Enthalpically Driven Cyclophane-Arene Inclusion Complexation: Solvent-Dependent Calorimetric Studies

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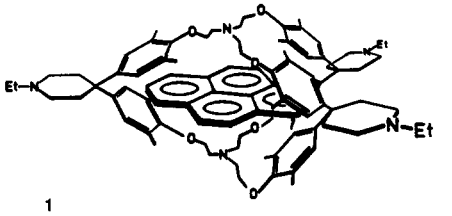
Abstract: The thermodynamic quantities characterizing tight apolar complexation processes have been investigated in a calorimetric study with cyclophanes as the receptors and benzene derivatives as well as pyrene as the substrates. In water, the inclusion complexation of benzene derivatives is strongly exothermic, accompanied by an unfavorable entropic term. A large part of the favorable enthalpy change results from solvent-specific contributions. Enthalpic data obtained from the van't Hoff analysis of ^{1}H NMR titrations are qualitatively in good agreement with the $\Delta H°$ values measured directly by calorimetry. Larger uncertainties in the van't Hoff data are a result of changes in the heat capacity $\Delta C_p°$. Negative heat capacity effects are characteristic for all inclusion processes in water and in methanol investigated in this study. The largest negative $\Delta C_p°$ values are measured for the complexation of benzene derivatives that possess a molecular dipole and hydroxy substituents and therefore interact strongly with their solvent cages. A calorimetric study in 12 solvents of different polarities shows that water is not special in providing an enthalpic driving force for apolar complexation. In all the solvents studied, the formation of a cyclophane-pyrene inclusion complex is enthalpically driven. The exothermicity generally increases from apolar solvents, to dipolar aprotic solvents, to protic solvents. A strong isoequilibrium relationship (R = 0.954) correlates the enthalpy and entropy for pyrene complexation in the different environments. A closer analysis reveals that an even stronger dual isoequilibrium relationship (R = 1.00 and 0.979) is expressed in the plot of enthalpy change against change in entropy. Stronger dual isoequilibrium relationships are also seen in the plots of the complexation free energy against the enthalpy and entropy changes, compared to single isoequilibrium relationships covering all solvents.

Introduction

Molecular complexation is the key event in most biological processes.[1,2] It is therefore not surprising that the driving forces that lead to the selective binding of a substrate to a receptor in aqueous solutions have been at the center of interest in numerous recent molecular recognition studies.[3-8] In many biotic complexation events, aromatic rings, e.g., the side chains of aromatic amino acids, are tightly incorporated into lipophilic niches, clefts, or pockets of a receptor.[1a] Flat aromatic substrates intercalate between base pairs in double-stranded DNA[4] or bind to the narrow, AT-rich segments of its minor groove.[9] We and others have shown that cyclophanes with lipophilic cavities are very suitable models, giving detailed analyses into these apolar binding events in aqueous solution.[7a,10-14] Studies in our laboratories demonstrated that apolar arene binding is not limited to water but occurs in solvents of all polarity.[15] For the stability of the pyrene complex **1**, a dramatic solvent dependency was observed.



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Upon changing from water, the most polar, to carbon disulfide, the least polar among 18 solvents, complexation free energies decrease from $-\Delta G° = 9.4$ kcal mol^{-1} to $-\Delta G° = 1.3$ kcal mol^{-1}. Apolar binding strength increases regularly from apolar solvents, to dipolar aprotic solvents, to polar protic solvents, and to water. A strong linear free energy relationship exists between the free energy of formation of complex **1** and the solvent polarity parameter $E_T(30)$ of the various solvents including water. What remained unanswered in this previous study was the important question about the nature of the complexation driving force, which strengthens with increasing solvent polarity.[15]

In another, variable-temperature ^{1}H NMR study, we showed that the inclusion complexation of para-disubstituted benzene derivatives by cyclophane **2** in water is entropically unfavorable and strongly enthalpy driven.[16,17] The same complexation in methanol is much weaker mainly as a result of a less favorable enthalpic component in this solvent. With similar inclusion geometries and, hence, host-guest interactions in both solvents,[18] a large portion of the more favorable complexation enthalpy seen in water had to result from specific solvent interactions. The thermodynamic characteristics measured for tight arene complexation by **2** in water (large negative $\Delta H°$ and negative $T\Delta S°$) are in sharp contrast to those measured for processes driven by the classical hydrophobic effect. The transfer of apolar solutes

