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Thermodynamics of the hydrophobic effect. II. Calorimetric measurement of enthalpy, entropy, and heat capacity of aggregation of alkylamines and long aliphatic chains

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Abstract

The thermodynamics of long aliphatic chain alkylamine aggregation in aqueous solution was studied by isothermal titration calorimetry (ITC). Protonated alkylammonium cations with linear aliphatic chains of 10–14 carbon atoms were fully soluble in aqueous solution at the beginning of titration, but practically insoluble after deprotonation by titrating with sodium hydroxide. The alkylamines aggregated and precipitated during the reaction, enabling direct measurement of the enthalpy of aggregation. The enthalpy of aggregation became increasingly exothermic upon increasing the chain length. Hydrophobic aggregation was enthalpy-driven and entropy-opposed for alkylamines with 12–14 carbon atoms at room temperature. Direct observation of hydrophobic aggregation by ITC at constant temperature and pressure provided more accurate thermodynamic parameters than obtainable from van't Hoff analysis. Aggregation into liquid or solid phases could be distinguished by ITC, but not by van't Hoff analysis of alkylamine solubility data.

Keywords: Dodecylammonium chloride; Thermodynamics of molecular interactions; van't Hoff enthalpy; Calorimetric enthalpy; Hydrophobicity; Isothermal titration calorimetry

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1. Introduction

The energetics and physical mechanism of hydrophobic interactions are important topics in biophysical chemistry. Hydrophobic interactions play a significant role in such important phenomena as protein folding, formation of biological membranes, and stacking of nucleotide bases in double-stranded DNA. Experimental and theoretical research on the thermodynamics of the hydrophobic effect continues to appear [1–3], adding to the seminal publications on the subject [4–6].

Association of long-chain aliphatic hydrocarbons cannot be directly observed by calorimetry. There are no conditions under which we could have hydrocarbons fully dissolved in water before a reaction and fully associated as an aggregate in aqueous suspension after the reaction. Instead, the reaction is usually studied by carrying it backwards. For example, the enthalpy of hexane association was determined calorimetrically by measuring the heat evolved upon adding a small amount of pure liquid hexane into water so that all hexane was dissolved [7]. Similarly, the enthalpies of dissolution in water of a series of alcohols [8] and of micellar surfactants [9] were determined calorimetrically by dilution of the associated compounds. Enthalpies of dissolution are — if the system is maintained at equilibrium — of equal magnitude but opposite sign to the enthalpies of aggregation. However, carrying the reaction in the forward direction, if possible, can check reversibility and enable direct observation of the aggregation of hydrophobic aliphatic chains and similar compounds.

Such aggregation reactions can be carried out employing compounds with functional groups that are altered during a reaction so that the compound becomes significantly less soluble at the end of the reaction. One of the more straightforward reactions is to begin with the highly soluble alkylammonium cation and end with practically insoluble alkylamine after deprotonation with hydroxide. Here we use this approach to investigate alkylamine aggregation by isothermal titration calorimetry (ITC), providing a full thermodynamic description of the hydrophobic interactions between long chain alkylamine molecules.

The preceding article [10] described the determination of the Gibbs free energy of alkylamine aggregation by measuring pK_a shifts for various alkyl chain lengths and derived aggregation thermodynamics parameters from van't Hoff analysis. Here we continue the discussion of the reliability of van't Hoff analysis [11], providing support for the findings [12–14] that calorimetry is much more precise than van't Hoff analysis.

The enthalpy of hydrophobic interactions is usually found to contribute negligibly to the association free energy. Hydrophobic interactions are usually considered to be entropy-driven [15], although at higher temperatures, the enthalpic contribution is increasingly favorable and significant. Still, there are no cases where entropy has been shown to be unfavorable for hydrophobic interactions at 25°C. Another important signature of the hydrophobic effect is that the heat capacity of hydrophobic aggregation is highly negative. It is thought that this results from hydrogen bonding and increased ordering of water molecules around the hydrophobic solute [16]. However, most experimental work and theoretical analysis has been done with low molecular weight alkanes and inert gases.

Here we are primarily interested in the aggregation thermodynamics of long aliphatic chains. Our results are quite unexpected. We find that association of long aliphatic chains at 25°C is driven by a large exothermic enthalpy, and opposed by an unfavorable contribution from entropy. However, the heat capacity of long chain aggregation is found to be basically consistent with the usual understanding of hydrophobic interactions. We speculate that the favorable enthalpy arises from extensive van der Waals interactions in the well-packed aggregates, while the decrease in entropy arises from conformational restrictions on the long chains in those aggregates.

The following article [17] compares our results to the literature on the thermodynamics of linear alkane, alcohol, and alkylamine dissolution and aggregation in water. The thermodynamics of aggregation is compared to the thermodynamics of vaporization, condensation, and hydration, allowing completion of the thermodynamic cycle.

2. Methods

2.1. Chemicals

Most alkylamines, including propylamine hydrochloride, propylamine, octylamine, nonylamine, decylamine, undecylamine, dodecylamine, tridecylamine, and tetradecylamine, were purchased from Aldrich Chemical Co. (Milwaukee, WI). Octylamine hydrochloride and dodecylamine hydrochloride were purchased from Acros Organics (currently obtainable through Fisher Scientific). All alkylamines were at least 98% pure, and were used without further purification. All other chemicals were of the reagent grade.

