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Thermotropic behaviour of myelin from multiple sclerosis affected brain

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Abstract

Myelin membrane has been extracted from the brains of individuals who during their life suffered from multiple sclerosis (MS). The thermotropic properties of these membranes and myelin membrane from brains unaffected by neurological disease were measured with a differential scanning calorimeter and compared. Two melting transitions were found, one near 40° and the other near 80°C. The transition at 80°C appears to be due to reversible denaturation of one of the membrane proteins, the proteolipid protein, and that at 40°C, to irreversible melting of the membrane sphingolipids. In MS myelins these transitions often occurred over a greater temperature range, and in many samples two separate melting processes could be clearly seen. The change in the higher temperature melting is evidently caused by a breakdown of proteolipid protein, as a broadened melting transition similar to that seen in MS myelins was also detected in disease-free myelin which had been stored above 0°C for several months. In addition, infrared spectroscopy showed that the ratio of the carbonyl/amide absorptions of the membrane (determined largely by the ratio of lipid to protein) increased in a sample which had a very ill-defined high-temperature melting. Loss of proteolipid protein from the membrane could account for such a change in the spectrum. The origin of the two-staged melting of the sphingolipids cannot be determined with any certainty at the moment, but it is possible that the range of lipid species which go to make up the membrane sphingolipids is greater in myelin from the MS-affected brains. © 1997 Elsevier Science B.V.

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1. Introduction

The white matter of the vertebrate nervous system consists of nerve fibres wrapped in thick sheaths of fatty membrane. This membrane is called myelin. Myelin insulates the nerve fibres, and in doing so greatly increases the rate of conduction of electrical impulses along them. Although myelin is morphologically an extension of the plasma membrane of the myelin forming cells, oligodendrocytes in the central nervous system (CNS), its composition differs greatly from the oligodendrocyte membrane and the membranes of other eukaryotes. Plasma membranes are made up of lipid and protein and in myelin the ratio of lipid to protein is higher and the lipid content is unusual. It is enriched in cholesterol, galactocerebrosides and ethanolamine phospholipids. The seven most common classes of lipid in myelin are:- cholesterol (40.9 mol% of total lipid), galactocerebroside (15.6%), cerebroside sulphate (4.1%), phosphatidylcholine (10.9%), ethanolamine phospholipids (13.6%), serine phospholipids (5.1%) and sphingo-

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myelin (4.7%). The sphingolipids, cerebroside, cerebroside sulphate and sphingomyelin contain sphingosine and long (typically C_{24}) fatty acids that are often hydroxylated [1].

In an earlier work from this laboratory [2], the thermotropic behaviour of bovine brain sphingolipids and their mixtures with cholesterol and the brain phospholipids was investigated with a differential scanning calorimeter. It was found that galactocerebrosides have very high gel–liquid crystal transition temperatures for biomembrane lipids. The galactocerebrosides with non-hydroxylated chains melted at 78.9°C. Melting temperatures for the myelin phospholipids are below 0°C. The galactocerebrosides formed solutions with cholesterol and phospholipids in the liquid crystalline state but phase separation occurred when these mixtures were cooled below the gel–liquid crystal transition temperature. The gel–liquid transition temperature for these mixtures lay between 35° and 55°C. When a sample of guinea pig CNS myelin, which had been equilibrated at 5°C for several weeks, was heated two endotherms were found in the heating scan – one near 40° and the other near 80°C. By analogy with the experiments conducted on the cerebroside mixtures, it was proposed that the endotherm at 40°C was the result of the melting of a sphingolipid gel phase which had separated from the other constituents of myelin on cooling.

Demyelination of nerve fibres occurs in a number of diseases of which multiple sclerosis (MS) is prototypical. Multiple sclerosis is an autoimmune disease in which myelin is destroyed while the nerve fibres, at least initially, are left intact. Epidemiological studies have shown that some individuals are more susceptible to this disease than others [3]. The work reported here compares the thermotropic properties of myelin from the brains of individuals who had suffered from multiple sclerosis with myelin from brains free of neurological disease. The intention was to see, if there was a difference in the myelin membrane of MS sufferers, either inherent or as a result of disease, whether this difference would be reflected in the membrane's thermotropic properties.