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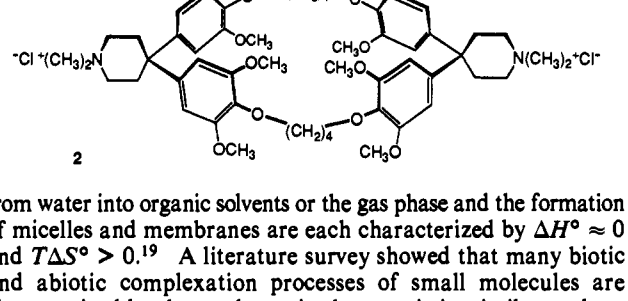

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from water into organic solvents or the gas phase and the formation of micelles and membranes are each characterized by $\Delta H^\circ \approx 0$ and $T\Delta S^\circ > 0$.[19] A literature survey showed that many biotic and abiotic complexation processes of small molecules are characterized by thermodynamic characteristics similar to those measured for the benzene complexes of cyclophane **2**. Tight binding of apolar aromatic substrates in hydrophobic pockets of enzymes and antibodies,[20] in the cavity of cyclodextrins,[21] and in the narrow AT-rich region of the DNA minor groove are all enthalpy-driven processes.[22]

This paper describes the application of microcalorimetric methods[23,24] to give direct, accurate measurement of the enthalpic driving force for the formation of benzene complexes by host **2**. The direct measurement of heats not only provides more accurate enthalpic data than the van't Hoff analysis of variable-temperature ^{1}H NMR titrations,[25] but it also allows the determination of experimentally measured heat capacity changes associated with the apolar binding processes.[17,26,27] In an unprecedented solvent-dependent calorimetric study, the thermodynamic driving force for the formation of complex **1** is determined in a total of 12 solvents of different polarity.[28] This study was designed to investigate whether the enthalpic driving forces seen for arene binding by **2** are a result of water or whether apolar complexation is also exothermic in organic solvents.[29]

Experimental Section

All enthalpic values were determined by using a Tronac 558 isoperibol/isothermal microcalorimeter. The runs were performed under isoperibol conditions where the surrounding bath temperature is held constant, and the titrant was added continuously.

The calorimeter consists of a 60-L water bath that maintains a constant-temperature environment within $\pm 1 \times 10^{-4}$ °C. The actual experimental accuracy is ±0.05 °C, which is the accuracy of the digital thermometer used to set the bath temperature. An insert containing the reaction vessel and buret allows the titrant and titrate to be fully submerged into the bath during temperature equilibration and also during a run. A 4-mL Dewar flask serves as the reaction vessel and is silver coated to reduce radiation losses. The buret delivers the titrant continuously, dispensing a total volume that is less than 10% of the reaction vessel volume to minimize changes in the system's heat capacity. A typical delivery time is 2 min with a volume of 0.3 mL. The system's detection limit is approximately 1×10^{-5} cal, depending upon the Dewar flask's volume. We typically measure 10^{-3}–10^{-2} cal for runs in organic solvents and 10^{-4}–10^{-3} cal for runs in aqueous solutions, and the thermal noise is on the order of 3×10^{-5} °C, which is below our usual temperature change of about 8×10^{-3} °C. The observable heat in water is smaller since the concentrations of the components are smaller for solubility reasons and since water has a large heat capacity. A digital voltmeter measures the heat, and the data is analyzed and stored via a computer.

To achieve a relatively large amount of heat, high concentrations of both host and guest were needed especially for complexations that have small equilibrium constants. An equally important criterion for obtaining meaningful enthalpic values is that the heat produced upon complexation is significantly larger than the heat of dilution. Therefore, cosolvents could not be used since the majority of heat produced came from the heat of dilution in these studies. For complexations in organic solvents that gave the largest exothermicity, e.g., $-\Delta H^\circ > 6$ kcal mol^{-1}, the titration heat was larger than the heat of dilution by approximately a factor of 10. For water and the other organic solvents, there was approximately a factor of 2 separating the heat produced by the titration run compared to the dilution run. An example of the high concentrations needed to obtain this factor of 2 is given by the formation of complex **1** in benzene, in which the total host concentration was 4.14×10^{-3} mol L^{-1} and the guest concentration in the titrant was 1.06×10^{-1} mol L^{-1}. It should be noted that performing heat of dilution runs for each specific set of host, guest, solvent, and temperature is critical since solutions can exhibit a wide range of heats of dilution including both exothermic and endothermic values.