2.2. Isothermal titration calorimetry

Isothermal titration calorimetry was performed with a Microcal (Northampton, MA) MCS calorimeter at 25.0–55.0°C temperatures by keeping the cooling circulating bath temperature constant at 20°C. The cell volume was 1.3438 ml. Compositions of solutions added to the cell and syringe were identical to the ones used for potentiometric titration described in the preceding article [10]. The only difference was that smaller volumes were used in the automatic titration calorimeter than for manual potentiometric titration with a burette. Two milliliters of 2.0 mM alkylammonium chloride solution (or other concentration) in 10 mM NaCl, with no buffer, were added to the calorimeter cell. Sodium hydroxide (10, 20, 50, and 100 mM in 10 mM NaCl) was injected in 40 aliquots of 6.25 μl or 20 aliquots of 12.5 μl with a 250-μl injection syringe, at 3-min intervals. Increasing the interval to 4.5 min had practically no effect on the data. Raw data curves were integrated by using standard Microcal Origin software available with the calorimeter [18]. Integrated ITC curves were simulated by the model described in Section 3. The areas under the integrated titration calorimetry curves were integrated a second time to obtain the enthalpies of binding/aggregation per mole of alkylamine.

2.3. Determination of the melting temperatures

Melting temperatures of several pure solid amines were determined with a precision of approximately ± 0.5°C by adding a solid sample to a standard capillary tube, placing it in a primary glass tube with water, and heating it slowly in a temperature-controlled water bath.

3. Results

3.1. Alkylammonium calorimetric titration with hydroxide

Positively charged *n*-alkylammonium cations with various alkyl chain lengths were titrated with sodium hydroxide using the isothermal titration calorimeter. There was no observable aggregation of short alkylamines, such as propylamine. However, long chain alkylamines, such as dodecylamine, aggregated in aqueous solution upon deprotonation. Reactions were essentially identical to those described in the preceding article [10]. The only difference was that the volumes needed for ITC were on the order of 2 ml vs. approximately 100 ml needed for potentiometric titration using a burette.

The Gibbs free energy of alkylamine aggregation, and its dependence on alkyl chain length, concentration, and temperature, was determined in the preceding article. The ITC experiments were needed to directly measure the enthalpy of alkylamine aggregation. The entropy of aggregation was obtained by subtracting Gibbs free energies of aggregation (from the pK_a shifts) from calorimetric enthalpies of aggregation. The heat capacity of aggregation was estimated from the temperature dependence of the calorimetric enthalpy.

Fig. 1 shows calorimetric raw data titration curves of deprotonation and aggregation of two alkylamines. The narrow line with large negative peaks is a titration of 1 mM dodecylammonium chloride (in 10 mM NaCl) with 10 mM NaOH (also in 10 mM NaCl). The broad line running along the base of the peaks of the narrow trace represents the control titration of 2 mM propy-

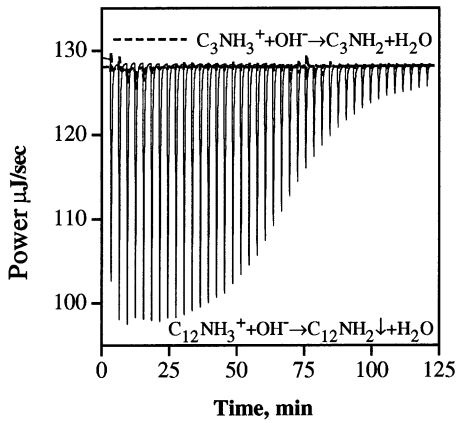

Fig. 1. Raw data calorimetric titration curves of propylammonium chloride (2 mM, broader line) and dodecylammonium chloride (1 mM, narrower line) with sodium hydroxide (20 or 10 mM, respectively) at 25°C. Propylammonium did not aggregate and the heat peaks were negligible. Dodecylammonium aggregated producing relatively large exothermic peaks.

lammonium chloride (in 10 mM NaCl) with 20 mM NaOH (in 10 mM NaCl). Several small positive and negative peaks are due to noise.

During the calorimetric titration at least the following four reactions could absorb or emit heat and affect the calorimetric curve:

$$RNH_3^+ \leftrightarrow RNH_2 + H^+ \quad (1)$$

$$H^+ + OH^- \leftrightarrow H_2O \quad (2)$$

$$RNH_2 \leftrightarrow RNH_2\downarrow \quad (3)$$

Dilution of NaOH during titration (4)

The enthalpies of reactions (1) and (2) are large. The enthalpy of propylammonium deprotonation at 25°C is equal to 57.91 kJ/mol [19]. The enthalpy of water formation from acid and base is −55.798 kJ/mol [19]. The heat of 20 mM NaOH dilution is approximately −2.5 kJ/mol (our data). Therefore, the enthalpies of reactions (1), (2), and (4) add to nearly exactly zero at 25°C. Thus, the observed enthalpy should be practically equal to the enthalpy of aggregation in reaction (3). In confirmation of this expectation, for the control experiment of propylammonium deprotonation, where there was no aggregation, the enthalpy was practically equal to zero throughout the titration at 25°C (Fig. 1).