In addition, the infrared spectrum of all myelin samples was recorded to assist the interpretation of the calorimetric data.

2. Materials and methods

2.1. Materials

Brain from MS patients was obtained from the MS Research Laboratory at Queen's University, Belfast, and disease-free brain from the MS Science Laboratory in the Department of Neurochemistry of the Institute of Neurology, London. Brains had been removed at autopsy within 12 h of death and stored at −78°C. White-matter samples were removed from areas free of gross plaques in which there were no visible signs of deterioration and the myelin isolated according to the method of King et al. [4]. White matter was homogenised in 0.29 M sucrose solution in a ratio of 10% w/v. The homogenate was layered over 0.85 M sucrose and centrifuged for 45 min at 82 500 g and 2°C. The flocculent layer left at the interface was collected, resuspended in 0.29 M sucrose solution and centrifuged again in the same manner. The interface layer was again collected and washed twice by mixing with distilled water and centrifuging at 80 000 g. The resulting myelin pellet was freeze-dried, weighed and stored at −20°C.

Sections from four brains (designated D255, 259, 261 and 276) were obtained in Belfast and from two brains (D287 and 292) in London. Four samples of myelin were extracted from each brain section, sufficient for analysis by both calorimetry and infrared spectroscopy.

2.2. Methods

2.2.1. Calorimetry

Freeze-dried myelin was packed tightly into high-pressure stainless steel pans. After a portion had been added, it was hydrated with excess buffer and the shrinkage in sample volume caused by addition of the buffer allowed the next portion to be added. In this manner, between 45 and 50 mg were packed into each pan. The pans were then hermetically sealed. The buffer used was phosphate-containing saline (0.1 M sodium chloride, 0.02 M phosphate, 0.0002 M sodium azide, pH 7.0). The weight of sample in the pans was determined by weighing the container of myelin before, and after each sample had been removed. The pans were then equilibrated at 5°C for six weeks. Heating and cooling scans were carried out on a

Perkin–Elmer DSC 7 differential scanning calorimeter, interfaced to a Perkin–Elmer 7700 computer via a TAC7 instrument controller. Heat flow vs. temperature data were digitised and stored on the computer's hard disc. The temperature, at which the heat flow in a transition was at a maximum (T_{max}), was calculated using software supplied by Perkin–Elmer. The temperature scale was calibrated using cyclohexane and indium as standards. All samples were scanned at 10°C/min.

2.2.2. *Fourier transform infrared spectroscopy (FTIR)*

Spectra were obtained using a Perkin–Elmer 1750 FTIR spectrometer equipped with a TGS detector and Perkin–Elmer 7300 computer for data acquisition and analysis. Myelin in buffer (10 mg/cm^3) was placed in thermostatted Beckman FH-01 CFT microcell fitted with CaF_2 windows and a 50 μm Teflon spacer. Temperature control was achieved by means of a cell jacket of circulating water. Infrared spectra were recorded at 20°C by signal averaging 400 scans at a resolution of 4 cm^{-1}. The spectrometer was continuously purged with dry air to eliminate water-vapour absorptions from the spectral regions of interest. A sample shuttle was used to permit the background to be signal-averaged concurrently with the sample. The buffer used for spectroscopy was deuterium oxide containing 0.1 M sodium chloride and 0.02 M phosphate. Its pH was 7.0. Buffer spectra were recorded in the same cell and under the same instrument conditions as the sample spectra. Difference spectra, the spectra of myelin free of buffer absorptions, were obtained by digitally subtracting solvent spectra from the corresponding sample spectra.