The Tronac 558 microcalorimeter uses an electrical calibration to convert the electrical signal into a heat quantity. An electrical resistance heater resides inside the reaction vessel, and the total power applied for calibration is chosen to closely match the power produced by the reaction. This calibration process is performed by a computer program before and after each titration and then averaged to give the "effective heat capacity" of the system. The program produces a thermogram and its corresponding power curve, which is a plot of the rate of voltage change against time. The heat produced is determined by integrating the power curve.

To obtain the complexation enthalpy, the heat of complexation needs to be separated from the total heat produced during the titration, which includes the heat of complexation, the heat loss to the surroundings, the heat associated with any temperature difference between the titrant and the titrate, and the heat of dilution. The heats that result from dilution and nonchemical processes are corrected for by performing multiple heat of dilution runs. This entails titrating the titrant into pure solvent under conditions similar to those of the complexation run, which includes using approximately the same concentration of titrant, volume in the Dewar flask, and equilibration time in the bath. The titrate's heat of dilution is negligible since its concentration is less than that of the titrant and since its relative volume change is much smaller. No specific correction is made for possible temperature differences between the titrant and the titrate, since both titrate and titrant are allowed to equilibrate to the same bath temperature. Once the heat of complexation has been determined, the change in enthalpy is obtained by dividing the measured heat by the molar amount of host-guest complex formed. Molar host-guest complex concentrations are calculated from known host-guest association constants K_a. For the complexes in this study, the K_a data had previously been obtained from accurate ^{1}H NMR and optical (opt) titrations.[15,16] These association constants are in very good agreement[25] with K_a values obtained in the evaluation of calorimetric (cal) titrations using a binding program provided by Tronac. For the formation of complex **1**, the free energies ΔG° at 303 K were measured in CH_3OH as −6.4 kcal mol^{-1} (cal and opt), in Me_2SO as −3.9 kcal mol^{-1} (cal and NMR), in acetone as −4.4 kcal mol^{-1} (cal) and −4.3 kcal mol^{-1} (NMR), in *N,N*-dimethylacetamide (DMA) as −4.4 kcal mol^{-1} (cal), and in DMA containing 10% (v/v) Me_2SO as −4.2 kcal mol^{-1} (NMR). With known K_a values, molar amounts of host-guest complex are easily calculated from the initial concentration of host and guest along with the titration rate and the solution volume in the Dewar flask.

An example of a typical run to determine enthalpies of complexation consisted of preparing a 1×10^{-3} M host (or guest) solution and placing approximately 3.5 mL into the Dewar flask. The buret was filled with a 2×10^{-2} M guest (or host) solution. For other titrations, similar titrate to titrant ratios were chosen, always in concentration ranges that ensured a high degree of complexation. In all runs, the volume of titrant was

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Table I. Thermodynamic Characteristics from Variable-Temperature ^{1}H NMR Titrations and Calorimetry for the Formation of Complexes between Cyclophane **2** and 1,4-Disubstituted Benzene Guests in Water and Methanol

guest	^{1}H NMR data		calorimetric data[a]				
	$\Delta G°_{293\ K}$,[b] kcal mol^{-1}	$\Delta H°_{293\ K}$, kcal mol^{-1}	$\Delta H°_{293\ K}$, kcal mol^{-1}	$\Delta H°_{299\ K}$, kcal mol^{-1}	$\Delta H_{310\ K}$, kcal mol^{-1}	$\Delta C_p°$,[c] cal mol^{-1} K^{-1}	$T\Delta S°_{293\ K}$, kcal mol^{-1}
Complexes in D_2O or H_2O[f]							
dimethyl *p*-benzenedicarboxylate	−6.81	−10.7 ± 1.0	−11.8	−11.9	−12.8	−60	−5.0
p-nitrotoluene	−6.01	−9.6 ± 3.0	−8.1	−8.8	−9.0	−50	−2.1
p-tolunitrile	−6.01	−9.8 ± 2.5	−8.1	−8.5	−9.2[e]	−70	−2.1
p-nitrophenol[d]	−5.86	−11.7 ± 1.5	−10.5	−10.9	−12.8[e]	−130 ± 20	−4.6
p-dimethoxybenzene	−5.38	−10.2 ± 2.5	−10.0	−10.1	−10.4[e]	−20	−4.6
p-xylene	−5.33	−7.4 ± 1.0	−7.2	−7.5	−7.6	−20	−1.9
p-dicyanobenzene	−5.23	−9.5 ± 1.0	−10.3	−10.7	−10.9	−30	−5.1
p-dinitrobenzene	−5.22	−9.5 ± 1.0	−9.8	−9.9	−10.4[e]	−40	−4.6
p-cresol	−4.71	−9.1 ± 1.0	−10.6	−11.0	−12.4	−110 ± 50	−5.9
hydroquinone	−3.69	−10.5 ± 1.0	−10.3	−11.2	−11.5	−60	−6.6
Complexes in CD_3OD or CH_3OH[g]							
p-dicyanobenzene	−1.86	−4.2 ± 1.5					
p-dimethoxybenzene	−1.20	−4.4 ± 1.5	−3.7 (293 K)	−3.6 (287 K)	−2.0 (281 K)		−2.5