The enthalpies of reaction (1) at 25°C for short alkylamines are practically independent of the alkyl chain length: ethylamine 57.36 kJ/mol, propylamine 57.91 kJ/mol, butylamine 58.49 kJ/mol, and hexylamine 58.95 kJ/mol [19]. For these short amines, the enthalpy of reaction (1) could be determined without interference from aggregation reaction (3). But Christensen et al. [19] does not provide values for longer alkylamines, presumably because the aggregation reaction interfered with the measurements. From the enthalpy values for ethylamine through hexylamine we can assume with confidence that the enthalpy of reaction (1) would also be 58 ± 5 kJ/mol for heptylamine through tetradecylamine and that the enthalpy of reaction (1) is practically independent of alkyl chain length.

Uncharged dodecylamine is quite insoluble in water, and it aggregated at the end of titration. The observed enthalpy was nearly exactly equal to the enthalpy of the aggregation reaction. The enthalpy of dodecylammonium deprotonation (1) was practically equal to that of propylammonium deprotonation because the heat of reaction (1) was practically independent of the alkyl chain length. Enthalpies of reactions (1), (2), and (4) added to zero for both propylammonium and dodecylammonium.

3.2. Dependence of $\Delta_{agg}H$ on the alkyl chain length at 25°C

Alkylammonium chlorides of various alkyl chain lengths from three to 14 carbon atoms were calorimetrically titrated with sodium hydroxide. Integrated calorimetric titration curves for propylammonium (C_3), undecylammonium (C_{11}), dodecylammonium (C_{12}), and tridecylammonium (C_{13}) are shown in Fig. 2. The enthalpies were practically equal to zero for alkylammoniums with chains shorter than 10 carbon atoms. However, alkylammoniums from 11 to 14 carbon atoms

Table 1
Long chain ($m = 10$–14 carbon atoms) alkylamine aggregation thermodynamic parameters at 25°C

m[a]	C[b] (mM)	$\Delta_{agg}H$[c] (kJ/mol)	$\Delta_{agg}G$[d] (kJ/mol)	$T\Delta_{agg}S$[e] (kJ/mol)	$\Delta_{agg}S$[f] (J/K-mol)	$\Delta_{agg}C_p$[g] (J/K-mol)
10	2	0 ± 2	-2.33 ± 0.4	2.3 ± 2.4	7.8 ± 8.1	–
11	2	-2.7 ± 1.5	-5.04 ± 0.6	2.3 ± 2.1	7.8 ± 7.0	–
12	2	-17.11 ± 1.0	-8.36 ± 0.6	-8.8 ± 1.6	-29.3 ± 5.4	–
13	2	-22.89 ± 1.2	-11.9 ± 0.6	-11.0 ± 1.8	-36.9 ± 6.0	-775 ± 150
14	2	-27.60 ± 1.5	-15.4 ± 1.4	-12.2 ± 2.9	-40.9 ± 9.7	–
12	0.5	-18.2 ± 1.6	-5.17 ± 0.7	-13.0 ± 2.3	-43.6 ± 7.7	–
12	1.0	-17.6 ± 1.1	-6.73 ± 0.8	-10.9 ± 1.9	-36.6 ± 6.4	–

Alkylamines C_{10} and C_{11} aggregate as liquids, and alkylamines C_{12}, C_{13}, and C_{14} aggregate as solids (separated by a blank line). Only the Gibbs free energy is a linear function of m through the phase change region because $\Delta_{transition}G = 0$. Last two lines show dodecylamine aggregation parameters at lower concentrations.

[a] Number of carbon atoms in alkylamine (e.g. $m = 12$, dodecylamine).

[b] Alkylamine concentration.

[c] Enthalpy of alkylamine aggregation determined experimentally by ITC, model-independent.

[d] Gibbs free energy of aggregation, determined by pK_a shift [10].

[e] Entropy of aggregation ($T\Delta_{agg}S$), obtained by subtracting Gibbs free energy from enthalpy.

[f] Entropy of aggregation ($\Delta_{agg}S$), obtained by dividing $T\Delta_{agg}S$ by temperature.

[g] Constant pressure heat capacity of aggregation, determined from the slope of enthalpy dependence on temperature in the range of 25–35°C.

yielded increasingly exothermic enthalpies upon increasing the chain length, because of the contribution from aggregation (Table 1).

3.3. Dependence of $\Delta_{agg}H$ on concentration at 25°C

We showed in the preceding article [10] that the Gibbs free energy of aggregation depends on the concentration of alkylammonium at the beginning of titration. Similar results were observed calorimetrically. The slope of the integrated calorimetric curves decreased upon decreasing the concentrations of both reactants but keeping the concentration ratio unchanged (Fig. 3), indicating the decrease of association constant of alkylamine aggregation. The molar enthalpies of aggregation obtained by integrating the experimental data in Fig. 3 were independent of the concentration of alkylammonium in the cell (Table 1). This independence is consistent with our previous van't Hoff analysis [10].

3.4. The model for fitting the calorimetric titration curves at 25°C

We fit the ITC curves according to the model developed in the preceding article [10], using eq. (15) of that paper, which relates added hydroxide concentration to total alkylamine concentration C, aggregation equilibrium constant A, K_w and K_{acid}, and the concentration of aggregate $[RNH_2\downarrow]$. We assumed that the evolved exothermic enthalpy was proportional to $[RNH_2\downarrow]$, so the aggregation enthalpy evolved at each injection as a function of y (mol NaOH added/mol alkylammonium) is:

$$\Delta_{agg}H(y) = \Delta_{agg}H_{int}\frac{\partial[RNH_2\downarrow]}{\partial y} \quad (5)$$

where $\Delta_{agg}H_{int}$ is the calorimetric integral molar enthalpy of an alkylamine aggregation.

