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Thermodynamics of the hydrophobic effect. III. Condensation and aggregation of alkanes, alcohols, and alkylamines

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Abstract

Knowledge of the energetics of the low solubility of non-polar compounds in water is critical for the understanding of such phenomena as protein folding and biomembrane formation. Solubility in water can be considered as one leg of the three-part thermodynamic cycle — vaporization from the pure liquid, hydration of the vapor in aqueous solution, and aggregation of the substance back into initial pure form as an immiscible phase. Previous studies on the model compounds n-alkanes, 1-alcohols, and 1-aminoalkanes have noted that the thermodynamic parameters (Gibbs free energy, ΔG; enthalpy, ΔH; entropy, ΔS; and heat capacity, ΔC_p) associated with these three processes are generally linear functions of the number of carbons in the alkyl chains. Here we assess the accuracy and limitations of the assumption of additivity of CH_2 group contributions to the thermodynamic parameters for vaporization, hydration, and aggregation. Processes of condensation from pure gas to liquid and aqueous solution to aggregate are compared. Hydroxy, amino, and methyl headgroup contributions are estimated, liquid and solid aggregates are distinguished. Most data in the literature were obtained for compounds with short aliphatic hydrocarbon tails. Here we emphasize long aliphatic chain behavior and include our recent experimental data on long chain alkylamine aggregation in aqueous solution obtained by titration calorimetry and van't Hoff analysis. Contrary to what is observed for short compounds, long aliphatic compound aggregation has a large exothermic enthalpy and negative entropy. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Aggregation in water; Solubility; Thermodynamics of molecular interactions; Heat capacity of hydrophobic interactions; Van't Hoff enthalpy; Calorimetric enthalpy

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1. Introduction

Hydrophobic interactions in aqueous solutions govern many biophysical reactions of chemical and biological significance, such as lipid aggregation, membrane formation, and protein folding. Since the publication of the classic works on the subject [1–3] a wealth of new experimental data and theoretical studies has appeared [4,5]. However, there is no concise recent review that organizes the data in terms of a simple model summarizing the processes of vaporization, hydration, and aggregation.

The three processes are linked in the form of a thermodynamic triangle (Fig. 1). Gibbs free energies of the three processes have been often illustrated in the form of such a triangle [6,7]. Vaporization begins with a pure liquid and ends with a pure gas involving a phase change of a single compound. The second process is the hydration of the gaseous compound by dissolving it in water. The last process, closing the thermodynamic cycle, is the process of aggregation, when fully hydrated compound precipitates from solution into an insoluble phase and returns to the initial pure liquid state.

Experimental data are available for three thermodynamic parameters (ΔG, ΔH, and ΔC_p) for all three processes, and the entropy can be calculated from $T\Delta S = \Delta H - \Delta G$. A large amount of data is available for alkanes and alcohols, but limited data is available for alkylamines. Precision and accuracy of the data varies considerably, and the various methods employed for the measurements are also of unequal reliability. Ideally, the sum of Gibbs free energy, enthalpy, entropy, and the heat capacity for all three processes in both the clockwise and the counterclockwise directions should be equal to zero. In practice, however, the sum of experimental data may deviate significantly from zero.

It was observed quite some time ago that the Gibbs free energy of alkane dissolution in water depends linearly on the number of carbon atoms in the aliphatic chain [8]. Similarly, the Gibbs free energies and other thermodynamic parameters

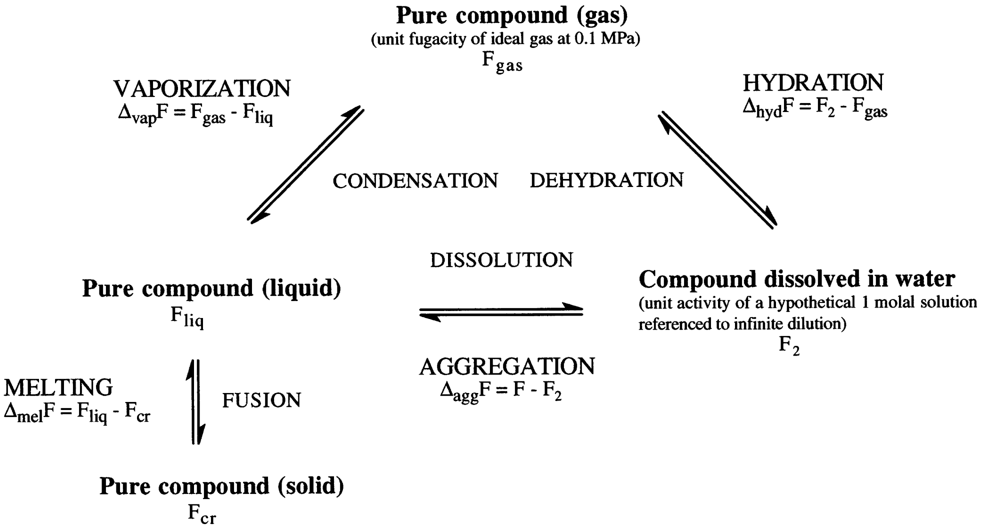

Fig. 1. Thermodynamic cycle of vaporization, hydration, and aggregation processes, and the equilibria between liquid and solid phases (fusion process). Standard state molar thermodynamic functions ($F = G$, H, S, or C_p) are shown under each form of the compound. Subscript 2 refers to the partial molar thermodynamic function of solute (component 2) in water (component 1). Thermodynamic parameters of the processes (shown with subscripts: vap, vaporization; con, condensation; hyd, hydration; agg, aggregation; dis, dissolution; fus, fusion; mel, melting) are equal to the differences between standard state thermodynamic functions of products and reactants.

(ΔG, ΔH, ΔS, and ΔC_p) are often linearly dependent on the number of carbon atoms in a molecule for all three processes. The validity of linear approximations has been questioned and experimental data sometimes contradicts such models [9]. A recent compilation of paraffin solubility [10] shows nearly the same solubility for hydrocarbons with 13–17 carbon atoms at various temperatures. These results appear to disobey linear relationships, but whether this is a real effect or due to experimental error when solubility is so low remains unclear. McAuliffe [8] had successfully shown earlier the validity of a linear model for hydrocarbons with nine and 10 carbon atoms when many experimental measurements contradicted it. In this paper we attempt to clarify the applicability and limitations of linear models.

We summarize literature data from various reviews on the thermodynamics of vaporization, hydration, and aggregation of aliphatic non-ionic compounds in terms of functional group contribution linear models. Only non-branched (linear) and saturated *n*-alkanes, 1-alcohols, and 1-aminoalkanes are considered in this study. Contribution of the methylene group (CH_2) is calculated for all processes, and the comparison between methyl (CH_3), hydroxyl (OH), and amino (NH_2) groups is provided similar to earlier compilations [11]. Alkylamine equilibria in water include ionization which complicates analysis of aggregation. This explains the small amount of experimental data for alkylamines in the literature. However, alkylamines are of great interest because they are part of biologically relevant polymers and therefore they are included in this study.

The model helps to compare various processes. It is especially important to compare the hydrophobic aggregation process in aqueous solution with the process of pure compound condensation from gaseous to liquid phase. Contributions of all thermodynamic parameters to these processes are qualitatively similar. The quantitative differences between these two processes allow us to evaluate the energetics of water participation in hydrophobic aggregation process.