[a] Calorimetric enthalpic data have an uncertainty of ±0.2 kcal mol^{-1}. [b] Free energies of complexation have an uncertainty of ±0.07 kcal mol^{-1} for complexes in D_2O and ±0.17 kcal mol^{-1} for complexes in CD_3OD. [c] Approximation numbers due to strong temperature dependence of $\Delta C_p°$. [d] $\Delta C_p°$ obtained by considering two additional $\Delta H°$ values: −10.0 (288 K) and −11.5 (303 K). [e] Measured at 308 K. [f] ^{1}H NMR in D_2O; calorimetry in H_2O. [g] ^{1}H NMR in CD_3OD; calorimetry in CH_3OH.

chosen to generate, at titration end point, less than 10% change in volume of the Dewar flask. The entire titration insert was lowered into the bath and allowed to equilibrate to bath temperature. The equilibration time depends upon the solvent and the bath temperature, and equilibration is reached when the voltmeter stabilizes. After the run, the complexation power curve was matched to the dilution power curve, enabling an accurate subtraction of the heat of dilution from the total heat produced. The power curve was integrated in 5-s intervals, and the left integration limit was placed at the maximum height of the power curve, which usually was obtained 10 s after the buret was turned on. Generally, the first three intervals gave the maximum heat produced, since heat loss became substantial at the end of the titration, and these values were usually consistent. These heats, after subtracting the heats of dilution and dividing by the molar amount of host–guest complex formed, were averaged and gave the complexation enthalpy.

To test the accuracy and precision of the calorimeter and our methodology, enthalpies of reactions obtained from the literature were measured.[30] A variety of thermometric titrations at 298 K produced enthalpies $\Delta H°$ for the following proton ionization reactions: THAM–HCl (THAM = tris(hydroxymethyl)aminomethane) gave −11.34 ± 0.03 kcal mol^{-1} (lit.[30a] −11.36 kcal mol^{-1}). THAM—acetic acid gave −11.32 ± 0.03 kcal mol^{-1} (lit.[30b] −11.36 kcal mol^{-1}). Glycine–acetic acid gave −10.72 ± 0.05 kcal mol^{-1} (lit.[30b] −10.64 kcal mol^{-1}). These results demonstrate the high accuracy of our system. The precision was also tested by running three imidazole–acetic acid titrations, which resulted in the highly precise value of $\Delta H° = -8.78 \pm 0.04$ kcal mol^{-1} (lit.[30b] −8.79 kcal mol^{-1}). The reported[30c] complexation enthalpy of 18-crown-6 with potassium chloride in water at 298 K was also replicated accurately, giving an average error of 0.9% in three independent runs. Such test runs were repeated at different times over the period of this investigation to ensure continued accuracy in the performance of the microcalorimeter. Most complexation enthalpies reported in this study were obtained in duplicate or triplicate runs, giving an average experimental error of ±0.2 kcal mol^{-1}.

Results and Discussion

A Strong Enthalpic Driving Force Exists for the Inclusion Complexation of Benzene Derivatives by Cyclophanes in Water. When we investigated in variable-temperature ^{1}H NMR studies the thermodynamic characteristics for the inclusion complexation of para-disubstituted benzene derivatives by cyclophane **2**, the van't Hoff analysis of the data showed a large enthalpic driving force as well as unfavorable entropic terms for all binding processes in water (Table I).[18] In methanol, overall complexation strength is significantly weaker mainly as a result of a strongly reduced enthalpic term. Since the complex geometries in both solvents are very similar (^{1}H NMR), the difference in the enthalpic driving force cannot be explained by differences in host–guest interactions. A large part of the favorable enthalpic effect in water must result from specific contributions by the solvent. In view of the importance of such findings for biotic and abiotic binding processes in water and the considerable uncertainties seen for the van't Hoff analyses (Table I), despite high linear correlation coefficients between $R = 0.955$ and 0.997, we redetermined the enthalpic driving force by using direct calorimetric measurements of the heats of complexation. Table I gives the calorimetric enthalpy changes at three different temperatures. Whereas the error analysis yields uncertainties varying from ±1.0 to ±3.0 kcal mol^{-1} for the van't Hoff data, much more accurate values are obtained in the calorimetric measurements. The uncertainty in all calorimetric $\Delta H°$ values included in Table I is ±0.2 kcal mol^{-1}. Generally in this study, where large enthalpic values are measured, the results from the van't Hoff analyses of variable-temperature binding titrations are in good qualitative agreement with the direct calorimetric data.

