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Calorimetric studies of macromolecular aqueous solutions

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Abstract

Both titration and differential scanning microcalorimetric techniques are shown to yield important information concerning the properties of macromolecules in aqueous solution. Application of titration calorimetry is examined in the context of deaggregation of cationic micelles (*e.g.* hexadecyltrimethylammonium bromide), of guest-host interactions and of enzyme-substrate interactions (*e.g.* CAT_{III} with chloramphenicol). Experimental data obtained using DSC are reviewed with reference to the thermal stability of enzymes (*e.g.* DNA-Gyrase) and to the gel to liquid crystal transition characterising vesicles formed by dialkylphosphates. In the latter, the dependence of the characteristic melting temperature T_m on chain length, counter cation and added solute is noted.

Introduction

Understanding the properties of aqueous solutions containing macromolecules presents an enormous challenge because these systems are very complicated. Nevertheless, these systems are extremely important in terms of wealth generation from an industrial standpoint, in terms of understanding life processes and in terms of the overall quality of life in the twentieth century. Here we review the application of two calorimetric techniques used at both the University of Leicester and the University of Groningen to study aqueous systems containing enzymes (1), ionic surfactants (2,3) and vesicles formed by dialkylphosphates (4-6). Using a titration microcalorimeter (MicroCal Ltd., USA) small aliquots (typically of the order 10^{-6} dm^3) of an aqueous solution are injected under computer control into a sample cell (volume typically approx. 1.4 cm^3) containing either water or another aqueous solution (7,8). The calorimeter records a series of exo- or endo-thermic pulses characterising a programme of injections. The differential scanning microcalorimeter (MicroCal Ltd., USA) compares the amounts of heat δq required to raise the temperature of sample and reference cells (volumes typically 1.4 cm^3) by δT over a range of temperature typically from 283 to 383 K (9,10).

Over a small temperature range where the macromolecular solute undergoes a change in structure/ organisation, the dependence of the differential isobaric heat capacity on temperature forms, in the textbook case, a bell-shaped plot. The maximum in the heat capacity occurs at a characteristic temperature (see below) and the area under the curve yields the enthalpy of the transition. In such cases, two states of the macromolecule are possible in solution. The analysis for more than one coupled equilibria (11) and for the often elusive cold-denaturation of enzymes (12) provides interesting challenges in data analysis.

Micelle Formation/Deaggregation

The phenomenon of micelle formation by surfactants in aqueous solution is well-documented (2,3,13) although thermodynamic description of the process of micelle formation remains a contentious issue (14), the competing merits of mass action and phase equilibrium models being hotly debated (15). Nevertheless, the reality of structural changes in the system is convincingly demonstrated by a titration microcalorimeter in which aliquots of a solution containing a surfactant at a concentration above the critical micellar concentration (cmc) are injected into a sample cell containing initially water. The results of a typical experiment are shown in Fig. 1 for hexadecyltrimethylammonium bromide (CTAB;aq) at 298.2 K. The break in pattern occurs at the cmc. When the concentration of surfactant in the sample

cell is below the cmc, the endothermic pulses accompanying injection of aliquots of solution having surfactant concentration above the cmc characterises the endothermic deaggregation of the CTAB micelles. But when the concentration of surfactant in the sample cell exceeds the cmc the enthalpy change accompanying injection is negligibly small because the only small contribution characterises dilution of micelles. We have attempted to model the injection processes by expressing quantitatively the enthalpies of the injected solution and the solutions in the sample cell before and after injection in terms of the apparent molar enthalpies of micellar and simple solutes in solution. Account was also taken of the fact that these complicated salt solutions are not ideal in a thermodynamic sense. As shown in Fig. 1, reasonable agreement was obtained (8) between observed and calculated titration plots. However, agreement between the two plots was not completely satisfactory when the composition of solutions are close to the cmc. In fact, the agreement became less satisfactory with decrease in alkyl chain length through the series from the C_{16} to C_{10} surfactants. The disagreement reflects the increasing importance of the role of ion-ion interactions as opposed to micelle deaggregation in determining the patterns emerging from the titration calorimetric plots.

