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# CALORIMETRY OF LIPID MODEL MEMBRANES

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## ABSTRACT

High sensitivity differential scanning calorimetry can be used to study the thermal behaviour of pure lipids and the phase behaviour of lipid mixtures. In addition it is possible to determine from the calorimetric traces the apparent molar heat capacities of the lipids provided the partial molar volumes are known. Mixing microcalorimetry can be applied to study the binding of ions to charged lipid membranes or the incorporation of proteins into lipid bilayers. Several applications of these two calorimetric methods are given.

Lipid bilayers are widely used as model systems for biological membranes. These lipid bilayers undergo a reversible phase transition from and ordered (gel) state to a more fluid (liquid crystalline) state. Differential scanning calorimetry (DSC) has been one of the standard methods to determine the transition temperatures ($T_m$) and transition enthalpies ( $\Delta H$) of this lipid phase transition (1). Biological membranes contain complex mixtures of phospholipids. DSC can also be used to study the mixing behaviour of lipids, i.e. to construct phase diagrams from the onset and end temperatures of the calorimetric endotherms (2,3). In the case of mixtures of phospholipids with identical fatty acyl chains but different polar groups phase diagrams normally indicate complete mixing in the gel as well as the liquid crystalline phase. This is also true for binary mixtures of dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidic acid (DMPA) at pH 6.5. DMPA is an anionic lipid at this particular pH, i.e. the head group is negatively charged and can bind divalent cations like $Mg^{2+}$ and $Ca^{2+}$. For pure DMPA binding of $Ca^{2+}$ leads to an increase of $T_m$ from 50 to 65°C with a slight reduction of the transition enthalpy. In DMPA/DMPC mixtures addition of $Ca^{2+}$ leads to a general increase in $T_m$ with a change of the shape of the isotherms. The phase diagram now indicates limited miscibility in the gel phase with a possible miscibility gap at high DMPA concentrations.

High sensitivity differential scanning calorimetry can also be used to determine apparent molar heat capacities of lipids in dilute dispersion provided the partial molar volume of the lipid is known (4.). The apparent molar heat capacities of a variety of lipids with different head groups were found to be

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higher than estimated from the heat capacities of liquid alkanes and group parameter values for the hydrophilic head groups or by comparison with a micelle forming lysolecithin. Intermolecular interactions, in particular with the surrounding water contribute to the observed heat capacities. An increase in the heat capacity is an indication for the existence of contributions from "hydrophobic hydration" of apolar residues of the lipid molecules. For detergent micelles it has been found before that up to 5 $CH_2$ groups of the alkyl chains must be in contact with water. The same conclusion can be drawn for bilayer membranes. That means that more water than previously estimated may be in contact with apolar parts of the bilayer. This finding should be discussed in connection with the high water permeability of bilayer membranes.

DMPA is a phospholipid which is negatively charged at neutral pH. A change of the pH to 12 leads to the dissociation of the second proton so that the head group becomes doubly charged resulting in decrease of $T_m$. Doubly charged DMPA was found to have a higher heat capacity than the singly charged form. An increase in "hydrophobic hydration" apparently overcompensates negative contributions to the heat capacity arising from the hydration of the doubly charged head group (4).

This effect can also be studied by mixing calorimetry using an LKB batch microcalorimeter with titration accessory. The heat of proton dissociation of DMPA was studied in the temperature range between 5 and 58°C by mixing a neutral DMPA dispersion with dilute NaOH. The titration experiment revealed that the heat of dissociation as well as the dissociation "constant" is a function of the degree of dissociation or surface charge. The heat of dissociation displays a strong temperature dependence from which the difference in heat capacities between the singly and doubly charged form can be determined. As found before in the DSC experiments the heat capacity of doubly charged DMPA is ca. 140 cal $\cdot K^{-1} \cdot mol^{-1}$ higher. Similar results can be obtained when a different phospholipid, namely dipalmitoyl phosphatidylglycerol (DPPG), which has a negatively charged head group at pH 8 is protonated by titration with dilute hydrochlorid acid. From the temperature dependence of the heat of protonation it is calculated that uncharged DPPG, which has a higher transition temperature than the charged form, has a heat capacity which is ca. 50 cal $\cdot K^{-1} \cdot mol^{-1}$ lower. These results for the protonation of lipid bilayers can be compared with data for the soluble molecule glycerophosphate. In this case the doubly charged glycero-phosphate has a heat capacity, which is 55 cal $\cdot K^{-1} \cdot mol^{-1}$ higher than the singly charged form. This supports the idea that changes in the extent of "hydrophobic hydration"

are responsible for the large opposite changes in heat capacity observed when charged lipids are protonated.

Similar experiments can be performed by titrating charged lipids with divalent cations. The binding of ions like $Ca^{2+}$ or $Mg^{2+}$ to DMPA leads to a reduction of the heat capacity. This is in contrast to the results obtained for the binding of $Mg^{2+}$ to linear polyelectrolytes like DNA where an increase in the heat capacity was observed. Binding of $Ca^{2+}$ to the negative head groups of DMPA is obviously different. From the decrease in heat capacity it can be concluded that not only water of hydration of the polar group is set free upon binding but also water in contact with hydrophobic groups is displaced leading to the formation of a more ordered gel phase.

Protein-lipid interactions in bilayer model membranes can also be studied by DSC. In most cases the incorporation of a hydrophobic protein into the lipid bilayer leads to a broadening of the transition and to a reduction of the transition enthalpy. A plot of $\Delta H$ vs. protein/lipid ratio can yield the number of lipid molecules withdrawn from the lipid phase transition, though in some cases non-linear plots are obtained indicating a more complicated behaviour. Depending on how the lipid molecules are perturbed by the protein $T_m$ is shifted to lower values or remains unchanged. An example for the first type of interaction is the protein bacteriorhodopsin (BR) incorporated into DMPA bilayers (5). In this case $T_m$ decreases and the endotherms broaden due to an aggregation of BR at lower temperatures. Melittin, a small peptide, is an example for the second type of proteins. Incorporated into DMPC bilayers it has no effect on $T_m$. Incorporation into DMPA bilayers even leads to a slight increase in $T_m$ indicating the presence of electrostatic effects. A plot of $\Delta H$ vs. protein/lipid ratio reveals distinct differences between the two systems. Particularly at low protein concentrations DMPC bilayers much more perturbed than DMPA bilayers.

The incorporation of melittin into bilayers can also be studied by mixing calorimetry. However, only in the vicinity of the phase transition is the kinetics of incorporation fast enough to get reliable results. The enthalpy of protein incorporation is negative just above $T_m$ and becomes positive just below $T_m$, indicating that the lipid molecules surrounding the protein are in some intermediate energetic state compared to the gel and liquid crystalline phase. Incorporation experiments with melittin into DMPC and DMPA bilayers again reveal distinct differences between the two systems as the enthalpy of melittin incorporation into DMPC below $T_m$ is larger than into DMPA bilayers, again indicating that DMPC is more perturbed by the protein than DMPA. In DMPC/DMPA mixtures melittin produced an increase

in the transition temperature which can be interpreted as evidence for a preferential association of this protein with charged lipids.

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