We proposed two explanations, more favorable changes in solvent cohesive interactions and a larger gain in dispersion interactions, for the stronger enthalpic driving force for apolar complexation in water compared to methanol and other solvents.[18,31] The differences in thermodynamic characteristics displayed by the tight binding of small apolar solutes by cyclophanes, cyclodextrins, enzymes, or antibodies ($\Delta H° \ll 0$, $T\Delta S° < 0$) compared to the formation of micelles and membranes ($\Delta H° \approx 0$, $T\Delta S° > 0$) are best accounted for in terms of differences in the tightness of the association. Strong host–guest interactions are primarily responsible for the large overall enthalpic driving force that is measured in the formation of a tight molecular complex. For both micellar association and molecular complexation, the desolvation of apolar surfaces provides a favorable entropic component, which is well known as the classical hydrophobic effect.[19] However, in a tight molecular complex, the degrees of freedom of the binding partners are considerably reduced, resulting in a strong entropic loss, which partially compensates for the enthalpic driving force and masks any gain in entropy from desolvation. In the micellar and membrane association processes, a major reduction in the degrees of freedom of the interacting components does not occur. Hence, the entropically favorable desolvation processes produce a positive entropic term that is measured. The interactions between the components in micelles and membranes are less tight than in molecular com-

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plexes, resulting in smaller overall enthalpic terms for the formation of these molecular aggregates.

Clearly, apolar binding and other association processes in water may be either enthalpically or entropically driven. Unfortunately, in the past, hydrophobic bonding has often been seen uniquely as an entropically driven process. Characterizing apolar binding processes in water as being hydrophobic is acceptable as long as no specific thermodynamic quantities are implied.

With accurate thermodynamic data now available, interesting molecular recognition phenomena can be discussed. The binding of three of the guests in Table I is characterized by significantly less unfavorable entropic terms. Negative $T\Delta S°$ values of around −2 kcal mol^{-1} are obtained at 293 K for the inclusion of the nonprotic toluene derivatives *p*-nitrotoluene and *p*-tolunitrile and for *p*-xylene, whereas the other guests complex with $T\Delta S°$ values around −5 kcal mol^{-1}. With their nonpolar methyl substituents, the toluene derivatives possess a more extended hydrophobic surface than the other guests, and therefore, their entropies of desolvation should be more favorable. However, the data suggest to us that differences in the degrees of freedom of the guests in the complexes provide a better explanation for the measured entropic terms. The guests that complex with a large unfavorable entropy change possess two polar substituents that prefer, for reasons of favorable solvation, to be oriented into the aqueous solution on both sides of the cavity. This functional group solvation requirement presumably leads to a more rigid axial complex geometry and generates a large unfavorable entropy contribution. In the complexes of *p*-nitrotoluene and *p*-tolunitrile, only one polar substituent needs to reach out into the solution. This can be achieved with a less rigid complex geometry, and CPK models demonstrate numerous favorable inclusion orientations in which the polar group of the guest should be favorably solvated. No geometry constraints resulting from functional group solvation are effective in the complex of *p*-xylene.