As we see in Figs. 2 and 3, the fitted curves represent the data reasonably well, indicating that

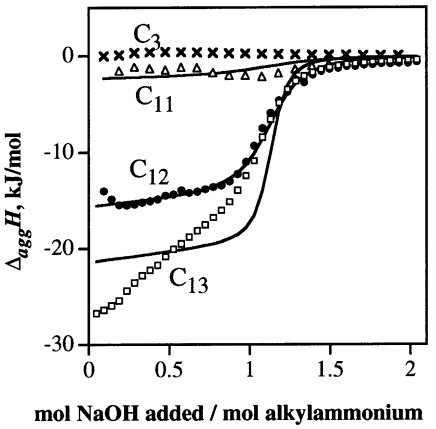

Fig. 2. Integrated calorimetric titration curves of propylammonium (×, C_3), undecylammonium (△, C_{11}), dodecylammonium (●, C_{12}), and tridecylammonium (□, C_{13}) titration with sodium hydroxide at 25°C. Datapoints are the experimentally observed results, and the lines are simulated according to the aggregation model and fitted to the data with the single fitting parameter — enthalpy. Alkylammoniums with longer alkyl chains produced increasingly large exothermic enthalpies upon aggregation.

the calorimetric curves reflect the formation of aggregate. A partial exception is tridecylammonium, whose titration curve had a slope at low y that could not be reproduced by the model. This behavior indicates that the enthalpy of tridecylammonium aggregation depends on reaction progress or the reaction is co-operative. However, we should bear in mind that the derivative curves plotted here are much more sensitive to error than the integrated curves. Especially good reproduction of calorimetric data was achieved for the dependencies on concentration shown in Fig. 3. A twofold decrease in concentration resulted in a reproducible decrease in slope of the curve, supports the aggregation model.

3.5. Gibbs free energy, enthalpy, and entropy of aggregation at 25°C

The Gibbs free energy of aggregation for the reaction (3) is equal to

$$\Delta_{agg}G = -RT\ln(AC + 1), \tag{6}$$

as discussed in the preceding article [10]. To obtain the entropy of aggregation we subtract the Gibbs free energy from the enthalpy:

$$T\Delta_{agg}S = \Delta_{agg}H - \Delta_{agg}G \tag{7}$$

These thermodynamic functions are plotted in Fig. 4 as a function of chain length. Decylamine and undecylamine aggregated as liquids, but longer alkylamines aggregated as solids at 25°C. The melting temperature of pure undecylamine is approximately 19°C and of dodecylamine is approximately 28°C. The dotted lines show the discontinuities in the enthalpy and entropy resulting from the phase change. However, there is no break in $\Delta_{agg}G$ because the free energy change of the phase transition is zero. The enthalpies and entropies of fusion can be estimated from the magnitudes of the discontinuities, as discussed below.

Enthalpies of aggregation of alkylamines shorter than 10 carbon atoms could not be reliably determined because the aggregation reaction proceeded to a progressively smaller extent, in-

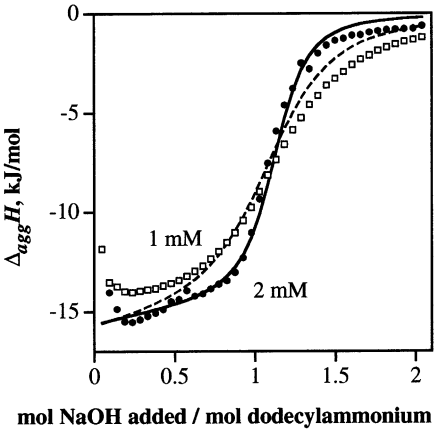

Fig. 3. Integrated calorimetric titration curves of 1 mM (□) and 2 mM (●) dodecylammonium (C_{12}) titration with sodium hydroxide at 25°C. Datapoints are the experimentally observed results, and the lines are simulated according to the aggregation model and fitted to the data with the single fitting parameter — enthalpy. The slope of the curve at the midpoint increased upon increasing concentration, consistent with the increased association constant of the reaction, but the enthalpy of aggregation was independent of concentration.

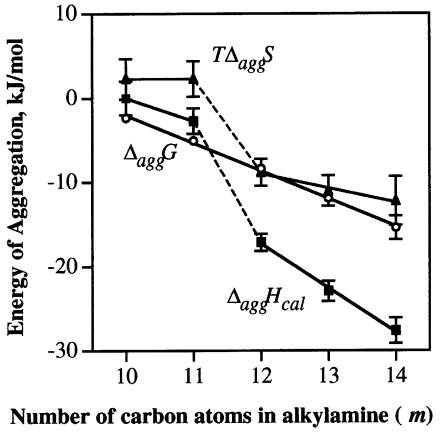

Fig. 4. Enthalpies (■), entropies (▲), and the Gibbs free energies (○) of long chain (10–14 carbon atoms) alkylamine aggregation at 25°C. Decylamine and undecylamine aggregate as liquids while the longer amines — as solids. Broken lines represent enthalpies and entropies of the phase change. There is no break in the Gibbs free energy line because $\Delta_{transition}G = 0$. Aggregation reaction is increasingly enthalpy-driven and entropy-opposed upon increasing aliphatic chain length.

creasing the error of measurement with decreasing chain length. Enthalpies of short alkylamine aggregation were determined by carrying out the backward dilution reaction as previously described [20]. Results will be discussed in the following article [17]. The calorimetric enthalpies we determined for decylamine and undecylamine aggregation were approximately equal to zero or slightly exothermic. Aggregation of these two liquid amines at 2 mM concentration was driven both by enthalpy and entropy.