3. Results

Myelin samples from the MS-affected brains, D255, 259, 261 and 276, showed broadly similar thermotropic behaviour. Like the guinea pig CNS myelin studied earlier [2], two melting processes were evident, one near 40° and the other near 80°C. However, unlike guinea pig myelin or myelin from disease-free white matter, melting transitions in MS myelins often took place in two stages. This behaviour is shown for the low-temperature transition in Fig. 1 and for the high-temperature transition in Fig. 2. Cooling curves, (b) in both figures, show that only the high-temperature transition was reversible. Fig. 3 contains heating scans which show the range of splitting of the low-temperature transition found in the sixteen samples examined from brains D255, 259, 261, and 276, from a minimum at (c) to maximum at (e). The T_{max}s for all these samples lay within 2°C of the T_{max} of myelin from D287 (trace (b)), a disease-free brain. Approximately 7°C below T_{max} in twelve samples there was either a shoulder or a fully resolved sub-transition. At maximum (e), the enthalpy change in this sub-transition was ≈50% of the total enthalpy change. Surprisingly, T_{max} for all samples of myelin from brain D292, the other disease-free brain, was 7°C

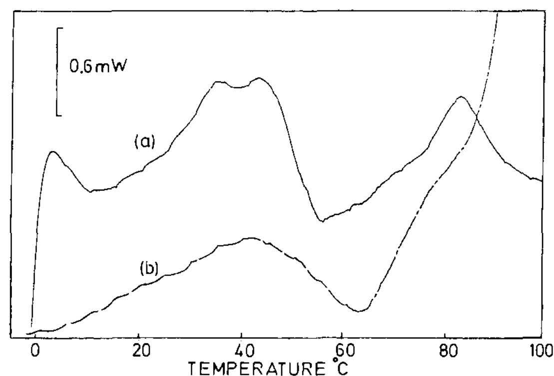

Fig. 1. Thermogram of a myelin sample from MS-affected brain, D255. (a) – heating scan, T_{max}s 35.0, 43.7 and 85.8°C, and (b) – cooling scan, T_{max} 63.4°C.

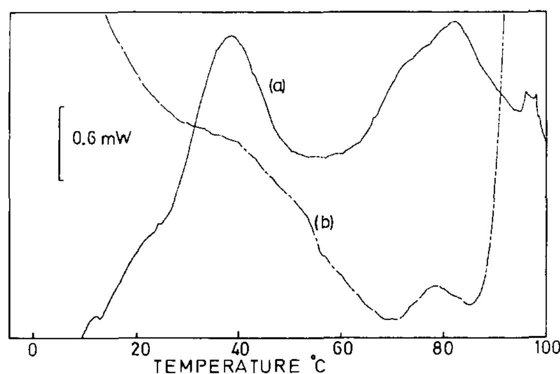

Fig. 2. Thermogram of a myelin sample from MS affected brain, D261. (a) – heating scan, T_{max}s 39.0 and 82.9°C, and (b) – cooling scan T_{max} 72.9°C.