The main goal of studying model systems, such as alkane solubility, is to apply the knowledge gained to biological polymers, such as proteins. It is often assumed that the thermodynamic parameters of atom–atom interactions (contact free energies, enthalpies, etc.) for model systems will carry over additively to complicated systems, such as proteins or DNA. Hydrophobic interactions are usually modeled by carrying out partitioning (octanol–water) or solubility (pure compound–aqueous solution) experiments. However, there are significant problems in the understanding and interpretation of currently available experimental results. For example, benzene partitioning from water to *n*-alkanes depends on the alkyl chain length of the alkane [12]. It is not straightforward to distinguish energetics of interactions from effects that affect only model systems, such as conformational changes of solvent alkane molecules.

Therefore, we believe that new experimental approaches are needed to study model systems of molecular interactions. The binding reactions of model compounds should be carried out in aqueous solution from beginning to end instead of by partitioning experiments between two solvents. Solubility experiments provide limited information at a single concentration. Unfortunately, it is not possible to carry out a reaction such as octane aggregation by beginning with octane dissolved in water and ending with octane that is aggregated (octane molecules bound to each other next to aqueous solution). However, it is possible to carry out similar reactions where an additional functional group is altered during the reaction. One such system is the alkylamines.

In the preceding articles [13,14] we have investigated aggregation of alkylamines containing long aliphatic chains (10–14 carbon atoms). Calorimetric titration of completely dissolved cationic alkylammoniums with sodium hydroxide produced practically insoluble alkylamines. Thermodynamic parameters of ammonium deprotonation, hydroxide protonation, hydroxide dilution, and alkylamine aggregation could be distinguished by varying concentrations and alkyl chain length of alkylamine. Such an approach enabled direct observation of aggregation reactions and produced energetic parameters of interactions between long alkyl chains in water that were unexpectedly quite different from the parameters observed by partitioning and solubility experiments.

Calorimetric enthalpies of aggregation reactions are usually determined by measuring the heat evolved upon dissolving liquid or solid compound and then extrapolating to zero concentration. Before the early calorimetric determinations [15,16] nearly all heats of solution were determined by van't Hoff analysis of solubility dependence on temperature. However, there are no measurements of the reverse reaction, i.e. starting with the compound that is fully dissolved in water and ending with the aggregate. As explained above, we carried out such reaction calorimetrically beginning with soluble alkylammonium cations and ending with insoluble alkylamines, and directly determined the enthalpy of aggregation of long alkylamines. There are several advantages of using titration calorimetry over van't Hoff analysis. First, the accuracy and precision of ITC is significantly greater because of the error and uncertainty of van't Hoff analysis when ΔG depends only slightly on temperature.

Second, phase changes can be observed only by ITC and are completely invisible to van't Hoff analysis. Alkylamines that exist as solids at 25°C form a solid aggregate in aqueous solution, while alkylamines that exist as liquids at 25°C form a liquid aggregate in aqueous solution. The enthalpies and entropies of aggregation into a solid phase differ significantly from the aggregation into a liquid phase. However, the Gibbs free energies of aggregation follow a straight line through the region of phase change. Therefore, only direct calorimetric measurements can detect this difference. Van't Hoff analysis of the Gibbs free energy dependence on temperature could not distinguish between the liquid and solid phases.

For comparison of our data with the results of partition and solubility experiments, we adopted the same standard states as in a comprehensive recent review of the experimental data of hydro-

Table 1
Equations for calculation of the dependence of the thermodynamics of vaporization, hydration, and aggregation of liquid phase linear alkanes, 1-alcohols, and 1-amino alkanes at 25°C on m, the number of carbon atoms in a molecule[a]

	Compounds	Vaporization	Hydration	Aggregation
ΔG (kJ/mol)	Alkanes	$2.86m - 13.10$	$0.72m + 13.79$	$-3.58m - 0.69$
	Alcohols	$2.86m + 0.20$	$0.72m - 14.53$	$-3.58m + 14.33$
	Alkylamines	$2.86m - 6.20$	$0.72m - 13.10$	$-3.58m + 19.30$
ΔH (kJ/mol)	Alkanes	$4.95m + 1.85$	$-3.70m - 11.10$	$-1.25m + 9.25$
	Alcohols	$4.95m + 32.30$	$-3.70m - 45.0$	$-1.25m + 12.70$
	Alkylamines	$4.95m + 15.30$	$-3.70m - 43.0$	$-1.25m + 27.70$
$T\Delta S$ (kJ/mol)	Alkanes	$2.09m + 14.95$	$-4.42m - 24.89$	$2.33m + 9.94$
	Alcohols	$2.09m + 32.10$	$-4.42m - 30.47$	$2.33m - 1.63$
	Alkylamines	$2.09m + 21.50$	$-4.42m - 29.90$	$2.33m + 8.40$
ΔS (J/mol-K)	Alkanes	$7.01m + 50.2$	$-14.82m - 83.5$	$7.81m + 33.3$
	Alcohols	$7.01m + 107.7$	$-14.82m - 102.2$	$7.81m - 5.5$
	Alkylamines	$7.01m + 72.1$	$-14.82m - 100.3$	$7.81m + 28.2$
ΔC_p (J/mol-K)	Alkanes	$-7.0m - 9$	$62.0m + 144$	$-55.0m - 135$
	Alcohols	$-7.0m - 35$	$62.0m + 70$	$-55.0m - 35$
	Alkylamines	$-7.0m - 42$	–	–

[a] For alkanes $m = 5–17$, for 1-alcohols $m = 1–12$, and for alkylamines $m = 3–11$. These equations describe the linear regressions shown in Fig. 2.

carbon and alcohol hydration [17]. As in that review, the molar concentration scale was used rather than the mole fraction scale.

2. Results and discussion

Fig. 2 shows selected literature data for the thermodynamics of the three processes at 25°C, 0.1 MPa, occuring in the clockwise direction of Fig. 1. Datapoints represent experimental data or their averages in the literature, and the lines represent linear regressions of the three processes. The slopes of the lines (i.e. the contribution per CH_2 group) for functionally different compounds (e.g. alkanes vs. alcohols) are constrained to be the same, a constraint seen to be accurately obeyed. Graphs are presented at the same scale for the three processes to enable visual comparison. Regression parameters are shown in Table 1. The sums of the model values for the three processes are equal to zero for all four thermodynamic parameters by default.

However, the enthalpies and entropies are usually obtained from the Gibbs free energy dependence on temperature. The Gibbs free energy of the phase change at a constant temperature is equal to zero. Quantities such as enthalpy and entropy, derived from the Gibbs free energy, do not contain any phase change information. Only the calorimetrically determined enthalpies reflect phase changes that take place during the reactions. Fig. 3 shows calorimetrically observable phase changes. It should be compared with Fig. 2 in which the phase changes are invisible to van't Hoff analysis.

