Modulation of thermotropic properties of cholesterol-free model myelin membranes by myelin basic protein

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Abstract: As a continuation of our research on model myelin membranes (MMM), in which we revealed that MMM that mimic normal (healthy) myelin are more fluid at 30 °C than those that mimic modified (diseased) myelin, we aimed to unveil the contribution of cholesterol in MMM thermotropic properties. Accordingly, we prepared MMM as lipid mixtures in the presence and absence of myelin basic protein (MBP), but excluding cholesterol (named MMM′). Using differential scanning calorimetry (DSC) and temperature-dependent UV-Vis spectroscopy, we determined the main phase transition temperature (T_m) of MMM′, i.e., the temperature of the gel-to-fluid phase transition of lipids in MMM′ systems. Besides the discrepancies obtained by these two techniques, we identified a difference of about 5 °C between normal and modified MMM′, independently of MBP. As evidenced by the maintenance and weakening of van der Waals forces at 30 °C and 60 °C, respectively, the fluidity of normal and modified MMM′ is comparable at a corresponding temperature. The modified MMM′ reflected different polar headgroup hydration than normal MMM′ at 30 °C, which is very likely associated with the stabilization of the gel phase in modified MMM′.

Keywords: model myelin membranes, cholesterol, myelin basic protein, thermotropic properties

1. Introduction

Myelin is a multilayered, lipid-rich membrane that surrounds the axon, providing structural support and ensuring the efficient transmission of neural impulses. One of the most notable features of myelin's composition is its high lipid content (70-85 %) in contrast to that of proteins (15-30%) [1]. Upon disruption of the myelin sheath in terms of unwinding of the sheath, the formation of vacuoles, or swelling of the bilayers due to the leakage of water and ions, commonly referred to as demyelination, the impaired transmission of neural impulses occurs and can produce clinical symptoms such as vision loss and muscle weakness, which are common features of multiple sclerosis (MS)

[1]. Studies on experimental autoimmune encephalomyelitis (EAE), an animal model of MS, have shown significant alterations in the amounts and ratios of key myelin lipids compared to those found in normal myelin. More specifically, diseased varieties displayed the reduced amount of sphingomyelin (SM) and phosphatidylcholines (PC), increased amount of phosphatidylethanolamines (PE), phosphatidylserines (PS), and cholesterol (chol), as well as reduced adhesive activity of myelin basic protein (MBP) [2].

As the molecular-level events underlying demyelination are poorly understood, we previously explored model myelin membranes (MMM) that mimic both normal and modified myelin membranes, with and without myelin basic protein (MBP). By examining the thermotropic properties of MMM with adsorbed MBP, we indirectly discriminated fluidity modulations of normal and modified MMM in the presence of MBP [3]. Given that cholesterol is one of the critical factors in (de)myelination [4], here we undertook the second step, i.e, we examined the thermotropic properties of both normal and modified MMM in the absence and presence of MBP, while excluding cholesterol (in the continuation of the text, they are referred to as normMMM'/modifMMM' ± MBP).

2. Results and Discussion

NormMMM'/modifMMM' ± MBP were prepared as multilamellar vesicles (MLV) according to established protocols [5] following lipid ratios in [2]. Their thermotropic properties were characterized using differential scanning calorimetry (DSC) and temperature-dependent UV-Vis spectroscopy. The former is a conventional approach that enables the detection of the main phase transition ($T_{m.}$), i.e. the transition of lipid bilayers from the gel to the fluid phase. The main discerning factor of these two phases refers to the weakening of van der Waals interactions between hydrocarbon chains of lipid molecules that results in a sudden decrease in the bilayer thickness, and is followed by the change in hydration of lipid polar headgroups [5]. The other technique relies upon a more recent approach that exploits the temperature-dependent change in turbidity of lipid suspensions, which is a direct function of the bilayer thickness. Accordingly, following chemometric approach [4] we determined T_m from the latter as follows: i) assuming that one component describes all temperature-dependent variability in the acquired spectra (Figure 1), we ii) decomposed the obtained spectra (**D**) as a product of two matrices: the one that that represents the concentrational profile of this one component (**C**) and another its spectral profile (**S**), respectively (**E** stands as a residuals matrix) (1), iii) owing to the sigmoidal character of the temperature-dependent concentrational profile, from the inflection point we determined T_m (Figure 2).

$$\mathbf{D} = \mathbf{C}\mathbf{S}^{\mathrm{T}} + \mathbf{E} \quad (1)$$

The molecular-level features that highlighted the change in van der Waals interactions are the FTIR bands (Figure 3) originated from the (anti)symmetric stretching of methylene goups of hydrocarbon chains ($\nu_{(a)s}CH_2$), whereas the changes in the bands

originated from the antisymmetric stretching of phosphate groups ($\nu_{as}PO_2^-$) suggested the differences in the hydration of normMMM'/modifMMM' ± MBP systems. [3]

3. Methodology

Temperature-dependent UV-Vis spectra (Figure 1), DSC curves (Figure 2a) and FTIR spectra of normMMM'/modifMMM' ± MBP (Figure 3) were acquired under the same conditions and analysed (Figure 3b) analogously to the data presented in [3] and [4]. The discrepancy between the values obtained from DSC and UV-Vis spectroscopy data (Table 1) originates from determining the change in turbidity at the beginning of the melting, which corresponds to the onset of the DSC curve, not its maximum (as determined here and in [3]).