The thermodynamic parameters were approximately linear functions of the alkyl chain length for C_{12}–C_{14} amines (Fig. 4). Within this range the contributions per mole of methylene group were: $\Delta\Delta_{agg}H = -5.25 \pm 0.5$ kJ/mol CH_2, $\Delta\Delta_{agg}G = -3.58 \pm 0.15$ kJ/mol CH_2, and $T\Delta\Delta_{agg}S = -1.67 \pm 0.65$ kJ/mol CH_2. The enthalpies of aggregation of these alkylamines were increasingly exothermic with increasingly chain length, while the entropy became increasing negative, opposing the aggregation process. This result indicates that enthalpy, rather than entropy, is the factor that drives hydrophobic interactions of long aliphatic chains.

As seen in Table 1, the molar enthalpy of aggregation did not depend on the concentration of alkylammonium. However, as discussed in the preceding article, equilibrium shifts toward the aggregate as C increases, thereby lowering $\Delta_{agg}G$ and making $\Delta_{agg}S$ less negative.

3.6. Dependence of $\Delta_{agg}H$ on temperature — the heat capacity

The heat capacity is an important characteristic of hydrophobic processes. We therefore carried out tridecylammonium deprotonation reactions at temperatures in the range of 25–55°C. Tridecylammonium was chosen because it is the longest amine that is soluble significantly above 2 mM where the experiments were carried out. Tetradecylammonium chloride is also slightly soluble above 2 mM, but within several days its solution becomes turbid and a precipitate appears within a week at 25°C.

Analysis of ITC data at temperatures higher than 25°C was significantly more complicated. Fig. 5 shows tridecylammonium and propylammonium titration curves at 39°C. A series of unusually large exothermic peaks can be seen near the middle of the titration of tridecylammonium, the most exothermic of which produced −190 kJ/mol enthalpy. This value is strikingly large — more than half the enthalpy of an average covalent bond. The evolution of such a large heat upon addition of a tiny amount of sodium hydroxide was puzzling, until we realized that tridecylamine aggregate changed its phase from liquid to crystalline, and the large enthalpy closely matched the heat of tridecylamine fusion. Crystallization occurred highly cooperatively when enough ammonium groups were neutralized, in a manner highly dependent on temperature.

Propylammonium titration, where there is no aggregation, evolved a relatively small amount of endothermic enthalpy equal to +3.28 kJ/mol at 39°C. This value represents the sum of heats of reactions (1), (2), and (4), i.e. the heats of propylammonium deprotonation, hydroxide protonation, and sodium hydroxide dilution. At 25°C the sum of these three heats coincidentally added up to zero. To obtain the enthalpy of aggregation of

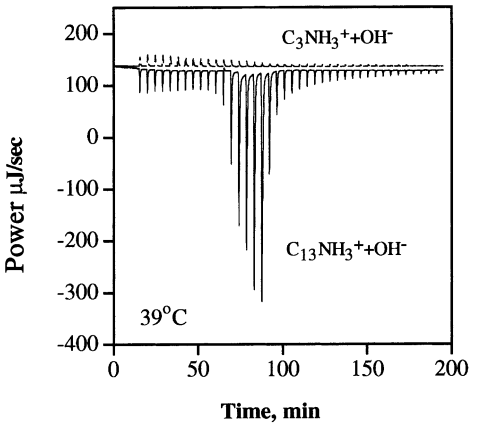

Fig. 5. Raw data calorimetric titration curves of 2 mM tridecylammonium chloride (solid line) and 2 mM propylammonium chloride (dashed line) titration with 20 mM sodium hydroxide at 39°C. Very large exothermic heats near the middle of titration are due to the fusion of tridecylamine precipitating into a solid phase. Relatively small endothermic heats were observed for propylamine which did not aggregate.

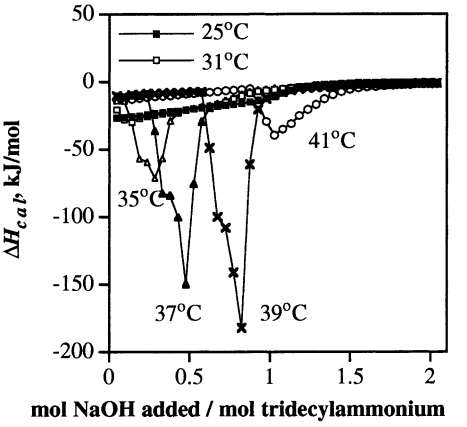

Fig. 6. Integrated titration calorimetry curves of 2 mM tridecylammonium chloride titration with 20 mM sodium hydroxide at various temperatures: 25°C (■), 31°C (□), 35°C (△), 37°C (▲), 39°C (×), and 41°C (○). The position of the large negative peak is highly temperature-dependent and could only be observed in a narrow temperature range. The peak is due to the large exothermic enthalpy of the tridecylamine phase change from liquid to solid aggregate.

tridecylammonium (reaction 3), one needs to subtract the heat of reactions (1), (2), and (4) for propylammonium from the observed enthalpy for the long chain alkylammonium. For example, the overall observed enthalpy of tridecylamine titration with hydroxide (solid line, Fig. 5) was equal to -41.84 kJ/mol. After subtraction we obtain the enthalpy of tridecylamine aggregation: $(-)41.84-(+)3.28=(-)45.12$ kJ/mol. Thus the enthalpy of tridecylamine aggregation is equal to approximately -45 kJ/mol at 39°C.