Fig. 3 compares thermodynamic parameters of condensation and aggregation measured calorimetrically. Condensation (Fig. 1) is the reverse of vaporization. All thermodynamic parameters for the two processes are equal but with opposite signs. It is important to compare condensation with aggregation because both processes involve binding of alkyl chains to each other: condensation is binding from gas phase and aggregation is binding from aqueous solution. Bold lines connect points for compounds that exist as solids at 25°C, and the narrow lines connect liquid or gaseous compounds. For example, dodecanol is liquid but tridecanol is solid at 25°C. Calorimetrically measured enthalpies of condensation and aggregation should have discontinuities due to the enthalpy and entropy of fusion. We have demonstrated this for the calorimetrically measured enthalpies of alkylamine aggregation [13].

The slopes of lines in Fig. 3 are the same as in Fig. 2 except for the solid compounds. Enthalpies and entropies of fusion (at the fusion temperature) were added for the solids. The discrepancy between the fusion temperature and 25°C could be neglected. Regression parameters of the condensation and aggregation of solid compounds are listed in Table 2. We now consider each thermodynamic parameter in greater detail.

2.1. Gibbs free energy of vaporization ($\Delta_{vap}G$) and condensation ($\Delta_{con}G$)

Precise data are available for hydrocarbons and alcohols [18]. Dependence on aliphatic chain length is linear within the error of experimental measurement. Slopes are nearly identical for all three classes of compounds with the unfavorable contribution of a methylene group of 2.86 kJ/mol. Alcohols have the largest $\Delta_{\mathrm{vap}}G$, i.e. it is most difficult to vaporize alcohols as compared to alkanes and alkylamines. Therefore there are no gaseous alcohols at room temperature. Alkylamines have intermediate $\Delta_{\mathrm{vap}}G$ because hydrogen bonds between amino groups are weaker and their number is lower as compared to alcohols. Thus, methylamine and ethylamine are gases at room temperature, with negative $\Delta_{\mathrm{vap}}G$ at 25°C. Interactions between alkane molecules are the weakest, and the first four alkanes — methane, ethane, propane, and butane — exist as gases at room temperature. The Gibbs free energy of condensation ($\Delta_{\mathrm{con}}G$) is the reverse of vaporization. There are no reliable data in the literature on condensation or vaporization free energy of solid alkanes, alcohols, or alkylamines. However, it is expected that the model can be extended linearly to the solids.

2.2. Gibbs free energy of hydration ($\Delta_{hyd}G$)

Experimental data for the hydration process of

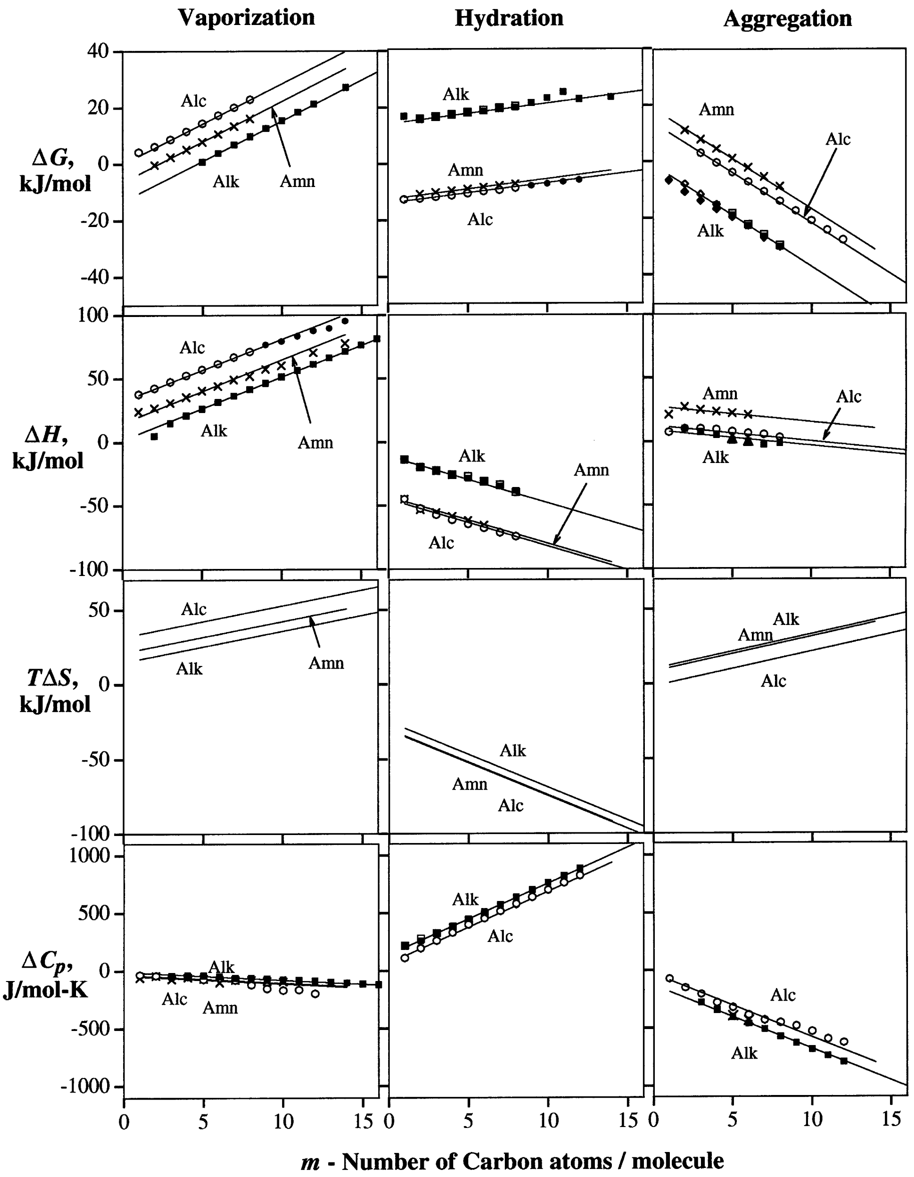

Fig. 2. Thermodynamic parameters of vaporization, hydration, and aggregation of alkanes (alk), alcohols (alc) and alkylamines (amn) without the distinction between liquid and solid phases (0.1 MPa, 25°C). Symbols represent experimental datapoints and lines represent regressions with the following constraints: (1) slopes for the three classes of compounds are the same; (2) vaporization, hydration, and aggregation processes sum up to zero. Regression equations are listed in Table 1. References for the sources of the datapoints are the following. $\Delta_{vap}G$: ■ and ○ — [17], and references therein, × — [6], $\Delta_{hyd}G$: ■ and ○ — [17], □, ×, and ● — [6] adjusted from mole fraction reference state by subtracting 9.96 kJ/mol ($RT\ln 55.56 = 9.96$ kJ/mol, where 55.56 — the number of moles of water in a liter), $\Delta_{agg}G$: ×, ○, and ◇ — [6] adjusted from mole fraction reference state by adding 9.96 kJ/mol, ■ — [23] from alkane solubility data ($\Delta_{agg}G = RT\ln\{\mathit{solubility},\ \text{molar}\}$), □ — [26] from solubility data, ♦ — [10] from octanol–water partition coefficient data ($\Delta_{agg}G = RT\ln K_{ow}$), $\Delta_{vap}H$: ■ — [19], ○ — [17], × and ● — [10], $\Delta_{hyd}H$: ■ and ○ — [17], □ and × — [6], $\Delta_{agg}H$: × — [27], ■, and ○ — [6], △ — [28], $\Delta_{vap}C_p$: ×, ■, and ○ — [10] by subtracting $C_{p\ liq}$ from $C_{p\ gas}$, $\Delta_{hyd}C_p$: □ and ● — [17], ■ and ○ — by subtracting $C_{p\ gas}$ [10] from $C_{p\ 2}$ [17], $\Delta_{agg}C_p$: ■ and ○ — by subtracting $C_{p\ 2}$ [17] from $C_{p\ liq}$ [10], × — [28].