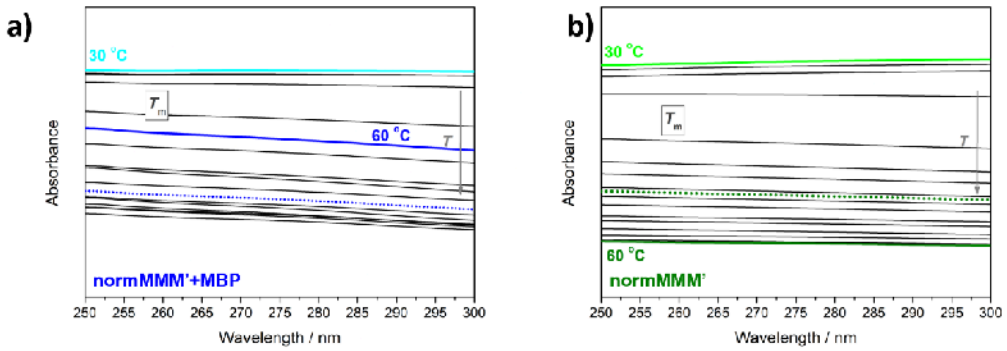

Figure 1. Temperature-dependent UV-Vis spectra (solid curves) and spectral profiles (dotted curves) of: a) normMMM'+MBP; b) normMMM'. The spectra acquired at 30 °C/60 °C are highlighted, as well as spectral profiles.

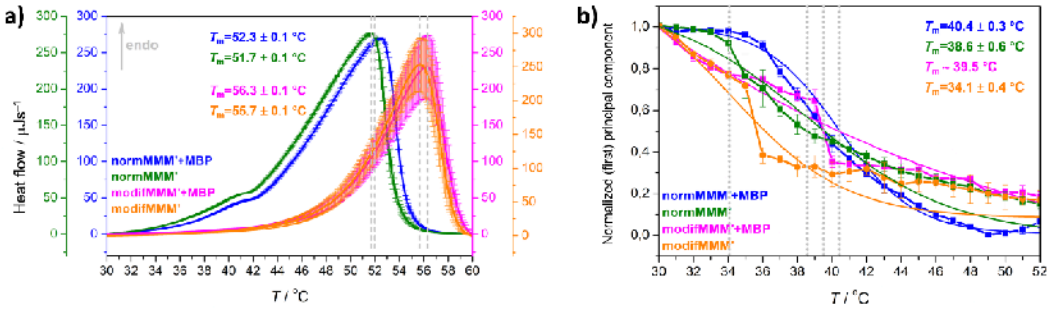

Figure 2. a) DSC curves of MMM' in the absence/presence of MBP; b) concentrational profiles of the (first) principal component accompanied with its fit on a single Boltzmann sigmoidal transition. The color associated with the spectrum of particular system is presented in the legend. Phase transition temperatures are highlighted with dashed lines and written on graphs.

Table 1. The main phase transition temperature (T_m) of normMMM'/modifMMM' ± MBP as obtained from DSC and from temperature-dependent UV-Vis spectra

system	T_m / °C

	DSC	UV-Vis
normMMM'+MBP	52.3 ± 0.1	40.4 ± 0.3
normMMM'	51.7 ± 0.1	38.6 ± 0.6
modifMMM'+MBP	56.3 ± 0.1	~ 39.5
modifMMM'	55.7 ± 0.1	34.1 ± 0.4

According to the FTIR spectra, in normMMM'/modifMMM', the adsorption of MBP does not modulate the fluidity of the MMM' as they do in the presence of chol [3], i.e., at 30 °C, all MMM' are in the gel phase, whereas at 60 °C are in the fluid phase (Figure 3a). The hydration differences are more pronounced between normMMM'/modifMMM' in the gel phase (at 30 °C, Figure 3b), probably due to the poorer hydration of phosphate groups in modifMMM' than in normMMM', irrespective of the MBP.

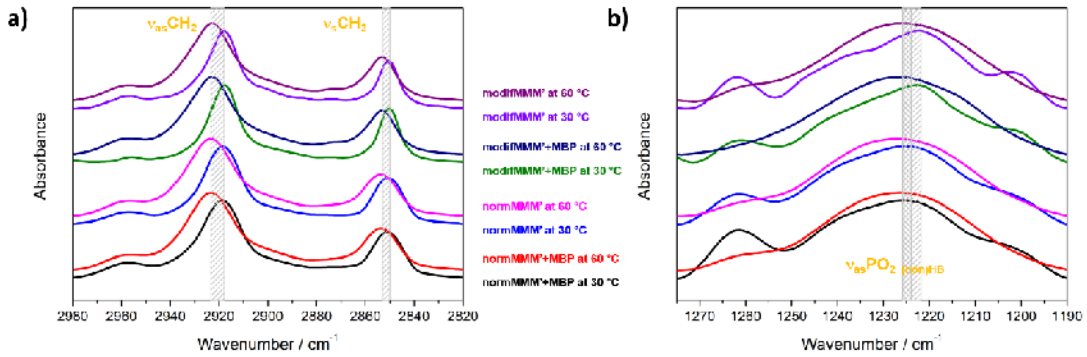

Figure 3. FTIR bands of normMMM'/modifMMM' ± MBP at 30 °C and 60 °C: a) $\nu_{(a)s}CH_2$; b) $\nu_{as}PO_2^-$. The color associated with the spectrum of a particular system is presented in the legend (in the middle of the figure).

4. Conclusions

In comparison with MMM, the absence of chol increases T_m, which is higher for modifMMM' than for normMMM', implying greater rigidity of the latter. In all systems, the adsorption of MBP does not lead to the reduction of van der Waals forces in chol-free systems. As evidenced by our previous data, the presence of chol in MMM is critical for the modulation of MMM fluidity in the presence of MBP.

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