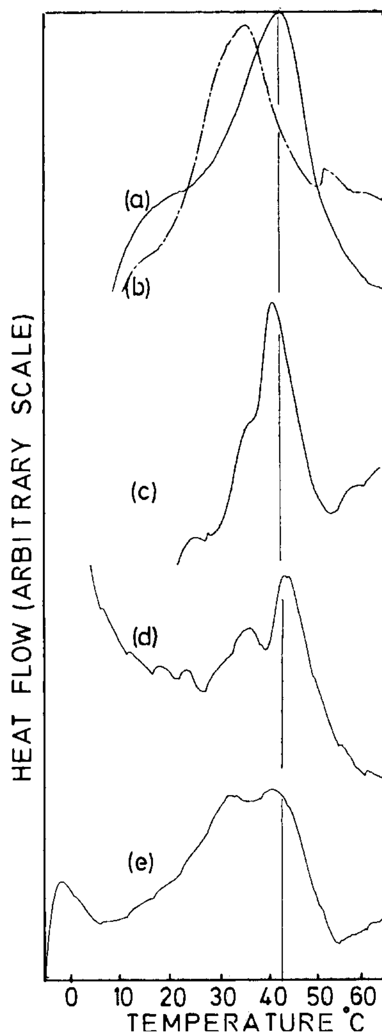

Fig. 3. Heating scans made on myelin samples from: disease-free brains (a) – D287, T_{max} 42.4°C, and (b) – D292, T_{max} 35.6°C: and MS-affected brains (c) – D261, T_{max} 40.0°C, and (d) D276, T_{max}s 36.4° and 43.6°C and (e) – D255, T_{max}s 35.0° and 43.7°C.

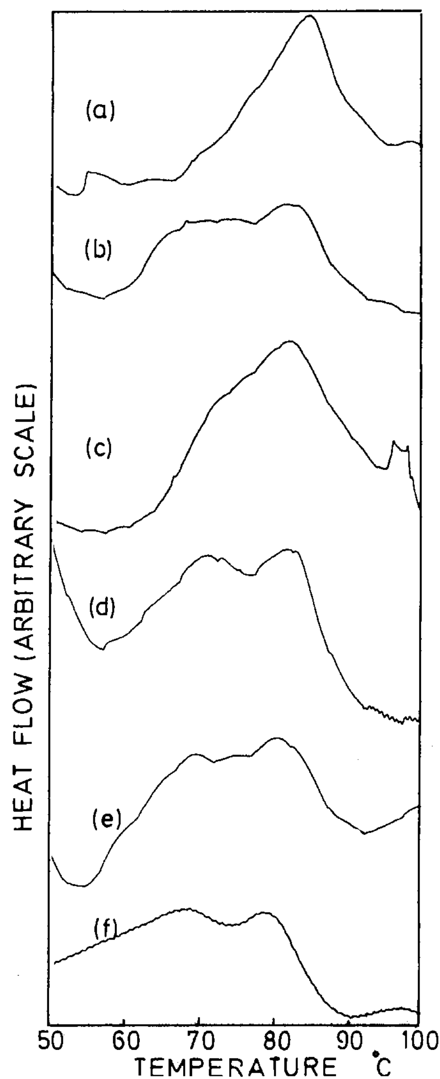

Fig. 4. Heating scans made on myelin samples from: (a) – disease-free brain D287, T_{max} 84.9°C; (b) – disease-free brain D287 after standing for six months at 5°C, T_{max}s 81.5° and 72.6°C; and myelin samples from: (c) – MS-brain D261, T_{max} 82.9°C; (d) – MS-brain D259, T_{max}s 82.1° and 70.7°C; (e) -D261, T_{max}s 80.0° and 70.0°C; and (f) – MS-brain D276, T_{max}s 78.7° and 68.7°C.

below T_{max} for D287, in the region of the sub-transition of the MS myelins.

Fig. 4 contains heating scans which show the degrees of splitting of the high-temperature transition in myelin from samples D255, D259, D261 and D276. T_{max} for this transition decreased as the splitting increased. At maximum (f), the bulk of the enthalpy change took place in the sub-transition, and T_{max} of the original transition was ≈6°C below T_{max} for this transition in the disease-free brain D287. Thirteen out of sixteen samples from these brains showed two-stage melting behaviour of this type. The high-temperature melting transitions for myelin samples from the two disease-free brains were not significantly different.