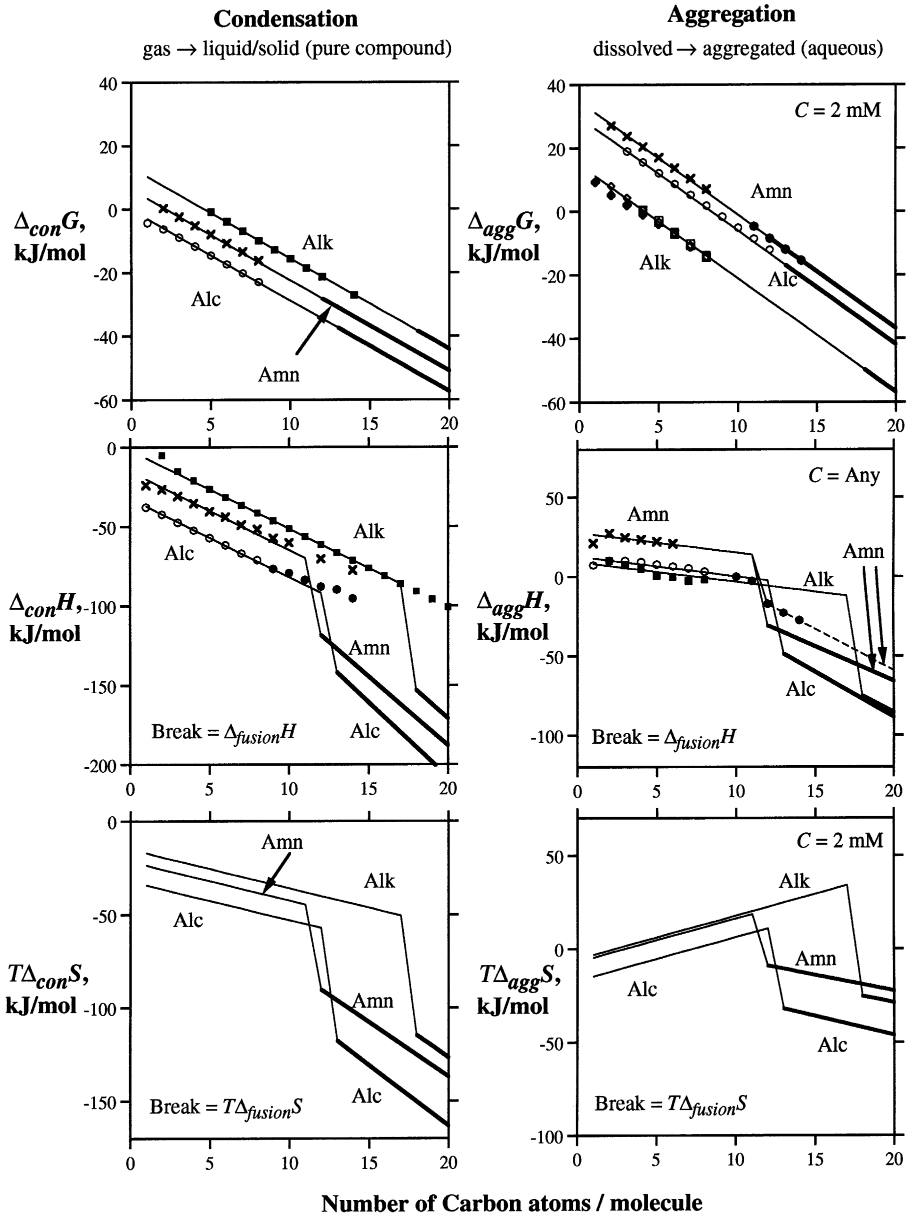

Fig. 3. Thermodynamic parameters of condensation and aggregation of alkanes (alk), alcohols (alc) and alkylamines (amn) with the distinction between liquid and solid phases. Symbols represent the same experimental datapoints as in Fig. 2, and narrow lines represent same regressions with equations listed in Table 1 (except $\Delta_{agg}G$ and $T\Delta_{agg}S$ that were adjusted to the 2-mM 'reference state' to be consistent with the experiment by the addition or subtraction of $RT\ln 0.002 = 15.41$ kJ/mol). Broad (bold) lines were calculated by adding enthalpies or entropies of fusion for the compounds that exist as solids at 25°C. Filled circles (●) in the $\Delta_{agg}G$ and $\Delta_{agg}H$ panels show our data obtained by pK_a shift ($\Delta_{agg}G$) [13] and titration calorimetry ($\Delta_{agg}H_{cal}$) [14]. Note the similarity and differences between the condensation and aggregation processes. Condensation is an enthalpy driven and entropy opposed process for all studied compounds, but the aqueous aggregation is a mixed process at 25°C. Note that datapoints for $\Delta_{con}H$ extend linearly beyond the phase change. This is because the data was obtained by van't Hoff analysis and the information about the phase change was invisible.

Table 2
Equations for calculation of the dependence of the thermodynamics of condensation and aggregation into a solid phase of linear alkanes, 1-alcohols, and 1-amino alkanes at 25°C on m, the number of carbon atoms in a molecule[a]

	Compounds	Condensation (into solid)	Aggregation (into solid)
ΔG (kJ/mol)	Alkanes	$-2.86m + 13.10$	$-3.58m - 0.69$
	Alcohols	$-2.86m - 0.20$	$-3.58m + 14.33$
	Alkylamines	$-2.86m + 6.20$	$-3.58m + 19.30$
ΔH (kJ/mol)	Alkanes	$-8.90m + 7.31$	$-5.20m + 18.41$
	Alcohols	$-9.37m - 19.92$	$-5.67m + 25.08$
	Alkylamines	$-8.70m - 13.80$	$-5.25m + 45.71$
$T\Delta S$ (kJ/mol)	Alkanes	$-5.94m - 7.62$	$-1.62m + 3.76$
	Alcohols	$-6.48m - 33.45$	$-2.06m - 5.09$
	Alkylamines	$-5.84m - 20.00$	$-1.67m + 11.00$
ΔS (J/mol-K)	Alkanes	$-19.9m - 25.6$	$-5.4m + 12.6$
	Alcohols	$-21.7m - 112$	$-6.9m - 17.1$
	Alkylamines	$-19.6m - 67$	$-5.6m + 37$

[a] For alkanes $m = 18–20$, for 1-alcohols $m = 13–20$, and for alkylamines $m = 12–20$. These equations describe the linear regressions shown in bold lines in Fig. 3. The slopes of the lines for the three classes of compounds are not constrained and the small variation shows the error of the estimates.

alkanes and alcohols were recently reviewed [17]. Linear models are good approximations of $\Delta_{\text{hyd}}G$. Alkanes are the most resistant to hydration with an unfavorable $\Delta_{\text{hyd}}G$. Alcohols and alkylamines are similarly hydratable with a slightly favorable $\Delta_{\text{hyd}}G$ because they can form hydrogen bonds with water. A methylene group contributes unfavorably approximately 0.72 kJ/mol to $\Delta_{\text{hyd}}G$.

