Reversible thermal transition of brain myelin proteolipid

A preliminary report on a high-sensitivity differential scanning calorimetry study

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Brain myelin proteolipid has been investigated using high-sensitivity differential scanning calorimetry (DSC) under various conditions. Crude proteolipid with a 40% (w/w) content of protein gave rise to a reversible transition, centered at about 60°C. The specific enthalpy of the transition was 50 ± 5 J·g^{-1} with a calorimetric to van't Hoff enthalpy ratio of 5.7 ± 0.5. To our knowledge this is the first intrinsic membrane protein in which a reversible thermal transition has been detected and investigated by DSC. Similar experiments were carried out using the recombinants of delipidated proteolipid and the pool of natural membrane lipids; in this case the transition was less enthalpic and showed lower cooperativity. The recombinants with lecithins, however, did not show any transition at 60°C.

Myelin *Basic protein* *Proteolipid* *DSC* *Enthalpy* *Reversible thermal transition*

1. INTRODUCTION

The thermal behaviour of natural and model biomembranes has been the subject of many studies carried out using DSC. Although the aim of many of these studies has been to investigate the protein-lipid interaction itself, in most calorimetric biomembrane investigations the emphasis of the research has in fact been directed more towards the influence of proteins on the thermal properties of the lipids than towards the proteins themselves [1,2]. DSC studies of membrane proteins are difficult with respect to problems of purification, occasional irreproducibility of calorimetric scans and the usual irreversibility of their thermal transitions when they do occur [3–6]. It is noteworthy that DSC is at present the most direct technique for obtaining the whole thermodynamic description of a protein, including its definition in terms of cooperative domains and their interactions, as in the case of several water-soluble proteins [7–10].

We present here a report on the thermal transition induced in an important membrane protein, the brain myelin proteolipid [11], by means of high-sensitivity DSC. This may be the first case of an intrinsic membrane protein undergoing a wholly reversible, temperature-induced, conformational transition. Some reconstituted PLP membranes also show this reversible effect, although the type and role of the lipids used in the reconstitution appear to be crucial.

Abbreviations: DSC, differential scanning calorimetry; PLP, myelin proteolipid; BMP, basic myelin protein; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine

2. EXPERIMENTAL

Crude PLP was obtained from bovine white matter according to Folch and Lees [12] with the modifications described by Monreal [13]. The

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preparation contained 60% (w/w) of lipid and less than 4% of BMP and other minor components. The delipidated PLP was obtained from the crude fraction as described by Aguilar et al. [14]. The pool of natural lipids we used was obtained in powder form by lyophilization of the pool of the water-*n*-butanol solutions, collected by dialysis and ultrafiltration during the delipidation procedure. The purity of the samples was routinely checked by SDS-polyacrylamide gel electrophoresis. Samples A and B refer to the lyophilized powders obtained from two separate PLP solutions during the delipidation according to Aguilar et al. [14]. Sample A was collected at the end of two consecutive dialyses against water-*n*-butanol. Sample B underwent, in addition, two ultrafiltration steps with the same solvent. Pure BMP was obtained by the method of Oshiro and Eylar [15]. The protein and phosphorus contents of all preparations were determined according to Lowry et al. [16] and Ames and Dubin [17], respectively.

Suspensions of crude PLP and samples A and B, as well as BMP solutions, were prepared by the addition of water, and their concentrations determined by weight. The recombinants of delipidated PLP with DMPC, DPPC and the pool of natural lipids were prepared by mixing stock benzene solutions of the lipid and protein in appropriate proportions and freeze-drying overnight. The resultant dry powder was dispersed in water at 40°C (or 50°C for DPPC) by vortex-mixing for 5 min. The final protein concentration was always between 0.5 and 2 mg/cm^3.

Calorimetric experiments were carried out in a high-sensitivity DSC DASM-1M [18] at a heating rate of 0.5 K/min. DSC thermodynamic parameters were calculated as in [10]. The M_r values used for PLP and BMP were 30000 and 18000, respectively, from their known sequences [19,20].

