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THERMOTROPIC PHASE TRANSITIONS IN NORMAL HUMAN MYELIN AS OBSERVED IN A SENSITIVE MICROCALORIMETER

M.A. MOSCARELLO, A.W. NEUMANN and D.D. WOOD

Department of Biochemistry, Research Institute, Hospital for Sick Children, and Department of Mechanical Engineering, University of Toronto, Toronto (Canada)

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Using a differential heat capacity calorimeter, phase transitions have been observed in normal human central nervous system myelin. Two large endothermic transitions at 33 and 77°C, respectively, and a smaller transition at 61–62°C, were routinely observed. The relatively simple protein composition of myelin has allowed us to assign the lower transition (33°C) to proteolipid-lipid interactions and the higher temperature transition (72°C) to basic protein-lipid interactions. The small transition at 61–62°C was not assigned, but is probably due to lipid. The abolition of the high temperature transition, after extraction of basic protein from myelin, the lack of phase transitions when a total lipid extract or the isolated proteins were studied, enabled us to assign the high temperature transition. Because the only other major protein was the proteolipid fraction, the low temperature transition (33°C) was assigned to proteolipid-protein interaction.

Introduction

Biological membranes consist of lipids and proteins primarily. The proportion of lipids and proteins varies widely from one membrane to another, e.g. red blood cells contain about 35% lipid by weight while myelin contains about 70% lipid by weight. All membranes contain a variety of lipids but the lipid composition varies widely, e.g. cholesterol varies from 9.4% of the total lipids of the nuclear envelope to 55.8% in intestinal microvillus membrane [1]. The lipids are generally believed to exist in the bilayer conformation. Organization of the lipids into groups or clusters, i.e. phase separation can be induced by various agents such as Ca^{2+} [2,3] and proteins [4], the former induces phase separation by binding to acidic lipids in a 1:2 molar ratio; proteins bind acidic lipids preferentially to their boundary layers [5]. Although membranes contain a large variety of lipids probably randomly mixed separation of specific lipids from the bulk by proteins results in the formation of a new protein-lipid complex, the properties of which may be quite different from those of either protein or lipid alone.

Phase transitions in membranes reflect specific structural properties. These can be most readily observed by calorimetry which detects processes having a significant energy change. Thermally induced transitions in membranes of *Mycoplasma laidlawii* were detected by calorimetric methods, a reversible transition attributed to the lipid components of the membranes and an irreversible transition at higher temperature attributed to protein denaturation [6,7].

Using conventional calorimeters, red cell ghosts have been examined for thermally induced phase

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

transitions [8] but none were found. However, thermally induced phase transitions in red cell ghosts have been reported more recently, using a much more sensitive differential heat capacity calorimeter [9]. At least four endothermic transitions were observed over a temperature range of 25–80°C. In contrast to the data reported for *Mycoplasma laidlawii* [6,7] in which the transitions were lipid phase transitions, those observed in the red blood cell were considered to be the result of protein denaturation [9].

Efforts to detect phase transitions in myelin, using conventional calorimetry have not been successful [10]. The failure to detect phase transitions in myelin was considered to be due to the high cholesterol content of this membrane (reported as 52.2% of the total lipids [1]). In the present communication, we report our studies on myelin in which we re-examine thermally induced phase transitions using a sensitive microcalorimeter similar to that used in the red cell work [9]. We observed two major and one minor transition in isolated human myelin. Since the major transitions are not reversible, they are not due to lipid components. However, neither of the two major proteins of myelin (lipophilin – a proteolipid component, nor basic protein) in isolated form show any cooperative phase transitions which could account for those observed with the isolated membranes. Although the nature of the transition cannot be identified clearly at the moment, protein components are definitely involved in a complex manner, probably in association with certain lipids.