2.3. Gibbs free energy of aggregation ($\Delta_{agg}G$)

The Gibbs free energy of aggregation quantitatively characterizes the hydrophobic interactions in an aqueous solution. Thus it is a very important parameter to determine unambiguously. Unfortunately, it is dependent on the reference state or the concentration of the hydrophobic compound. There is a number of such standard states used to define Gibbs energies and entropies of aggregation, among which the most popular are molar [17] and mole fraction [2,6] standard states. Here we list the values as actually measured at different concentrations. In the preceding articles we described this for alkylamine aggregation [13]. Fig. 2 shows $\Delta_{\text{A}}G$ for the molar standard state, and Fig. 3 shows $\Delta_{\text{agg}}G$ for 2-mM solutions and include our experimental datapoints. The actual value of $\Delta_{\text{agg}}G$ without the reference state or concentration is meaningless. $\Delta_{\text{A}}G$ is equal to the ΔG of dissolution for molar standard state, but with opposite sign. There is a wealth of solubility data for alkanes and alcohols in the literature, but relatively little for alkylamines. The free energies of dissolution can also be estimated from octanol–water partition coefficients (K_{ow}) using the standard relationship $\Delta G = -RT\ln K_{\text{ow}}$, where R is the universal gas constant, and T is the absolute temperature in Kelvin.

Linear models represent data well within the error of the experiment. Models are linearly extendable for the solids as shown by our experimental datapoints in Fig. 3. The methylene group contribution is highly favorable and equal to -3.58 kJ/mol. This energy by definition exactly compensates the sum of vaporization and hydration energies ($2.86 + 0.72 = 3.58$). Saturated hydrocarbon chains dislike being in water so much, that it is easier to vaporize them than to dissolve them in water. Addition of one functional group, however, makes such hydrocarbon much more soluble. Interestingly, the amino group interacts with water stronger than the hydroxy group. This difference is not due to the fact that amines protonate to a little extent upon their dissolution in water. Alkylamine dissolution experiments were carried out in 0.1 M NaOH where there is practically no protonation upon dissolution. Furthermore, alkylamine solubilities determined from the $\mathrm{p}K_{\mathrm{a}}$ shift nicely follow the straight line (Fig. 3). Our analysis separates protonation and aggregation equilibria [13].

2.4. Enthalpy of vaporization ($\Delta_{vap}H$) and condensation ($\Delta_{con}H$)

Compilations of the enthalpy of vaporization appear to be the most precise and accurate of all thermodynamic parameters in this study. Enthalpies of vaporization for alkanes from pentane to eicosane ($C_{20}H_{42}$) fit to a nearly perfect straight line. Some data were correlated and smoothed, however [19]. $\Delta_{\mathrm{vap}}H$ for methane could not be experimentally measured because the critical temperature of methane is 190.58 K, below the standard temperature of 25°C. $\Delta_{\mathrm{vap}}H$ for ethane, propane, and butane fall significantly below the straight line that best describes other alkanes. The methylene group contributes 4.95 kJ/mol of unfavorable enthalpy to $\Delta_{\mathrm{vap}}H$ (liquid) and approximately 8.9 kJ/mol to $\Delta_{\mathrm{vap}}H$ (solid). It is one of the largest methylene group contribution of all processes and thermodynamic parameters in this study. Similarly to the $\Delta_{\mathrm{vap}}G$, alcohols have the largest and alkanes the smallest $\Delta_{\mathrm{vap}}H$ with alkylamines in the middle.

However, most data were obtained from the T-dependence of the Gibbs free energy, and most estimation methods rely on the vapor pressure–temperature curve [18]. Such a method hides the large enthalpy of fusion that evolves upon condensing gaseous compound into a solid. This discrepancy is emphasized in Fig. 3 ($\Delta_{\mathrm{con}}H$). Datapoints were obtained by van't Hoff analysis and extend linearly beyond the chain length where the solid phase is formed. Directly observed enthalpies should follow the lines that account for $\Delta_{\mathrm{fus}}H$.

2.5. Enthalpy of hydration ($\Delta_{hyd}H$)

Enthalpies of hydration of gaseous alkanes, alcohols, and alkylamines are negative (favorable), with the methylene group contribution of -3.70 kJ/mol (liquid). Alcohols are nearly indistinguishable from amines, and alkanes have significantly less favorable $\Delta_{\mathrm{hyd}}H$. Notice that $\Delta_{\mathrm{hyd}}H$ compensates for 75% of the $\Delta_{\mathrm{vap}}H$ thus leaving a small contribution from $\Delta_{\mathrm{agg}}H$. Linear models well approximate $\Delta_{\mathrm{hyd}}H$ within the error of measurement.

2.6. Enthalpy of aggregation ($\Delta_{agg}H$)

Before microcalorimeters became available, the $\Delta_{\mathrm{agg}}H$ or ΔH of dissolution was measured by the dependence of solubility on temperature and employing the van't Hoff relationship. This method introduced relatively large errors, thus making it appear that the data can be accurately represented by a straight line as shown in Fig. 2. When calorimetric measurements of the heats of dissolution became available it became obvious that the enthalpy of dissolution does not depend exactly linearly on the aliphatic chain length for compounds with short chains. For example, ΔH of dissolution is non-linear between methanol and propanol [20]. A linear model well approximated the behavior of alcohols with chain lengths greater than four carbon atoms with the contribution of a methylene group of -1.54 kJ/mol. The linear fit with the constraints of the thermodynamic cycle including data for methanol through propanol gave the contribution of methylene group to the $\Delta_{\mathrm{agg}}H$ of -1.25 kJ/mol (liquid only). The discrepancy between the two values is relatively insignificant. However, it shows that a linear model does approximate ΔH of dissolution of short chain compounds.

Enthalpy of aggregation into a solid precipitate could only be determined calorimetrically. Filled circles and the extended dashed line in Fig. 3 ($\Delta_{\mathrm{agg}}H$) show our experimental results for alkylamine aggregation obtained by titration calorimetry [14]. There is a significant discrepancy between the model (bold line) and the linear fit of calorimetric data (dashed line). The measured enthalpies of decylamine and undecylamine were approximately zero, significantly below the expected values (extended solid line). This discrepancy indicates that the fusion enthalpy of alkylamines in aqueous solution may be significantly lower than expected for the pure alkylamines. However, the methylene group contribution to $\Delta_{\text{agg}} H$ (solid) was approximately -5.25 kJ/mol.

Contrary to the Gibbs free energy of aggregation, the enthalpy of aggregation is independent of the standard state or concentration. In other words, if 1 mol of a compound aggregates, the enthalpy is always the same, no mater at which concentration the experiment was carried out. This property of enthalpy makes it a convenient thermodynamic parameter to be used for functional group additivity purposes.

