Application of titration calorimetry to study binding of ions, detergents, and polypeptides to lipid bilayers

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Abstract: The binding of ions, the incorporation of detergents and polypeptides into lipid bilayers, and the CMC and heat of micellization of detergents were studied by titration calorimetry. The heat of dissociation of dimyristoylphosphatidic acid (DMPA$^-$ + OH$^-$ → DMPA$^{2-}$ + H$_2$O) was investigated as a function of temperature covering the phase transition of singly and doubly charged DMPA. The intrinsic pK$_0$ for the dissociation was determined from the titration curves applying the Gouy-Chapman theory. pK$_0$ decreases with temperature from ca. 6.2 at 11°C to 5.4 at 54°C. The temperature dependence of the dissociation enthalpy $\Delta H_{\text{Diss}}$ was combined with DSC data on the transition enthalpies $\Delta H_{\text{Tran}}$ for DMPA in its two ionization states to construct a complete enthalpy vs. temperature diagram. Titration calorimetry was also used to determine the CMC and the heat of micellization of the detergents SDS, octylglucoside, Na-cholate, and Na-deoxycholate. From the temperature dependence of the CMC and the heat of micellization the thermodynamic functions $\Delta G$ and $\Delta S$ were determined as a function of temperature. The interaction of Na-deoxycholate with lipid bilayers was studied at a temperature where the heat of micellization was zero. Complex titration peaks were observed, indicating incorporation reactions on different time scales. The heats of reaction depended on the nature of the phospholipid. Titration calorimetry was also used to study the binding of melittin to lipid vesicles. The sign of the heat of incorporation depended on whether the lipid was in the gel or the liquid-crystalline state and on the chemical nature of the phospholipid.

Key words: Titration calorimetry — lipid bilayers — ion binding — detergents — peptide binding

Introduction

Differential scanning calorimetry has been widely used to study phase transitions in lipid bilayers [1, 2]. Reaction and titration calorimetry has only recently become applicable due to the availability of sensitive instruments [3]. We have used titration calorimetry in the titration and the batch mode employing the Microcal OMEGA titration calorimeter to test this method for its suitability to study a variety of reactions using lipid vesicles and detergents.

Heat of Dissociation of DMPA

Phospholipids such as dimyristoylphosphatidic acid (DMPA) show a pH-dependent ionization and transition behavior, which has been studied in quite some detail [4, 5]. At lipid concentrations of ca. 1 mM the apparent pK for the dissociation DMPA$^- + OH^- → DMPA^{2-} + H_2O$ is ca. 10–10.5. This apparent pK can be shifted by changing the ionic strength and thus the extent of counter ion condensation at the lipid bilayer surface [5]. The dissociation of the second proton can be induced by the addition of NaOH. As the permeation of $H^+$/OH$^-$ through lipid bilayers is within the dead time of the titration calorimeter, all head groups can be titrated. The observed heats of reaction $\Delta H_R$ will be the sum of several heat effects, namely, the dissociation enthalpy $\Delta H_{\text{Diss}}$, the enthalpy of neutralization of water $\Delta H_{\text{Neut}}$, the heats of dilution of NaOH, and
additional enthalpic contributions caused by changes in head-group hydration, head group interactions, and rearrangements of the fatty acyl chains. The heat of dilution of NaOH and the heat of neutralization of water can be measured separately or taken from tabulated values. After subtraction of these from $\Delta H_R$, the residual value for $\Delta H_{\text{Diss}}$ still contains the non-separable contributions. Figure 1 shows the calorimetric heat signals observed when titrating a 1 mM vesicle dispersion of DMPA$^-$ with 0.1 M NaOH [6]. The integrated heat of reaction is also shown in Fig. 1. The curves for 11°C and 53.6°C correspond to the dissociation of DMPA$^-$ in the gel state and liquid-crystalline state, respectively. At 48.7°C the dissociation induces a transition from the gel into the liquid-crystalline state. This contribution leads to the effect that the first heat signals are endothermic. The titration curves can be simulated using the Gouy-Chapman theory [6, 7]. At low temperature the intrinsic $pK_a$ for the DMPA$^-$ dissociation is 6.2, whereas at higher temperature it was determined to 5.4. At intermediate temperatures no simulations could be performed due to the pH induced gel to liquid-crystalline phase transition. The total dissociation enthalpy was also determined by a batch experiment titrating a 0.01 M NaOH solution with a 1 mM DMPA vesicle dispersion. The results of the temperature dependence of $\Delta H_{\text{Diss}}$ are shown in Fig. 2. The sudden jump in the dissociation enthalpy between 23 and 52°C is caused by the OH$^-$ induced phase transition. Thus, in this temperature range $\Delta H_{\text{Diss}}$ includes the transition enthalpy $\Delta H_{\text{Trans}}$. The dissociation enthalpies are positive above 52°C and below 23°C. In the intermediate temperature range the dissociation enthalpy increases with temperature, indicating a positive $\Delta C_p$. This shows that during the transition into the doubly charged liquid-crystalline state more hydrophobic surface of lipid molecules is exposed to water [6]. The titration data could be combined with the differential scanning calorimetry data to construct a complete enthalpy vs. temperature diagram of DMPA in its two ionization states (see Fig. 2) [6, 8].

Heat of demicellization of detergents

The CMC and the heat of demicellization of detergents can be easily studied by titration calorimetry, providing the CMC is not too low, i.e., below $10^{-4}$ – $10^{-5}$ M. The experiments are carried out by diluting a micellar solution of the detergent in buffer or water to concentrations below the CMC. The observed enthalpic effects are then