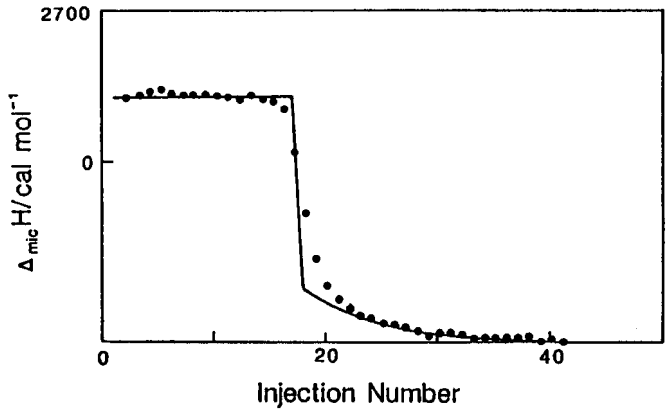


Fig. 1. Titration calorimetric plot for CTAB (aq; 1.54×10^{-2} mol dm^{-3}) in 5×10^{-9} m^3 aliquots into a sample cell containing initially water: cmc = 0.95×10^{-3} mol dm^{-3}; $\Delta_{mic}H^{\infty} = -2.45$ kcal mol^{-1}.
[The points are experimental whereas the full line is calculated; see text.]

The clear-cut pattern shown by the titration calorimetric data for CTAB(aq) has prompted a number of studies probing the effects of added salts, neutral solutes (16,17) and surfactants on the cmc and enthalpy of micelle formation for CTAB(aq). At constant total surfactant concentration, the apparent cmc of a mixture of CTAB and DOTAB decreases with increase in mole fraction of DOTAB although the enthalpy of micelle formation expressed in terms of one mole of CTAB is rather insensitive (18). Rather more dramatic changes in the titration calorimetric plots are observed when an aqueous solution containing both CTAB and 1-pentanol are injected into a sample cell containing a dilute solution of 1-pentanol in water. The first few injections are exo- rather than endo-thermic (19) which we interpret as a combination of endothermic micelle deaggregation and exothermic transfer of pentanol from the hydrophobic core of the micelles into the aqueous solution. The sign reversal when pentanol is replaced by 2-butoxyethanol is not observed but a new feature emerges in the region where the composition of the sample cell approaches the cmc. As shown in Fig. 2, the titration plot shows a hump pointing to slightly greater endothermicity in the region of the cmc. We attribute (20) this feature to a *"mopping-up"* operation of the strongly hydrophobic organic co-solute (21,22) by CTAB micelles injected into the solution held in the sample cell (20).

Host-Guest and Enzyme-Substrate Interactions

In a number of important experiments the titration calorimeter is used to study a range of interactions between solutes. For example, aliquots of a solution containing a guest are injected into a solution containing a host. An example is highlighted in Fig. 3 in which the host is α-cyclodextrin and the guest is the anion p-nitrophenolate (at pH 9). The classical binding curve (23) shows that almost all of the injected guest is initially bound by the host. At high injection numbers, all host sites are occupied and the titration calorimeter simply records the small dilution effect of the guest solution. In Fig. 3 we report the titration curve and the best-fitted line based on the formation of 1:1 host-guest complex.

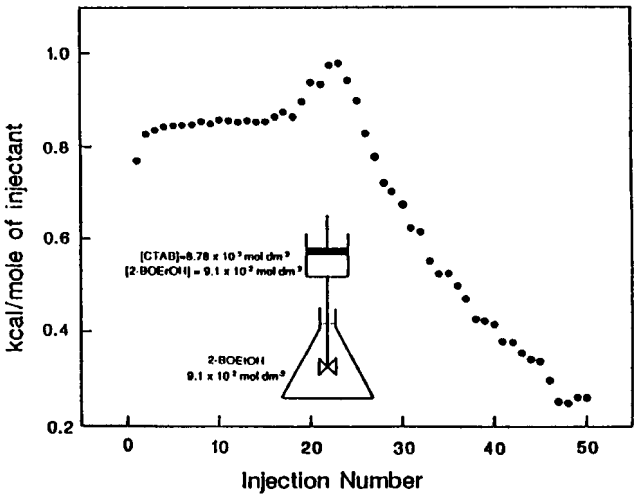


Fig. 2. Titration Calorimetry - Injection of aliquots of (CTAB + 2-butoxy- ethanol)(aq) into 2-butoxyethanol(aq) at 298.2 K.

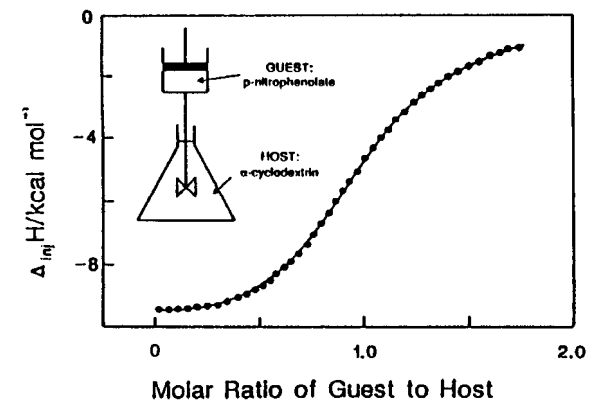


Fig. 3. Titration calorimetric plot for binding of p-nitrophenolate (aq; pH 9); 6×10^{-3} mol dm^{-3}) by α-cyclodextrin (8×10^{-3} mol dm^{-3}; aq): K_B = 1.9×10^3 mol dm^{-3}; $\Delta_B H^{\infty} = -10.2$ kcal mol^{-1}.