No splitting of either high- or low-temperature transitions was found in any myelin sample from

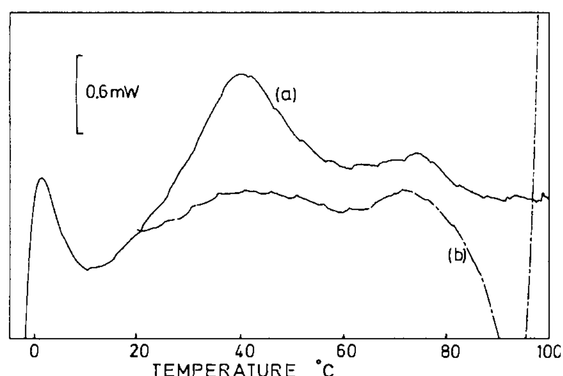

Fig. 5. Thermogram of a myelin sample from MS-affected brain, D276: (a) – heating scan, T_{max}s 40.1° and 76.6°C; and (b) – cooling scan, T_{max} 60.5°C.

disease-free brains D287 and 292 that had been equilibrated at 5°C for six weeks.

Fig. 4(b) shows a heating scan carried out on a myelin sample from disease-free brain D287 that had been held at 5°C for six months. Clearly, this treatment has caused a broadening of the high-temperature transition which is now very similar to that observed in most of the MS myelins. However, this treatment had no effect on the low- temperature melting, which was still single-staged and had an unchanged temperature of maximum heat flow.

In general, enthalpy changes for comparable transitions in the samples described previously were very similar. No attempt has been made to calculate precise enthalpy changes because the nature of species responsible for the transitions are not known with certainty, nor are the compositions of any of the myelin samples. In one sample from brain D276, there was clearly a significant decrease in the enthalpy change of the high-temperature transition. Heating and cooling scans for this sample are shown in Fig. 5.

Fig. 6 shows FTIR spectra of samples of myelin from the two disease-free brains and MS brain D276 in the 1600–1800 cm^{-1} wave number range. The largest and most reproducible variation found in the FTIR spectra was in the ratio of the magnitudes of the carbonyl ($\sim$1733 cm^{-1}) and amide I ($\sim$1650 cm^{-1}) absorptions [5]. This ratio for samples from the same disease-free brain was nearly constant, as can be seen by comparing the results from D287, (d) and (e) in the figure. In D292, (c), the ratio of the carbonyl/(amide I) absorptions was slightly greater. Most of the MS

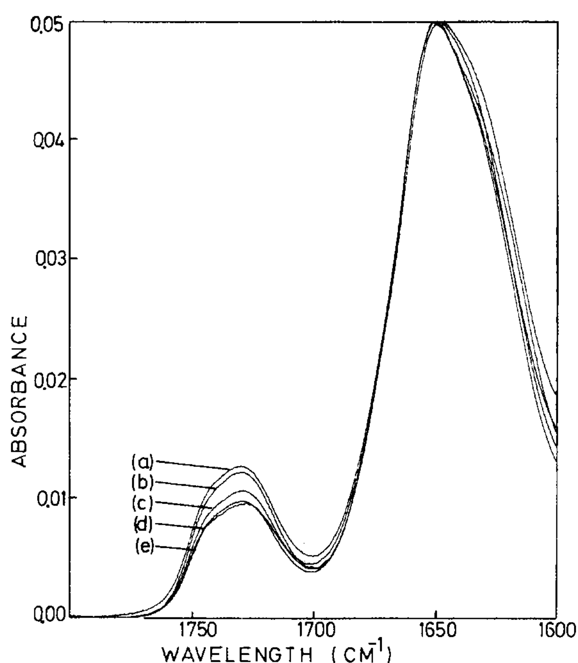

Fig. 6. Fourier transform infrared spectra between 1800 and 1600 cm^{-1} of myelin samples from MS-brain D276 (a) and (b), disease-free brain D292 (c), and disease-free brain D287 (d) and (e).

myelins had a carbonyl/amide ratio which was similar to the value for D292. However, two samples in particular from D276 had significantly greater ratios, (a) and (b). Just like the fall in the amide I absorption relative to the carbonyl absorption, there was a similar relative fall in the magnitude of the carbon-hydrogen stretching vibration at 2 820 cm^{-1}. However, the magnitude of this decrease was not as large as that seen in the amide I absorption.