2.7. Entropy of vaporization ($T\Delta_{vap}S$) and condensation ($T\Delta_{con}S$)

Entropy changes for all three processes were calculated by subtracting linear models for the Gibbs free energies from linear fits for the enthalpies ($T\Delta S = \Delta H - \Delta G$). Thus, entropies carry both errors and uncertainties of enthalpy and Gibbs free energy. Furthermore, there is no 'direct' way to measure ΔS. Therefore, the entropies are least reliable of the three parameters.

Entropies of vaporization ($T\Delta_{\text{vap}}S$) are highly positive (favorable), since molecular disorder increases upon vaporization and additional degrees of freedom are gained. The contribution of the methylene group is equal to 2.09 kJ/mol (liquid) and 6.09 kJ/mol (solid) for all three classes of compounds. Alcohols have the most positive and alkanes the least positive $T\Delta_{\text{vap}}S$. This difference reflects the fact that alcohols break the greatest number of interactions and gain the most degree of freedom upon vaporization.

2.8. Entropy of hydration ($T\Delta_{hyd}S$)

Entropies of hydration $T\Delta_{\text{hyd}}S$ are strongly negative and unfavorable for the hydration reaction. Their magnitudes exceed entropies of vaporization. $T\Delta_{\text{hyd}}S$ are nearly identical for all three classes of compounds. The contribution of the methylene group is strongly unfavorable and equal to -4.42 kJ/mol (liquid). This value reflects how much freedom an aliphatic gaseous molecule loses upon hydration by water and perhaps even more importantly, how much water is structured around the dissolved aliphatic molecule.

2.9. Entropy of aggregation ($T\Delta_{agg}S$)

Entropy of aggregation in an aqueous solution is important for the understanding of the hydrophobic effect. Similarly to the Gibbs free energies, and contrary to the enthalpies, the entropies of aggregation are dependent on the standard reference state or the concentration. Entropies in Fig. 2 are shown for the 1-M standard state and those in Fig. 3 are calculated for an experiment where the starting concentration of an aggregating compound is 2 mM.

$T\Delta_{\text{agg}}S$ appears to be positive for all three classes of compounds (Fig. 2). However, at a low concentration the entropies of aggregation of some liquid and all solid compounds are negative at 25°C. The contribution from the methylene group for liquids is favorable and equal to 2.33 kJ/mol, while for solids it is unfavorable and equal to -1.67 kJ/mol. The favorable contribution of $\Delta_{\text{agg}}H$, as described above, is smaller and equal to -1.25 kJ/mol of methylene groups (liquid). Thus, the aggregation of aliphatic chains in water may be partially driven by the gain in entropy or opposed by the loss in entropy depending on the phase, concentration, and the chain length of the aggregate.

2.10. Heat capacity of vaporization ($\Delta_{vap}C_p$) and condensation ($\Delta_{con}C_p$)

Heat capacity describes the dependence of enthalpy on temperature. $\Delta_{\text{vap}}C_{\text{p}}$ is approximately equal to zero, when compared to hydration and aggregation, for alkanes, alcohols, and alkylamines in this study. The heat capacity of aliphatic chains slightly decreases upon vaporization indicating a small loss in enthalpy fluctuations. The contribution of the methylene group is relatively small, negative, and equal to -7.0 J/(mol-K) (liquid). The heat capacity of solid compound vaporization should include the heat capacity of fusion. There is little literature data on the heat capacity of long aliphatic compounds, therefore we excluded heat capacity from Fig. 3.

2.11. Heat capacity of hydration $(\Delta_{hyd}C_p)$

The heat capacity of alkane and alcohol hydration is highly positive. The contribution of the methylene group is equal to 62.0 J/(mol-K) (liquid). This large increase in the heat capacity of aliphatic chains upon hydration is explained by the behavior of water molecules located near aliphatic chains [21].

2.12. Heat capacity of aggregation $(\Delta_{agg}C_p)$

Heat capacity of aggregation is highly negative. The methylene group contribution is equal to −55.0 J/(mol-K). This property is even considered a 'signature' of the hydrophobic effect. A strongly negative heat capacity is observed, for example, when hydrophobic interactions are formed during protein folding [22]. Despite its importance, the heat capacity is determined from the enthalpy dependence on temperature (from ITC), and not 'directly' by differential scanning calorimetry (DSC), since it is difficult to carry out DSC experiments on aqueous aggregate suspensions. It was shown that deriving enthalpy from the Gibbs free energies lead to large errors and uncertainties. Similar dangers are associated with the derivation of the heat capacity from enthalpy, especially if the enthalpy was derived from the Gibbs free energy.

2.13. Functional group contributions

The summary of the functional group contributions to the thermodynamics of all three processes is given in Table 3. These equations can be applied for liquid and solid compounds. At 25°C the

Table 3
The methylene group contributions to the thermodynamics of several processes, and comparison between methyl, hydroxy, and amino terminal group contributions at 25°C

Thermodynamic parameter	Group	Vaporization (liquid)	Condensation (liquid)	Hydration	Aggregation (liquid)	Aggregation (solid)	Condensation (solid)
ΔG (kJ/mol)	CH_2	2.86	−2.86	0.72	−3.58	−3.58	−2.86
	$CH_3 \rightarrow OH$[a]	10.44	−10.44	−29.04	18.60	18.60	−10.44
	$CH_3 \rightarrow NH_2$[b]	4.04	−4.04	−27.61	23.57	23.57	−4.04
ΔH (kJ/mol)	CH_2	4.95	−4.95	−3.70	−1.25	−5.25	−8.99
	$CH_3 \rightarrow OH$[a]	25.50	−25.50	−30.20	4.70	4.79	−25.4
	$CH_3 \rightarrow NH_2$[b]	8.50	−8.50	−28.20	19.70	18	−9.2
$T\Delta S$ (kJ/mol)	CH_2	2.09	−2.09	−4.42	2.33	−1.67	−6.09
	$CH_3 \rightarrow OH$[a]	15.06	−15.06	−1.16	−13.90	−13.8	−15.0
	$CH_3 \rightarrow NH_2$[b]	4.46	−4.46	−0.59	−3.87	−5.6	−5.2
ΔS (J/mol-K)	CH_2	7.01	−7.01	−14.82	7.81	−5.60	−20.4
	$CH_3 \rightarrow OH$[a]	50.51	−50.51	−3.89	−46.62	−46.3	−50.3
	$CH_3 \rightarrow NH_2$[b]	14.96	−14.96	−1.98	−12.98	−18.8	−17.4
ΔC_p (J/mol-K)	CH_2	−7.0	7.0	62.0	−55.0	–	–
	$CH_3 \rightarrow OH$[a]	−19	19	−136	−155	–	–
	$CH_3 \rightarrow NH_2$[b]	−26	26	–	–	–	–

Condensation and aggregation into solid and liquid phases are compared.

[a] Effect of the terminal methyl group change into hydroxyl group.

