo* remains to be determined.

The different localization of the MBP-isoforms is still an intriguing phenomenon. Although all of them are highly charged, they can be found at different sites in the oligodendrocyte. The involvement of selective MBP mRNA transport is obvious. Yet it will be interesting to compare the interaction of the different isoforms of MBP with artificial membranes. Moreover, such a study can also be of clinical interest, because different isoforms of MBP can be involved in (re)myelination.

Recently it was shown that there are other proteins present in oligodendrocytes and myelin, which may be important in the biogenesis of myelin and myelin structure [5]. Characterization of these compounds and examination of their interaction with membranes will give insight in the mechanism by which these proteins may be involved in determining and defining the myelin structure.

Since long, the involvement of the major myelin proteins MBP and PLP in MS has been recognized, and it is now well established that both proteins play an important role in the immunological response which accompanies MS. In this thesis, some preliminary data are given concerning involvement of distinct MBP charge isomers in the biochemical composition of MS myelin. Although a direct correlation may not exist between C-8 and remyelination, the relative increase of C-8 vs. non-citrullinated MBP may be related directly to the disease. This important observation needs further research in which MS model systems other than EAE [6,7] may be very helpful.

It will also be relevant to further define the role of the heterogeneity of the proteins. Possibly, distinct isomers may play a prominent role during particular the myelination process e.g. in stages in which structural dynamics of myelin is needed. Thus changes in MBP heterogeneity may be also important in relation to the occurrence of demyelinating diseases, such as multiple sclerosis. This emphasizes the necessity for continuous research of fundamental aspects concerning the biogenesis and the structure of myelin.

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