Materials and Methods

Myelin was isolated from human white matter by the method of Lowden et al. [11]. For calorimetry studies, 50 mg of lyophilized myelin were suspended in 1.2 ml of Hepes buffer, pH 7.4, (2 mM Hepes, 10 mM NaCl, 0.1 mM EDTA) and homogenized gently to obtain a uniform suspension. The suspension was dialysed overnight against three changes of Hepes buffer at 4°C to remove traces of sucrose which were left behind from the sucrose gradient used in the isolation of myelin. One ml of the suspension was accurately measured into the sample cell of the microcalorimeter (Microcal MC-1, Amherst, MA) and an equal volume of buffer was measured into the reference cell. After equilibration, the heating rate was chosen and the colorimetric run was begun. Instrument calibration was carried out at regular intervals, using glass-distilled water in both reference and sample cells. Before samples were put into cells, the solutions were de-aerated under vacuum.

Phosphorus was determined by the method of Bartlett [12] on each myelin sample so that the same amount of myelin was used in each run even though no quantitative interpretation was made of the data.

Total lipid extract from myelin was prepared from 300 mg of isolated myelin after extraction of basic protein. The residue was chromatographed on an LH-20 column in chloroform/methanol (1 : 1, v/v) with 5% 0.1 M HCl [13] as described previously. The lipid fractions were pooled, neutralized with NH_3, dialysed against water to remove solvents and salts, lyophilized and stored under nitrogen at −20°C. For calorimetric runs, 1/6 of the total material was suspended in Hepes buffer, pH 7.4, as for the myelin samples.

Results

Normal human myelin

The thermogram obtained when 50 mg of myelin were heated from 12–90°C is shown in Fig. 1. Three endothermic transitions (Fig. 1a) were observed, one centered at 33°C, a small transition at 61–62°C, and a third transition at 72°C. If the sample was cooled to 5°C after heating to 90°C and then reheated at the same scanning rate used in the original run, no endothermic phase transitions were observed. Thus, the endothermic transitions observed in Fig. 1a are probably not lipid phase transitions because these should be reversible. If the sample shown in Fig. 1 was heated to 45°C so that only the first endothermic transition was recorded (Fig. 1b), cooled to 5°C immediately and then reheated, a broad rounded transition was observed (Fig. 1c). Therefore, the first transition was not reversible after heating to 45°C.

Although the temperatures and general features of all transitions were totally reproducible from run to run, ΔH values for the transitions are not reported because the broadness of the transitions made it difficult to estimate the beginning and

ending of the transitions. Thermograms were obtained for 34 different myelin samples from nine different individuals. The transitions were reproduced in every sample.

The thermograms for bovine brain sulphatide and cerebroside are shown in Fig. 1 d and e. Netiehr of these lipids show phase transitions which coincide with those observed in myelin. The total lipid extract from the equivalent of 50 mg myelin is shown in Fig. 1f. No sharp endothermic transitions were observed but rather a broad non-cooperative melting over the whole temperature range. We concluded that the transitions observed in myelin are probably not due to lipid components per se.