On the other hand, another toluene derivative, *p*-cresol, shows the second largest unfavorable $T\Delta S°$ value for its inclusion by **2**. The driving force for *p*-cresol complexation, $\Delta G°$, is among the least favorable ones despite having one of the largest negative enthalpic components ($\Delta H°_{298}$ = −10.6 kcal mol^{-1}). We explain these data by the formation of a more structured complex as a result of an intermolecular hydrogen bond formed between the guest's hydroxyl group and a proximate methoxy group of the host. Such an additional binding force is clearly apparent from *p*-cresol's enthalpic term, which is ~2.5–3 kcal mol^{-1} more negative than the enthalpies measured for the other toluene derivatives or for *p*-xylene. However, the increased enthalpic driving force is not reflected in a higher free energy since it is compensated by the entropic loss resulting from the geometric constraints imposed by the additional hydrogen bond. The substantial differences between the thermodynamic characteristics for inclusion complexation of *p*-cresol and *p*-xylene are important in view that these substrates can be used as models for the aromatic side chains of the amino acids tyrosine and phenylalanine, respectively. It is noteworthy that this difference is only revealed in a tight complexation event such as the one described above but not in phase-transfer studies such as those previously reported by Tanford et al.[32]

The thermodynamic characteristics for hydroquinone inclusion provide another example of a large enthalpic gain, which as a result of large entropic compensation, is not reflected in the measured free energy. It is the weakest binding guest but has among the largest enthalpic driving force (Table I). An attractive explanation for the large favorable enthalpy for hydroquinone inclusion is the formation possibly of two hydrogen bonds to two host methoxy groups on opposite sides of the cavity. Such an additional 2-fold oriented bonding, however, imposes high geometric constraints on the complex, and this is reflected in the very large $T\Delta S°$ value of −6.6 kcal mol^{-1}.

Negative Heat Capacity Changes Accompany Cyclophane-Arene Complex Formation. Heat capacity changes $\Delta C_p° = (\partial \Delta H°/\partial T)_p$ have become an important thermodynamic quantity in the investigation of biological association processes.[26,33] They are best determined by measuring heats of association as a function of temperature in a calorimeter. The majority of protein–protein, protein–ligand, and protein–DNA interactions show large negative $\Delta C_p°$ values.[20b] These values have been used to measure the extent of temperature-induced structural changes occurring in the solvent, in the solvated free components, and in the solvated complex. Negative heat capacity changes in the range of 20–200 cal mol^{-1} K^{-1} also accompany the inclusion binding of apolar solutes by cyclodextrins in water.[27] Recently, Dougherty et al. in a statistical analysis of variable-temperature ^{1}H NMR titration data showed that negative $\Delta C_p°$ values of similar magnitude are also characteristic for the inclusion complexation by cyclophanes in water.[17] In addition, they also calculated negative heat capacity changes in chloroform, which however, were of smaller magnitude compared to those in water.

Table I shows the calorimetric enthalpy change measured at variable temperatures for the complexes of cyclophane **2**. A linear regression analysis of the plots of three enthalpic values as a function of temperature in a 20 °C interval provided the heat capacities. The $\Delta C_p°$ value for *p*-nitrophenol was obtained from a total of five enthalpies. For all complexation processes by **2** in water and in methanol, negative heat capacity changes are measured. With values of −20 to −130 cal mol^{-1} K^{-1}, their magnitudes are in the range expected from previous work.[17,27] The complexation of *p*-nitrophenol and *p*-cresol in water experiences the largest change in enthalpy with temperature, and the resulting larger $\Delta C_p°$ values have higher accuracy than the other, smaller $\Delta C_p°$ values. The uncertainties of the smaller heat capacity effects are greater for several reasons. The study was executed in the limited temperature range of 20 °C in which the K_a values of the complexes had previously been determined.[16] As discussed in the Experimental Section, the complex concentrations that are needed to transform measured heats into enthalpies are calculated from these association constants. Most importantly, for most binding processes, a strong nonlinearity exists in the plots of enthalpy change as a function of temperature.[34] This significant dependence of $\Delta C_p°$ on temperature suggested that a more accurate linear regression analysis would not be obtained by measuring heats in a larger temperature interval. Some of the $\Delta C_p°$ values in Table I had been qualitatively predicted by a statistical analysis[17] performed by Professor D. A. Dougherty on our variable-temperature ^{1}H NMR data. For example, a heat capacity effect of −153 cal mol^{-1} K^{-1} was calculated for *p*-nitrophenol complexation.[35] The changes in the heat capacity presumably are responsible for the large uncertainties in the complexation enthalpies (Table I) that we had obtained previously by using van't Hoff analyses of variable-temperature ^{1}H NMR binding data.[16]