3. RESULTS AND DISCUSSION

The DSC profile corresponding to crude PLP is shown in fig.1. The process is highly reproducible and reversible except for the small shoulder centered at about 73°C, which disappears on reheating the sample. DSC control experiments of the natural lipids included in the crude PLP do not show any type of transition and, since PLP is responsible for more than 90% of the protein content in that crude preparation, the above transition practically corresponds to the thermal unfolding of PLP. The shoulder in fig.1 has been assigned to the presence of a low percentage of BMP by carrying out DSC crude PLP experiments with additional BMP (not shown), whereupon the shoulder becomes a small irreversible peak, also centered at about 73°C.

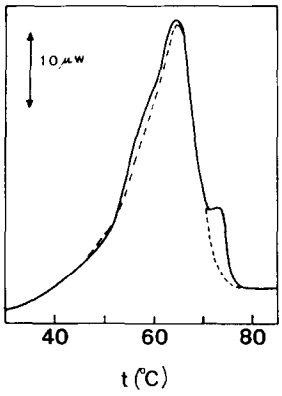

Fig.1. Original calorimetric recording of crude PLP in water. (——) First heating, (– – –) reheating of sample. c = 1.1 mg/cm^3.

The specific enthalpy of the main transition, once the shoulder contribution is eliminated, has been found to be 50 ± 5 J · g^{-1} (averaged over 6 independent measurements). The calorimetric to van't Hoff molar enthalpy ratio calculated is 5.7 ± 0.5. There are few DSC studies of membrane proteins in the literature to date [3–6] and the thermal transitions in these have always been found to be irreversible. The enthalpy value found here is nearly twice that of all water-soluble proteins with a similar melting temperature, except for collagen, which is about 75 J · g^{-1} at 60°C [9]. We have proposed a model for PLP in the myelin membrane [21] where there are 5 hydrophobic transmembrane helical segments. The high enthalpy expected for the breaking of one hydrogen bond in such non-polar media [9] should give rise to an extremely high enthalpy, which could easily explain the value found here. It is also tempting to relate the calorimetric to van't Hoff enthalpy ratio of about 6 to the 7 theoretical domains which are envisaged in our PLP model, i.e. the 5 transmem-

brane segments and the 2 hydrophilic regions, the extracellular and cytoplasmic one [21].

All these results are apparently in disagreement with the absence of any protein transition reported by Curatolo et al. [22] when studying DMPC-PLP recombinants using DSC. In fact we obtained the same results as Curatolo et al. when we extended their studies on the influence of PLP in both DMPC and DPPC order-disorder transitions, including high protein/lipid ratios [23]. To determine whether PLP suffered some irreversible alteration during its purification, we followed the PLP purification procedure from crude PLP using DSC at each stage. We found that preparation A (see section 2) gave a temperature-induced, reversible, conformational transition at 60°C also but with lower specific enthalpy and cooperativity. DSC investigation of preparation B and of the further steps in PLP purification showed no trace at all of thermal transition.

There is, however, an alternative explanation which requires the presence of some specific lipids for PLP to undergo the transition. To clarify this point we carried out DSC experiments with PLP recombinants using the pool of natural lipids (see section 2) removed during PLP purification from crude PLP. Remarkably this recombinant did show a thermal transition centered at around 60°C with an enthalpy value and cooperativity similar to those of sample A, both being completely reversible. It is then evident that the presence of certain natural lipids, perhaps in minimal quantities, is a prerequisite for the PLP reversible transition.

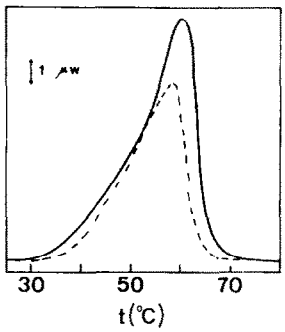

Fig.2. Thermal DSC profile of the recombinant of pure delipidated PLP with the pool of natural lipids in water. (——) First heating, (– – –) reheating of sample. c = 1.1 mg/cm^3.

We would finally like to emphasize the fact that to our knowledge this is the first intrinsic membrane protein in which a reversible thermal transition has been detected and investigated by DSC. We have also shown how some natural lipids are necessary for this membrane protein to undergo a given conformational transition.

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