[b] Effect of the terminal methyl group change into amino group.

following compounds are liquids: alkanes $m = 5–17$, alkanols $m = 1–12$, and alkylamines $m = 3–11$. To obtain aggregation or condensation thermodynamics for solid compounds, the energy of fusion should be added. However, this is an approximate estimate for aqueous solutions, and the fusion temperature is generally different than 25°C. If this difference is significant then the heat capacity should be used to estimate the enthalpy or entropy of fusion at 25°C. The methylene group contribution is easily determined from the slope of thermodynamic parameter dependence on the aliphatic chain length. The slopes in Tables 1 and 2 are equal to methylene group contributions listed in Table 3.

Energetics of the terminal methyl, amino, and hydroxy groups are also compared to each other. Their absolute contributions cannot be accurately determined without varying their number. Therefore we only compare amino and hydroxy groups to the methyl group. For example, we want to compare aggregation Gibbs free energy of heptane and hexanol at 25°C. Both compounds have nearly the same molecular volume and molecular weight. The only difference is that the terminal methyl group of heptane is changed into the hydroxy group of hexanol. The Gibbs free energy of aggregation for hexanol is 18.60 kJ/mol greater than that of heptane (Table 3). In molecular terms, this energetics is explained by the formation of hydrogen bonds between hexanol and water making it unfavorable to aggregate as compared to heptane.

Despite an apparent good match between experimental data and the linear models we should keep in mind that linear models are only the first empirical approximation to estimate thermodynamic parameters. Very short aliphatic chains (1–4 carbon atoms) often behave quite differently than expected from linear approximations. For example, the enthalpies of vaporization of short alkanes are clearly non-linear. Most notably, the $\Delta_{\mathrm{vap}}H$ of methane is not 6.8 kJ/mol as the linear model predicts, but it cannot even be measured or estimated because of the critical point issue as discussed above. The experimental $\Delta_{\mathrm{vap}}H$ of ethane is 5.16 kJ/mol and linear model would estimate 11.75 kJ/mol. However, from pentane through eicosane the discrepancy between the model and experimental data does not exceed 0.1 kJ/mol.

Similarly, $\Delta_{\mathrm{agg}}H$ of alcohols from methanol through butanol does not follow the linear model [20]. However, the authors say that linear models perfectly predict $\Delta_{\mathrm{agg}}H$ of alcohols from pentanol through octanol at four tested temperatures. The equation at 25°C in this reference was $\Delta_{\mathrm{agg}}H = -1.54m + 15.67$. Our best fit for alcohols (including methanol through butanol) gave $\Delta_{\mathrm{agg}}H = -1.25m + 12.70$. The discrepancy is significant due to non-linearity of the first four homologous alcohols and again reminds that the linear model is valid only for long chain alcohols.

Despite the above mentioned limitations of the linear models, sometimes they help question the accuracy of experimental data. For example, tabulated data on long chain (greater than 12 carbon atoms) alkane solubility may be unreliable [10]. The databook lists the solubilities of all hydrocarbons from 13 through 20 carbon atoms to be between 0.1 and 0.2 nM. The difference between these values is much smaller than expected from the linear dependence of $\Delta_{\mathrm{agg}}G$ on the number of carbon atoms as first determined by McAuliffe [2] and plotted by Tanford [23] for hydrocarbons from four to eight carbon atoms. It is highly unexpected that the slope of $\Delta_{\mathrm{agg}}G$ on the number of carbon atoms would change so much to explain solubilities between 0.1 and 0.2 nM for the long chain alkanes. Actually, these solubilities are so low that the existing experimental methods may not be able to measure them accurately.

2.14. Thermodynamic parameters at elevated temperatures

We now examine how the Gibbs free energies and enthalpies of alkane and alcohol vaporization, hydration, and aggregation depend on temperature in the biologically relevant temperature range of approximately 15–65°C.

The standard way of calculating thermodynamic parameters at various temperatures is by using the following equations [24]:

$$\Delta H^{\circ}(T_2) = \Delta H^{\circ}(T_1) + \Delta C_{\mathrm{p}}^{\circ}(T_2 - T_1)^{\circ} \quad (1)$$

$$\Delta S^{\circ}(T_2) = \Delta S^{\circ}(T_1) + \Delta C_{\mathrm{p}}^{\circ}\ln(T_2/T_1) \quad (2)$$

$$\begin{aligned}\Delta G^{\circ}(T_2) = \Delta G^{\circ}(T_1) + \Delta C_{\mathrm{p}}^{\circ}(T_2 - T_1 \\ - T_2\ln(T_2/T_1)) \\ - \Delta S^{\circ}(T_1)(T_2 - T_1)\end{aligned} \quad (3)$$

These standard equations assume that heat capacity is known and that it is independent of temperature in the temperature range of interest. Furthermore, the heat capacity should be measured calorimetrically and not as a second derivative of the Gibbs free energy. In reality, however, the latter method is frequently used. We argue that such analysis may lead to erroneous conclusions. Consider the aggregation of tridecylamine as an example. Fig. 4 shows the temperature dependence of Gibbs free energy, enthalpy, and entropy of aggregation of tridecylamine. Compare the enthalpies of aggregation that were measured by the titration calorimetry (left panel) at a constant temperature [14] with the estimate by van't Hoff analysis (right panel) [13]. It appears first that the $\Delta_{\mathrm{agg}}H_{\mathrm{vH}}$ data is more accurate and less scattered than $\Delta_{\mathrm{agg}}H_{\mathrm{cal}}$. However, after analyzing the error of estimating $\Delta_{\mathrm{agg}}G$ as a function of temperature, we conclude that the error is greater than the whole scale of Fig. 4. Even the sign of the enthalpy at any temperature could not be determined unambiguously. This is because the $\Delta_{\mathrm{agg}}G$ variation with temperature is so small. Furthermore, the $\Delta_{\mathrm{agg}}H_{\mathrm{vH}}$ does not contain any information about the phase change, i.e. that tridecylamine aggregates as liquid at $t > 43°\mathrm{C}$ and as solid at $t < 29°\mathrm{C}$. The exact temperature of the phase change in aqueous suspension could not be precisely determined. The range may actually be wider in aqueous suspension than for pure tridecylamine.

Another reason why the Gibbs free energy should not be used to obtain derived $\Delta_{\mathrm{agg}}H_{\mathrm{vH}}$ is because $\Delta_{\mathrm{agg}}G$ depends on concentration. Even