The magnitude of $\Delta C_p°$ is strongly dependent on the nature of the guest. The largest negative values (−110 to −130 cal mol^{-1} K^{-1}) are obtained for dipolar guests with a hydroxy group (*p*-nitrophenol and *p*-cresol). A negative change in heat capacity $\Delta C_p°$ means that the difference in enthalpy $\Delta H°$ between the state of the solvated free components and the state of the solvated complex increases with temperature. The guests *p*-nitrophenol and *p*-cresol having a molecular dipole and a hydrogen-bonding group interact strongly with their solvent cage and enhance structuring. When the temperature is raised, this interaction becomes weaker and the enthalpy of the state of solvated free components rises. Assuming that the host–guest complex is more rigid and is less temperature labile than the guest–solvent cage assembly, the state of solvated complex is not raised as much with increasing temperature, and therefore, a larger negative $\Delta H°$ value is measured with increasing temperature. Correspondingly, for the complexation of guests that do not interact as strongly with water, e.g., the nonprotic symmetric guests *p*-xylene, 1,4-di-

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Table II. Enthalpic ($\Delta H°$) and Entropic ($T\Delta S°$) Contributions to the Free Energies of Formation $\Delta G°$ of Complex **1** in Solvents of Different Polarity

run	solvent	$\Delta G°$,[a] kcal mol^{-1}	$\Delta H°$, kcal mol^{-1}	$T\Delta S°$, kcal mol^{-1}
1	2,2,2-trifluoroethanol	−7.8 ± 0.1	−20.0 ± 0.2	−12.2 ± 0.2
2	methanol	−6.4 ± 0.1	−12.0 ± 0.2	−5.6 ± 0.2
3	ethanol	−6.1 ± 0.1	−11.0 ± 0.2	−4.9 ± 0.2
4	*N*-methylacetamide	−5.8 ± 0.1	−9.0 ± 0.2	−3.2 ± 0.2
5	*N*-methylformamide	−5.1 ± 0.1	−5.6 ± 0.1	−0.5 ± 0.1
6	*N*,*N*-dimethylacetamide[b]	−4.4 ± 0.1	−2.0 ± 0.4	+2.4 ± 0.4
7	acetone	−4.3 ± 0.1	−6.6 ± 0.4	−2.3 ± 0.4
8	dimethyl sulfoxide	−3.9 ± 0.2	−6.4 ± 0.2	−2.5 ± 0.2
9	*N*,*N*-dimethylformamide	−2.9 ± 0.2	−3.7 ± 0.2	−0.8 ± 0.2
10	tetrahydrofuran	−2.7 ± 0.2	−3.0 ± 0.2	−0.3 ± 0.2
11	chloroform	−2.3 ± 0.2	−3.1 ± 0.2	−0.8 ± 0.2
12	benzene	−1.5 ± 0.2	−0.8 ± 0.2	+0.7 ± 0.2

[a] The $\Delta G°$ values in runs 8–12 were obtained in deuterated sovlents, whereas all calorimetric data result from protonated solvents. The amounts of 1% (v/v) Me_2SO (in run 1) and 10% (v/v) Me_2SO (in runs 4 and 5) were cosolvents in binding titrations to determine $\Delta G°$, which introduces a nondetermined minor error into the concentrations used to transform measured heats into enthalpies. [b] $\Delta G°$ value from calorimetric titration.

methoxy-, 1,4-dinitro-, and 1,4-dicyanobenzene, smaller negative $\Delta C_p°$ values (−20 to −40 cal mol^{-1} K^{-1}) are measured.

Solvent Dependency of the Enthalpic Driving Force for Apolar Complexation. After it was determined that a strong enthalpic driving force exists for the complexation of benzene derivatives by cyclophanes in water and a much less favorable enthalpic term was found for similar binding events in methanol, we were interested in exploring whether apolar inclusion complexation in other organic solvents is also enthalpically controlled or whether water adopts a unique role. For this solvent-dependent calorimetric study, complex **1** was chosen. Previously, it was explained in detail why complex **1** is a favorable choice for a binding analysis across solvents of all polarity, with the results being that the free energy of formation of **1** decreases in a predictable way when changing from water, to polar protic organic solvents, to dipolar aprotic solvents, and finally to apolar solvents.[15] A strong linear free energy relationship between $\Delta G°$ and the empirical solvent polarity parameter $E_T(30)$ was also valid for water, which demonstrated that the binding free energy in water along with the other solvents can be rationalized on the basis of their physical properties.