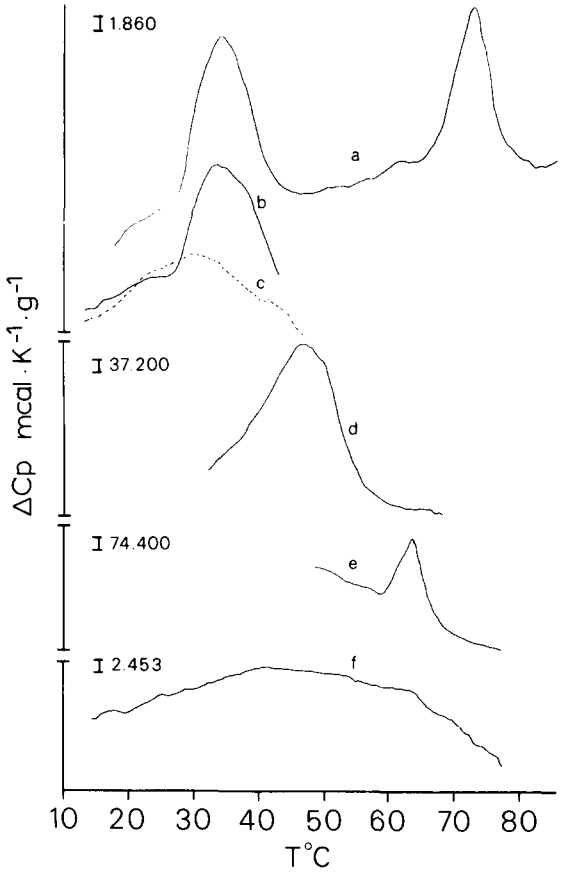

Fig. 1. Thermotropic phase transitions in normal human myelin and some lipids. (a) Normal human myelin (50 mg/ml) in Hepes buffer, pH 7.4. Scan rate 120 K/h, range 30 μV, sensitivity 8×. (b) Normal human myelin (50 mg/ml) in Hepes buffer, pH 7.4. Scan rate 120 K/h, range 30 μV, sensitivity 8×. (c) Same sample as (b) reheated after cooling from 45°C. (d) Bovine brain sulfatide (5 mg/ml) in Hepes buffer, pH 7.4. Scan rate 60 K/h, range 30 μV, sensitivity 8×. (e) Cerebroside (5 mg/ml) in Hepes buffer, pH 7.4. Scan rate 30 K/h, range 30 μV, sensitivity 8×. (f) Total myelin lipid extract from 50 mg myelin in Hepes buffer, pH 7.4. Scan rate 60 K/h, range 30 μV, sensitivity 16×.

Effect of pH on transitions

The effect of pH on the endothermic phase transitions was studied by obtaining thermograms at different pH values, from pH 3.2 to 10.4 (Fig. 2). At pH 3.2 only, the low temperature transition at about 30°C was observed. At pH 5.4, both the transition at 30°C and the one at 65°C were observed, while the transition at 72°C was not observed. At pH 7.2, all three transitions were seen

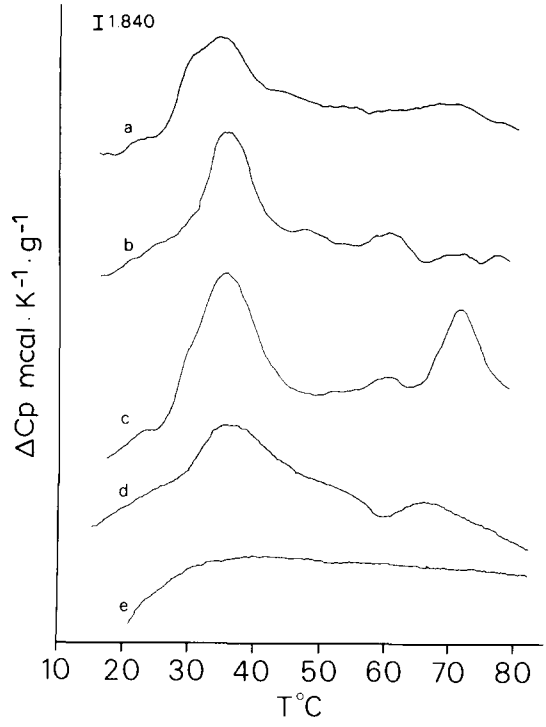

Fig. 2. Effect of pH on phase transitions observed in normal human myelin. (a) Normal human myelin (50 mg/ml) in glycine-HCl buffer, pH 3.2. Scan rate 60 K/h, range 30 μV, sensitivity 16×. (b) Normal human myelin (50 mg/ml) in sodium acetate buffer, pH 5.4. Scan rate 60 K/h, range 30 μV, sensitivity 16×. (c) Normal human myelin (50 mg/ml) in Hepes buffer, pH 7.4. Scan rate 60 K/h, range 30 μV, sensitivity 16×. (d) Normal human myelin (50 mg/ml) in glycine buffer, pH 10.4. Scan rate 60 K/h, range 30 μV, sensitivity 16×. (e) Same sample as (d), allowed to cool slowly overnight and reheated the next day at the same calorimeter settings.

as in Fig. 1a. At pH 10.4, all three transitions were observed but of considerably reduced enthalpy. When cooled slowly overnight and then reheated under the same conditions, no phase transitions were found (Fig. 2e).