Integrated experimental curves of tridecylammonium deprotonation at various temperatures are shown in Figs. 6 and 7. The unusual negative peak is first visible at 29°C (not all data is shown in Figs. 6 and 7 for clarity). It gradually increases and reaches a maximum at 39°C, then decreases until no peak is visible at 43°C. At higher temperatures (Fig. 7) at approximately 50°C there is a relatively small positive peak visible near the point of stoichiometric neutralization of tridecylammonium. Note that the scale of Fig. 7 is much smaller than of Fig. 6. We did not attempt to fit these curves to a model. However, they demonstrate how much information can be obtained by ITC.

The integrated curves, such as shown in Figs. 6 and 7, were integrated again to obtain the molar enthalpies of tridecylamine aggregation at various temperatures. As explained above, the analysis at

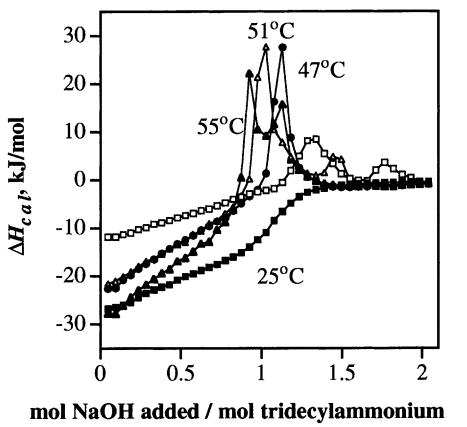

Fig. 7. Integrated titration calorimetry curves of 2 mM tridecylammonium chloride with 20 mM sodium hydroxide at various temperatures: 25°C (■), 43°C (□), 47°C (●), 51°C (△), and 55°C (▲). The positive peaks are due to the tridecylamine phase change from liquid to solid aggregate. Note that the scale of Fig. 7 is much smaller than of Fig. 6.

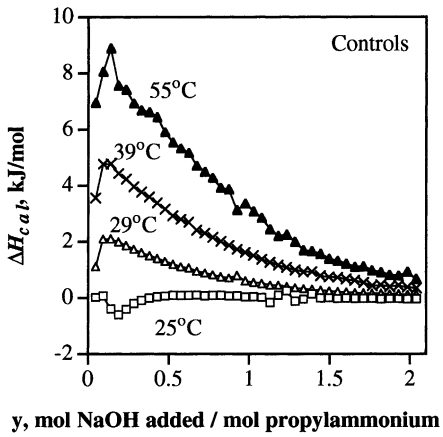

Fig. 8. Integrated titration calorimetry curves of 2 mM propylammonium chloride with 20 mM sodium hydroxide at various temperatures: 25°C (□), 29°C (△), 39°C (×), and 55°C (▲). These curves demonstrate that in the absence of aggregation only small endothermic heats are produced. At 25°C the heat of this control reaction was coincidentally equal to zero. The areas under such curves were subtracted from the areas under curves in Figs. 6 and 7 yielding calorimetric enthalpies of tridecylamine aggregation, shown in Fig. 9. Note that the scale of Fig. 8 is much smaller than of Figs. 6 and 7 and the subtraction only slightly affects the calorimetric enthalpies of aggregation.

temperatures greater than 25°C was more complex because the enthalpies of reactions (1), (2), and (4) did not add up to zero as was the case at 25°C. Control experiments with propylammonium at all temperatures were carried out and several are shown in Fig. 8. Enthalpies observed during propylammonium deprotonation were relatively small (as compared to tridecylamine aggregation enthalpies), positive, and increased slightly with temperature. To obtain the enthalpies of aggregation, these enthalpies representing reactions (1), (2), and (4) should be subtracted. However, subtraction had only a minor effect on the overall results. Note that the scale of Fig. 8 is much smaller than of Figs. 6 and 7.

By subtracting observed molar enthalpies for propylammonium from those for tridecylammonium, we obtained the enthalpy of tridecylamine aggregation, plotted in Fig. 9 as a function of temperature. The enthalpy slightly decreased with increasing T until there was an abrupt change at approximately 40°C to a much less exothermic value. The enthalpy then gradually decreased again with increasing temperature. The abrupt change is due to the aggregation into solid below 29°C and into liquid above 43°C. An explanation of this phase change follows.