4. Discussion

A previous study [2] with lipids extracted from bovine brain showed that the 40°C transition in myelin was due to melting of sphingolipid phase which separated from the other components of the myelin membrane on cooling. It was postulated that melting took place at the boundary between the sphingolipids and the remainder of the membrane. In this case, the splitting of the melting endotherm of the sphingolipids of the MS myelins could either occur because these

lipids crystallised in two phases with different compositions, or else because sphingolipid crystallites were present in a heterogeneous membrane environment. The large difference between the low- temperature melting processes in the samples from disease-free brains D287 and D292 was unexpected. However, there was also a significant difference between the spectra of myelin from these two brains. The infrared absorption at $1733\,cm^{-1}$ in myelin is from the carbonyl groups of the membrane glycerolipids, the phosphatidylcholines, phosphatidylethanolamines and phosphatidylserines, the $1650\,cm^{-1}$ absorption mainly from protein with a small contribution from the sphingolipids [5]. An increase in the carbonyl/amide ratio implies an increase in glycerolipids relative to the amide-containing components of the membrane, protein and sphingolipid. The agreement between the melting temperature of myelin from the disease-free brain D292 and the sub-transition of the MS myelins is also noteworthy, but establishing its significance will have to await the collection of more data on disease-free myelin.

Given that the myelin membrane consists of protein and lipid it might be anticipated that the source of the transition at 80°C was denaturation of one of the membrane proteins. However, Figs. 1 and 2 show this transition to be reversible, although protein denaturations are not usually reversible [6], at least not on the time scale of the experiments described here. Cortijo et al. [7–9] have detected a similar transition in myelin from the bovine brain, T_{max} 80.3°C, and assign this transition to the denaturation of myelin proteolipid and DM20 protein. However, this transition was irreversible. It can hardly be a coincidence that melting transitions occur at identical temperatures in both bovine and human myelin. Thus, the transition in human myelin was apparently the result of proteolipid protein denaturation. Why it was reversible in human myelin remains unclear. Trace (b) in Fig. 4 shows that the changes detected in this transition in MS myelin can be simulated by prolonged storage above 0°C. Prolonged storage under these conditions will cause chemical breakdown and proteolysis of the protein component of the membrane. Therefore, the decrease in T_{max} and splitting of the high-temperature transition in MS myelin was probably due to breakdown of membrane protein.

The increase in the carbonyl/amide ratio seen in samples from D276, spectra (a) and (b) in Fig. 6, and the near absence of any sign of a protein denaturation in one of these samples, Fig. 5, suggests that in some regions of this brain proteolysis had gone far enough for protein fragments to be lost from the membrane.

5. Conclusion

There are clear differences between the thermal properties of myelin from disease-affected and disease-free white matter. In principle, the changes in the structure or chemical composition of myelin which give rise to these differences could either have existed during life, or may have occurred between death and autopsy or else during storage after autopsy. Since all brain specimens were stored at −80°C and there was no correlation between time in storage and the extent of the changes in thermal properties, we can discount the third possibility. The second possibility is plausible since some of the changes observed in myelin from MS-affected brain take place in myelin from disease-free brain if it is not stored at a very low temperature. It may be that the level of endogeneous proteases are higher in the MS-affected brains. However, the loss of protein from one MS-affected brain, suggested by both calorimetry and spectroscopy, could only have occurred during their life. The differences in membrane composition or structure which are responsible for the two-stage melting of the sphingolipids also seem to have occurred during their life. It is possible that the range of sphingolipid types in myelin from the MS-affected brain is greater than that found in disease-free samples.

Differences in myelin composition, which were present before death, could be hereditary and be among the factors responsible for the predisposition of some individuals to develop MS. However, although white-matter samples were removed from areas free of visible deterioration, the possibility cannot be excluded that changes in myelin properties revealed by calorimetry occur during the very earliest stages of the interaction of the immune system with the myelin membrane, before deterioration becomes apparent.

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