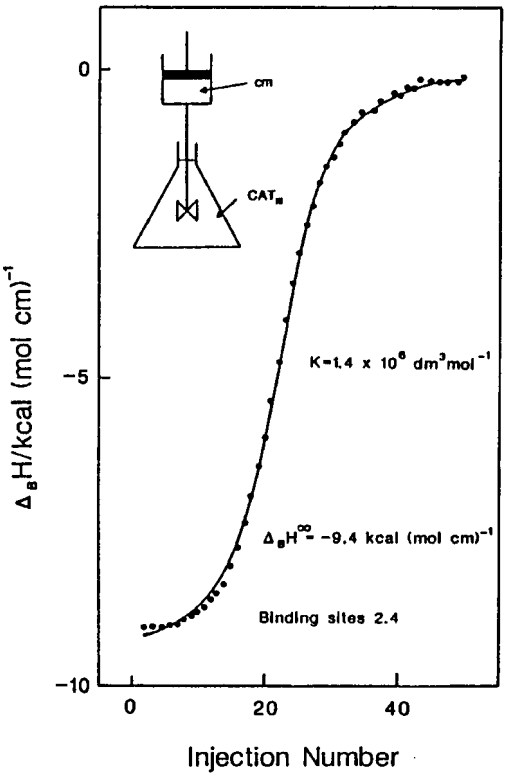
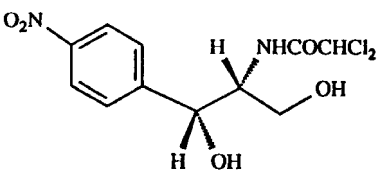


Fig. 4. Titration calorimetric plot; titration of aliquots of chloramphenicol(aq) into chlor – amphenicol acetyltransferase(aq).

The latter simple example forms the background to one of our main interests, namely the thermodynamics of binding of substrates to enzymes.

Bacterial resistance to the antibiotic chloramphenicol (CM; I) stems from acetylation of the 3-hydroxyl group by acetyl coenzymes in a process (24,25) catalysed by chloramphenicol acetyltransferase, CAT_{III}.



(I)

The latter enzyme comprises a trimer; each subunit has a relative molecular mass of 25000. The monomer is inactive catalytically. The three active sites are formed between the subunits of the trimer; each binding site comprises a tunnel, length 25×10^{-10} m, extending through the protein. The results of a typical calorimeter run are shown in Fig. 4 in which aliquots of CM(aq) are injected into an aqueous solution containing CAT_{III}(aq). The well-formed S-shaped curve indicates reasonably strong binding of CM to the enzyme.

Thermal Stability of Enzymes

The enzyme CAT_{III} despite the fact its structure involves three clustered subunits has a high thermal stability, a single maximum being recorded (26,27) near 84 Celsius with an enthalpy of denaturation equal to 290 kcal mol^{-1}. A more complicated DSC scan is recorded for DNA gyrase (28). The latter enzyme catalyses the introduction of negative supercoils into closed circular DNA in a process driven by the hydrolysis of ATP. The enzyme is an A_2B_2 tetramer. The intact enzyme can be cleaved into a number of fragments including 64 kDa and 33 kDa fragments of Gyr A and a 43 kDa Gyr B N-terminal domain. DSC scans for these fragments allow components of the scan for the intact enzyme to be assigned; Fig. 5.