To the best of our knowledge, there is no data in the literature about the melting temperature or enthalpy of fusion of tridecylamine. However, the values for dodecylamine ($T_m = 28.32$°C) and tetradecylamine ($T_m = 38.19$°C) are available [21]. We measured the melting temperatures of all three amines, obtaining $T_m = 28.2 \pm 0.5$°C for dodecylamine, 29.5 ± 1°C for tridecylamine, and 38.0 ± 1°C for tetradecylamine. The melting temperature followed a zig-zag line as a function of alkyl chain length, probably due to packing differences between even and odd chain lengths. Crystalline tridecylamine melted near 30°C. In aqueous solution, however, the phase change appeared to take place at approximately 40°C, with indications of a transition extending from 29 to

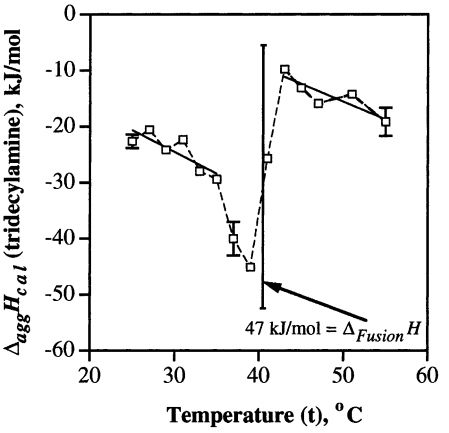

Fig. 9. Overall integrated molar enthalpies of tridecylamine aggregation as a function of temperature (□). The $\Delta_{agg}H$ values are directly experimentally observed and independent of the aggregation model. Vertical line is equal in length to 47 kJ/mol which is the approximate enthalpy of pure tridecylamine fusion at 30°C. In aqueous solution the fusion temperature is apparently broadened and shifted to a higher temperature of approximately 40°C. The lines with midpoints at 30 and 50°C are linear fits of experimental values within the 5°C range and have slopes ($\Delta_{agg}C_p$) of (−)775 and (−)630 J/mol-K, respectively.

Table 2
Estimated tridecylamine aggregation thermodynamic parameters as a function of temperature

Temperature[a] (°C)	$\Delta_{agg}H^{b}_{cal}$ (kJ/mol)	$\Delta_{agg}G^{c}$ (kJ/mol)	$T\Delta_{agg}S^{d}$ (kJ/mol)	$\Delta_{agg}S^{e}$ (J/K-mol)	$\Delta_{agg}C^{f}_{p}$ (J/K-mol)
25	-22.6 ± 1.2	-12.3 ± 0.6	-10 ± 2	-35 ± 7	–
30	-26 ± 2	-12.2 ± 0.8	-14 ± 3	-46 ± 10	-775 ± 150
35	-32 ± 3	-12.1 ± 0.9	-20 ± 4	-65 ± 13	–
40	-50 ± 5	-11.8 ± 1.0	-38 ± 6	-122 ± 20	–
45	-13 ± 1.5	-11.5 ± 1.0	-2 ± 3	-5 ± 10	–
50	-15 ± 2	-11.1 ± 1.1	-4 ± 3	-12 ± 10	-630 ± 200
55	-19 ± 2.5	-10.6 ± 1.1	-8 ± 4	-26 ± 13	–

Errors include standard deviations and uncertainties of the estimate. A blank line separates solid and liquid tridecylamine aggregation. Tridecylamine aggregation is enthalpy-driven and entropy-opposed process within this temperature range, independent of phase formed.

[a] Estimates for these temperatures were made by polynomial regressions of experimental data.
[b] Estimates from experimental data shown in Fig. 9.
[c] Estimates from the data in Mantulis and Bloomfield [10] ($C = 2$ mM).
[d] Calculated by subtracting $\Delta_{agg}H_{cal} - \Delta_{agg}G$ ($C = 2$ mM).
[e] Calculated from $T\Delta_{agg}S$.
[f] Estimated from $\Delta_{agg}H_{cal}$ dependence on temperature within ± 5°C range.

43°C, a range much broader than that of the pure solid. The reported enthalpies of fusion are $\Delta_{fus}H(C_{12}) = 43.5$ kJ/mol and $\Delta_{fus}H(C_{14}) = 51.0$ kJ/mol [21]. The enthalpy of tridecylamine fusion is expected to be somewhere in this range. Fig. 8 shows a vertical bar of 47 kJ/mol near 40°C, a value in the expected range for $\Delta_{fus}H(C_{13})$.

We conclude that tridecylamine aggregates formed in aqueous solution are liquid above 43°C and solid below 29°C. The slopes of $\Delta_{agg}H$ vs. T (the heat capacity) in Fig. 8 are consistent with this interpretation. The solid line between 25 and 35°C is the linear regression of the points in that range; it yields a heat capacity of -775 J/mol-K, in reasonable agreement with the value estimated from literature data for similar linear aliphatic compounds (see Matulis [17] for a comparison with literature). Similarly, $\Delta_{agg}H$ vs. T between 43 and 55°C has a slope of -630 J/mol-K. The heat capacity of a liquid is usually greater than that of a solid, and the heat capacity of a liquid usually increases with increasing temperature. This is consistent with our data that show that more heat capacity is lost upon forming a solid than upon forming a liquid aggregate.