The effect of pH may be quite complex since both ionizable groups on the proteins and lipids are affected. Ionization of the polar headgroup of lipids has a marked effect on the transition temperature (T_c) of lipids [6]. In general, the transition temperature is increased at lower pH values. Therefore, the absence of the high temperature transition (about 70°C) at pH 3.2 and 5.4 are probably not due to lipid. Similarly, the T_c of the low temperature transition (about 30°C) is unaffected by changes in pH and is probably not due to lipid either.

Since basic protein of myelin can be readily extracted with acid [11], it is likely that the acidic conditions of pH 3.2 resulted in loss of most of the basic protein from the membrane. Thus, the high temperature transition was assigned to basic protein.

This conclusion was supported by extraction of basic protein from myelin under acid conditions [11]. The thermogram for this extracted myelin is shown in Fig. 3a. The thermogram for basic protein in Hepes buffer (5 mg/ml, which is equivalent to the amount of basic protein in 50 mg myelin) is shown in Fig. 3b. No phase transitions were observed over the whole temperature range. Therefore, the high temperature transition is due to basic protein in association with some lipid component or components and is not due to merely unfolding of the protein. Since the transitions are not reversible, extensive protein-lipid interactions are implied.

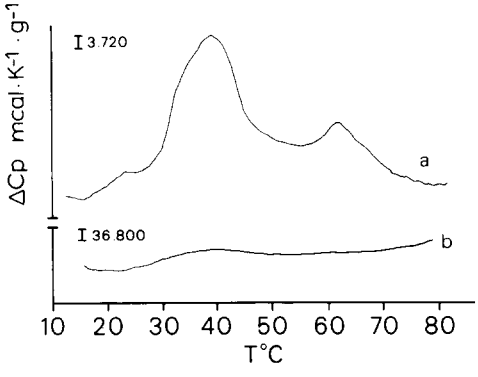

Fig. 3. (a) Normal human myelin from which basic protein had been extracted (50 mg/ml) in Hepes buffer, pH 7.4. Scan rate 60 K/h, range 30 μV, sensitivity 8×. (b) Normal human myelin basic protein (5 mg/ml) in Hepes buffer, pH 7.4. Heating rate 30 K/h, range 30 μV, sensitivity 16×.

Discussion

Thermal transitions in myelin have not been observed previously [10] using conventional calorimeters. However, two large and one small endothermic transitions have been observed in isolated human myelin with a sensitive microcalorimeter.

The protein composition of myelin is relatively simple since two protein fractions, the proteolipid and basic proteins make up about 80% of the total protein of myelin [14]. Therefore, the two major endothermic transitions must be related in some way to these two proteins since they have been shown not to be related to the lipid components of myelin (the transitions were not reversible as expected for a lipid transition and a total lipid extract of myelin did not show any endothermic phase transitions). The isolated proteins did not show evidence of protein unfolding throughout the temperature range studied.

Extraction of basic protein from myelin in the usual way [11] abolished the high temperature transition (72°C) but not the low temperature transition (32°C). Thermal analysis at pH 3.2 of isolated myelin (conditions under which basic protein can be isolated) abolished the high temperature transition (72°C). Therefore, we concluded that the high temperature transition (72°C) involved basic protein. Since we have no evidence that basic protein unfolds at this temperature, a basic protein-lipid complex is probably responsible for this transition.

Since two proteins make up 80% of the total myelin protein, and the high temperature transition has been shown to involve basic protein, the low temperature transition (32°C) must involve the myelin proteolipids which make up about 50% of the total myelin protein. The isolated protein (lipophilin, a component of the proteolipid fraction) showed no thermal transitions over the temperature range used in this study. Earlier studies with circular dichroism [15] showed no evidence of

unfolding up to 40°C and then a highly noncooperative unfolding at higher temperatures. Therefore, this transition cannot be due to protein unfolding either but must reflect protein-lipid interactions.

Both major phase transitions observed with the differential heat capacity calorimeter probably represent the melting of a protein-lipid complex. Further studies of these transitions are in progress, using X-ray diffraction techniques. Wide angle X-ray diffraction studies have shown that the transition at 61–62°C is probably related to a phase transition involving the hydrocarbon chains of the lipids (Chia, L.-S., Thompson, J.E. and Moscarello, M.A., unpublished data). Therefore, tentative assignment of all three calorimetric transitions has been made.

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