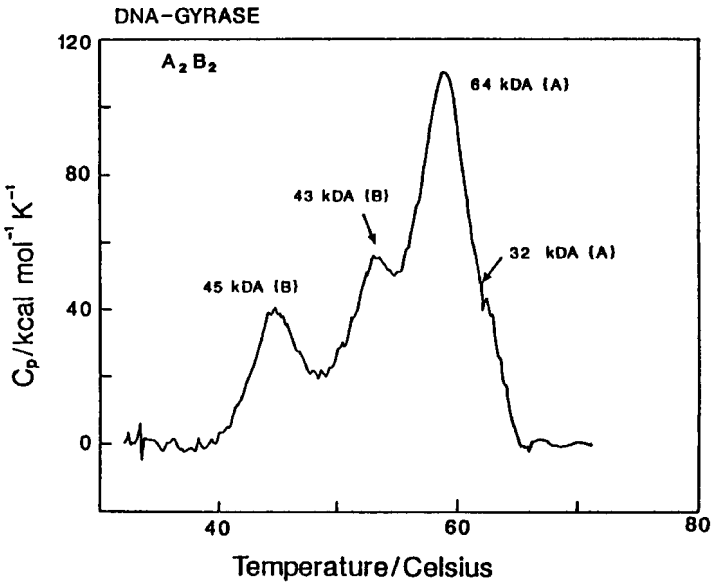


Fig. 5. DSC scan of *E. coli* DNA gyrase, an A_2B_2 protein; assignments based on DSC scans for DNA gyrase A, two fragments of the gyrase A and a fragment of gyrase B (see text).

Gel to Liquid-Crystal Transitions in Vesicles

In aqueous solution many surfactants having two long hydrophobic alkyl chains (4-6) form closed bilayer structures – vesicles. These vesicles are interesting both in their own right and as models for natural lipid systems and for applications as drug delivery systems (29). The properties of vesicles in aqueous solutions are critically dependent on the method of preparation. Indeed, methods which use solutions of surfactants in organic solvents mixed with water have been shown (30,31) to be particularly suspect.

In our recent research we have concentrated attention on vesicles formed by dialkylphosphates in aqueous solution. The pattern is set by vesicles formed by sodium di-n-dodecylphosphates. For these systems the gel to liquid-crystal transition occurs near 348 K. The melting involves a co-operative melting not throughout the whole vesicle but in patches comprising a fluctuation of around 150 monomers; the melting process is accounted for in terms of a simple two-state equilibrium describing the transformation for a patch of monomers from the gel to liquid-crystal states (32); Fig. 6.

The transition temperature T_m (at the maximum of molar heat capacity) depends on the counter cation decreasing when sodium cations are replaced by tetramethylammonium cations (33). The transition temperature (for sodium salts) increases (31) on going from R = n–$C_{12}H_{25}$, through R = n–$C_{14}H_{29}$, R = n–$C_{16}H_{33}$ to R = n–$C_{18}H_{37}$. Detailed studies of vesicles formed by mixtures of symmetric and asymmetric dialkylphosphates point to the importance of steric factors in governing the packing of alkyl chains in the vesicle bilayers (34). DSC results for vesicles formed by (sodium) di-alkylphosphates with two C_{18} alkyl chains and unsaturation at C_8 show the importance of chain flexibility and stereochemistry on the gel to liquid-crystal transition (35).

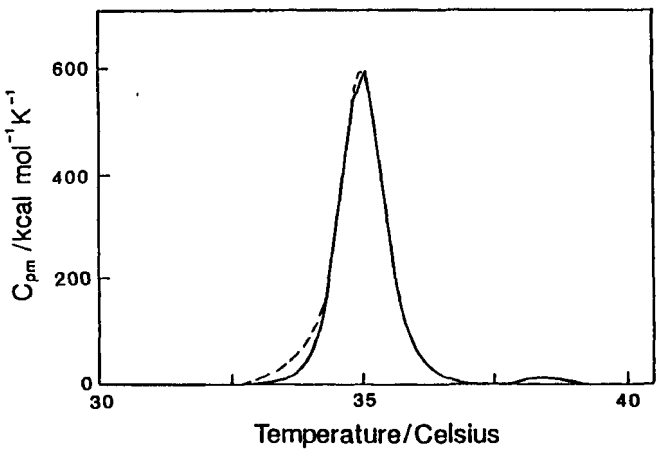


Fig. 6. Dependence of molar heat capacity on temperature for vesicles formed by sodium di-n-dodecylphosphate (aq; 8.4×10^{-3} mol dm^{-3}). Comparison of observed and calculated dependences based on melting of a patch comprising 168 monomers.

Recently we have turned our attention to the effect of added solutes on the parameters characterising the gel to liquid-crystal transition. In fact, the DSC scan pattern is a sensitive indicator concerning the nature of added solute - vesicle interactions. The contrast between added NaCl and $CaCl_2$ on vesicles formed by (sodium) DDP is particularly striking (36). Thus added NaCl shifts the measured T_m to higher temperatures whereas a complex scan is recorded when $CaCl_2$ is added, indicative of binding of Ca^{2+} ions to the phosphate groups at the vesicle surface. The impact of added solutes as recorded by DSC scans for vesicular systems has the potential for contrasting the impact of binding of added solutes to the surface and of incorporation of added solutes within the bilayer. The effect of adding surfactants such as CTAB to vesicles formed by di-n-octadecyltrimethylammonium bromide (DOAB) has indicated that information from DSC scans is important in understanding these phenomena (37).

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