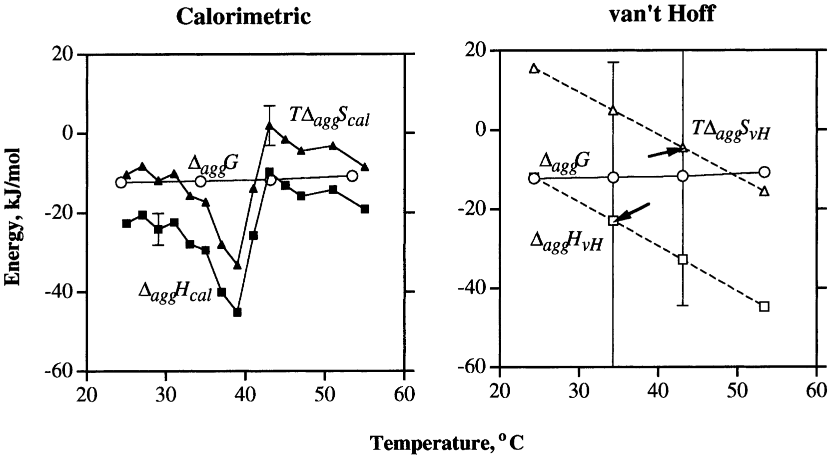

Fig. 4. Dependence of tridecylamine aggregation thermodynamics on temperature. Solid lines connect data obtained by calorimetric analysis (cal, left panel), and the dotted lines connect data obtained by van't Hoff analysis (vH, right panel). Symbols represent our obtained experimental datapoints: ■ — calorimetric enthalpy of aggregation $\Delta_{\mathrm{agg}}H_{\mathrm{cal}}$ [14], ○ — Gibbs free energy of aggregation from pK_{a} shift $\Delta_{\mathrm{agg}}G$ [13], □ — van't Hoff enthalpy of aggregation ($\Delta_{\mathrm{agg}}H_{\mathrm{vH}}$) derived from the $\Delta_{\mathrm{agg}}G$ dependence on temperature, ▲ — calorimetric entropy of aggregation ($T\Delta_{\mathrm{agg}}S_{\mathrm{cal}}$) obtained by subtracting $\Delta_{\mathrm{agg}}G$ from $\Delta_{\mathrm{agg}}H_{\mathrm{cal}}$, △ — van't Hoff entropy of aggregation ($T\Delta_{\mathrm{agg}}S_{\mathrm{vH}}$) derived from the temperature dependence of $\Delta_{\mathrm{agg}}G$. Tridecylamine aggregates as a solid at temperatures below 29°C and as liquid above 43°C. This distinction is visible only in the calorimetric analysis of the aggregation reaction. The standard errors and uncertainties of calorimetric and van't Hoff analyses are compared (arrows show the points for which error bars are given).

though the $\Delta_{\mathrm{agg}}H_{\mathrm{vH}}$ values are concentration-independent when derived from $\Delta_{\mathrm{agg}}G$ that were obtained at various concentrations, it adds uncertainty to the analysis of the thermodynamics. Entropy also should not be derived from the Gibbs free energy. The uncertainties are much smaller when the entropy is obtained by subtraction of $\Delta_{\mathrm{agg}}G$ from the $\Delta_{\mathrm{agg}}H_{\mathrm{cal}}$. For example, the $T\Delta_{\mathrm{agg}}S_{\mathrm{vH}}$ was unreasonable at 25°C. Van't Hoff analysis would lead to a conclusion that the entropy change is positive, significantly driving the aggregation reaction. Instead, the calorimetric analysis shows that entropy is actually lost during such aggregation reaction and disfavors aggregation. The difference between the two entropy values in Fig. 4 could be largely accounted by the phase change. However, we should not be misled by the apparent high accuracy of the $T\Delta_{\mathrm{agg}}S_{\mathrm{vH}}$. It is only a coincidence in Fig. 4 that $T\Delta_{\mathrm{agg}}S_{\mathrm{vH}}$ is similar to $T\Delta_{\mathrm{agg}}S_{\mathrm{cal}}$ at higher temperatures. The error of $T\Delta_{\mathrm{agg}}S_{\mathrm{vH}}$ is so large that it spans the entire Fig. 4. This is because the $\Delta_{\mathrm{agg}}G$ determination had a standard error of approximately 0.9 kJ/mol and the value of $\Delta_{\mathrm{agg}}G$ varied by 1.5 kJ/mol in the temperature range of 25–55°C. Attempts to derive entropy from such experimental data could easily lead to erroneous conclusions. Because of insufficient amount of calorimetric data we did not extend linear analysis (as in Fig. 2) to higher temperatures.

2.15. Molecular interpretation and application to systems of biological significance

Based on our results in the previous two articles [14], we believe that several important assumptions about the hydrophobic interactions should be reconsidered and new molecular models are necessary to explain them. First, it is important to distinguish between the liquid and solid phases that are formed after the binding reactions. For example, if a membrane bilayer is in a solid form, then the exothermic enthalpy of the membrane formation is likely to be significantly greater as compared to a liquid membrane. The energetic reason for a formation of such membrane is likely to change from entropic to enthalpic upon the fusion of the membrane. Similarly, in the case of lipid binding to protein, if the lipid remains liquid-like in the complex, then the enthalpic contribution is expected to be significantly smaller than if the lipid in the complex becomes solid-like. So the question of phase is very important for the anticipation of the binding energetics.

Second, even though we showed that solid aggregates of aliphatic chains form due to favorable enthalpy and unfavorable entropy, it does not essentially change the conventional view of the hydrophobic effect. The favorable enthalpy largely evolves due to the close regular packing of aliphatic chains and the formation of many van der Waals contacts between molecules, which cannot be formed in the liquid phase. This also leads to the large loss of entropy due to the restriction of aliphatic chains upon fusion. But the loss is due to fusion and not due to binding itself.

Third, the methylene group contributions to the energetics of alkyl chain binding to each other (in water or in gas phase) are suitable for application to complicated systems, such as alkylamine binding to DNA (our preliminary data). The contributions are likely to be applicable to such systems as biomembranes and lipid binding to proteins. These contributions quite precisely describe the behavior of methylene group in long aliphatic chains with respect to aqueous solution, and liquid and solid phases. Contrary to earlier suggestion that solubility and partitioning experiments significantly underestimate the magnitude of the hydrophobic effect [25], we believe that the addition of a CH_2 group to an alkane increases its chemical potential by approximately 3.58 kJ/mol [0.856 kcal/mol, 124 J/(mol-Å^2)]. These values are consistent with conventional solubility and partition experiments.

Fourth, it appears easier to provide molecular interpretation to observed enthalpies and heat capacities than to Gibbs free energies and entropies, because only molar enthalpies and heat capacities are independent of concentration used in the experiment. Therefore it should also be emphasized that such phenomena as 'enthalpy–entropy compensation' or 'enthalpy-driven reaction' should be carefully analyzed in terms of the concentrations at which such phenomena are observed.

3. Conclusions

1. Ionizable group pK_a shifts may occur due to aggregation. Such shifts in alkylamines provide a convenient tool to study hydrophobic aggregation.
2. Direct observation of hydrophobic aggregation by isothermal titration calorimetry yields energetic parameters that are significantly more precise than van't Hoff analysis of solubility and partitioning data.
3. Determination of the phase of the bound complex is essential in estimating binding energetics. Aggregation into a solid phase was found to evolve significantly more exothermic enthalpy than aggregation into a liquid precipitate. It is impossible to distinguish liquid and solid phases in van't Hoff analysis.
4. Enthalpy and heat capacity are independent of experimental concentration, while entropy and Gibbs free energy are dependent on concentration. In other words, entropy and Gibbs free energy of aggregation depend on the reference state which is totally arbitrary. For example, $\Delta_{agg}H$ of heptylamine is equal to approximately 19 kJ/mol at both 2-mM and 1-M concentrations, while $\Delta_{agg}G$ of heptylamine is equal to approximately $+10$ kJ/mol at 2 mM and -6 kJ/mol at 1-M concentration. Observable properties, such as pK_a shift, will be affected by the concentration.

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