The thermodynamic parameters of tridecylamine aggregation as a function of temperature are summarized in Table 2. The enthalpies of aggregation decrease slightly with increased temperature yielding a negative heat capacity characteristic of the hydrophobic effect. The abrupt change in the enthalpy above 40°C represents the phase change of the aggregate. Below approximately 42°C the aggregate is solid, and the large exothermic enthalpy of fusion is observed. Above approximately 42°C the aggregate is liquid, and the enthalpy of aggregation represents the formation of liquid precipitate. Gibbs free energies were obtained by the pK_a shift method as explained in the preceding article [10]. Subtraction of Gibbs free energies from enthalpies yields entropies of aggregation. The entropies of aggregation were negative at all tested temperatures of 25–55°C. These findings are in apparent contradiction with the conventional understanding of the hydrophobic effect. However, until now the enthalpies and entropies of alkane aggregation have been obtained by van't Hoff analysis of the Gibbs free energies of dissolution. There is a large error in such analysis when the Gibbs free

energy is nearly independent of temperature. Therefore, we believe that our calorimetric results are significantly more accurate than those based on van't Hoff analysis.

Despite our best effort to determine the enthalpies of aggregation at many temperatures with greatest possible precision, the derived values of the heat capacity still had a significant error and uncertainty. As mentioned above, for the range of 25–35°C, the heat capacity was -775 ± 150 J/mol-K. It is important, however, that the value was obtained by titration calorimetry experiment in aqueous solution.

4. Discussion

We have shown that we can interpret the heat changes upon titration with base, as measured by isothermal titration calorimetry, to determine the thermodynamics of aggregation of long aliphatic chain alkylamines in aqueous solution. The enthalpy of aggregation became increasingly exothermic upon increasing the chain length. In contrast with usual expectations for hydrophobic reactions, aggregation was enthalpy-driven and entropy-opposed for alkylamines with 12–14 carbon atoms at room temperature and higher temperatures up to 55°C.

However, there are systems where the enthalpy has been shown to play an important driving role in long aliphatic chain aggregation. For example, consider the results of the micellization of long chain alkyltrimethylammonium bromides [9]. The enthalpy of tetradecyltrimethyl-ammonium bromide micellization at its cmc (determined as the enthalpy of infinite micelle dilution) was -4.88 kJ/mol, and that of hexadecyltrimethylammonium bromide was -9.76 kJ/mol at 25°C. The average contribution of a methylene group to the enthalpy of micellization for chain lengths of 10, 12, 14, and 16 carbon atoms was approximately -1.6 kJ/mol.

Another major result of this study is that determination of thermodynamic parameters by van't Hoff analysis of the temperature dependence of the equilibrium constant or Gibbs free energy may be considerably less accurate than direct calorimetric measurement, despite the apparent high precision of the ΔG vs. T data. This situation arises for two main reasons. First, competing enthalpy and entropy effects, especially in phase transitions, largely compensate each other and may be hidden when analyzing ΔG alone. Second, the T-dependence of ΔG is often very small. Very high accuracy and precision of the measurements are then essential to obtain meaningful derivatives of the data. This difficulty is especially obvious for hydrophobic interactions, where the $\Delta_{agg}G$ temperature dependence is very small.

As an example, we compare the calorimetric results obtained in this study with the van't Hoff analysis of the same reactions described in the preceding article [10]. The calorimetrically measured enthalpies of dodecylamine and tridecylamine aggregation were equal to -17.11 ± 1.0 and -22.89 ± 1.2 kJ/mol, respectively (Table 1). The enthalpies of the same processes by van't Hoff analysis [10] were -22.47 and -12.00 kJ/mol, respectively (all at 25°C). Thus, the calorimetric analysis in the present paper shows that the van't Hoff analysis incorrectly determined the relative exothermicities of tridecylamine and dodecylamine aggregation. The errors of the results obtained by differentiating $\Delta G/T$ with respect to T were at least 10 times greater than the errors of calorimetric measurements (Fig. 4). The situation is all the more misleading because the results of van't Hoff analysis appeared to be quite precise, and the second derivative (heat capacity) was consistent with the usual view of hydrophobic interactions.

These results cast doubt on the accuracy of the dodecane aggregation thermodynamics [21] shown in Figs. 8 and 9 of the preceding article [10]. Measurement of the dependence of alkane solubility on temperature may be insufficient to obtain a full picture of the thermodynamics of hydrophobic interactions. Unfortunately, however, it is not possible to carry out an ITC investigation of alkane aggregation in aqueous solution. It is

necessary to have functional groups, such as amino, carboxyl, or other group to make the hydrocarbons soluble in water. Therefore we believe that our calorimetric analysis of long chain alkylamine aggregation provides the best estimates currently available of long hydrocarbon chain aggregation in water.

Unfortunately, a similar calorimetric analysis could not be used to obtain reliable enthalpies of short alkylamine aggregation, because the aggregation reaction proceeded to a very small degree. Decylamine was the shortest amine for which the enthalpy of aggregation was determined with reasonable confidence. Still, we believe that the thermodynamics of short aliphatic chain aggregation should be experimentally reinvestigated using calorimetric methods, instead of the van't Hoff analysis usually employed.

In addition to the issues of accuracy and precision, van't Hoff analysis cannot provide any information on phase changes. By definition, the Gibbs free energy difference is zero for a phase change. However, the phase change is often accompanied by large changes in enthalpy and entropy that exactly cancel each other to yield zero Gibbs free energy difference. Taking a temperature derivative of such Gibbs free energy does not provide the underlying enthalpy and entropy. Exactly such a problem was encountered when analyzing alkylamine aggregation by van't Hoff method. The enthalpy and entropy of tridecylamine fusion below approximately 42°C were completely invisible by van't Hoff analysis [10], but could be quite accurately determined by calorimetric analysis.

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