The heat of formation of complex **1** was determined by microcalorimetry in a total of 12 solvents that encompass a wide range of polarities. Table II shows the previously measured free energies[15] together with the newly obtained complexation enthalpies as well as the calculated changes in entropy. Unfortunately, a calorimetric study in water was not possible since not enough heat was produced at $c = 10^{-7}$ mol L^{-1}, the limiting solubility of the nonprotonated macrobicyclic host. The formation of **1** is enthalpically driven in all solvents (Table II), and complexation entropies are unfavorable except in benzene and *N*,*N*-dimethylacetamide (DMA). Table II shows that complexation in alcohols exhibits the largest exothermicity and, in general, the enthalpic driving force decreases from polar protic solvents, to dipolar aprotic solvents, and to apolar solvents. Correspondingly, the complexation entropy becomes increasingly less favorable as the exothermicity increases, resulting in a strong isoequilibrium relationship (see below). For the formation of **1** in methanol, significant changes in $\Delta H°$ with temperature were observed. From the data obtained at five different temperatures between 288 and 308 K, a negative heat capacity change of $\Delta C_p° = -120 \pm 20$ cal mol^{-1} K^{-1} was calculated (Figure 1).

Table II clearly shows that the enthalpic and entropic terms can differ dramatically for the complexation in solvents in which similar binding free energies are measured. The free energies for formation of **1** in the three dipolar aprotic solvents acetone, Me_2SO, and DMA differ only by 0.5 kcal mol^{-1}. Binding in the two former solvents is strongly enthalpy driven ($\Delta H° \approx -6.5$ kcal mol^{-1}) and entropically unfavorable ($T\Delta S° \approx -2.4$ kcal mol^{-1}).

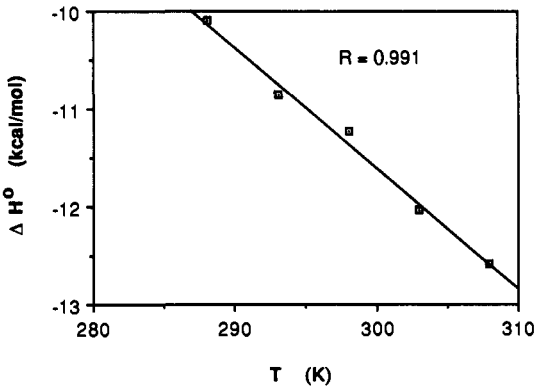

Figure 1. Enthalpy for formation of complex **1** in methanol as a function of temperature to determine changes in the heat capacity $\Delta C_p°$.

In contrast, the enthalpic driving force for binding in DMA is more than 4 kcal mol^{-1} smaller, and inclusion in this solvent is characterized by both favorable enthalpic ($\Delta H° = -2.0$ kcal mol^{-1}) and entropic ($T\Delta S° = +2.4$ kcal mol^{-1}) contributions of similar magnitude. A favorable entropic term for apolar inclusion binding in an amide solvent has also recently been observed with cyclodextrins in *N*,*N*-dimethylformamide (DMF).[36]

Whereas we had observed a strong correlation between $E_T(30)$[37] and the free energy of formation of **1** ($R = 0.934$), only very weak correlations exist between $E_T(30)$ and $\Delta H°$ ($R = 0.739$) and between $E_T(30)$ and $\Delta S°$ ($R = 0.567$).

Clearly, the combination of the results in Tables I and II suggests that the enthalpic driving force for tight apolar inclusion complexation increases with increasing solvent polarity, becoming strongest in polar protic solvents, and ultimately in water. What is still missing from a complete picture of a liquid-phase apolar binding process is the separation of the intrinsic host–guest interaction term from any solvent effects. It has been shown that the changes in thermodynamic quantities seen in various solvents are predominantly solvent effects, since the geometry of complex **1** and, hence, the host–guest interactions are very similar in all environments. What cannot be answered yet is whether solvent effects are endothermic in the less polar solvents and exothermic in the more polar solvents, particularly in water, or whether all solvent effects are endothermic. In the latter case, solvent effects would always subtract off from the intrinsic host–guest interaction enthalpy. According to Tables I and II, polar protic solvents would provide the least endothermic solvent effect and benzene the most endothermic solvent effect. It is our hope that experimental gas-phase measurements or computational studies on complex **1** or other complexes by apolar receptors eventually will provide accurate thermodynamic data for the intrinsic apolar host–guest interaction term. Only with this information will the enthalpic solvent effect become known, which will determine whether water, and possibly other solvents, provide a true enthalpic driving force for tight apolar complexation.

Isoequilibrium Relationships for Solvent-Dependent Apolar Complexation. It is evident by the data in Table II that, generally, as the formation of complex **1** becomes more exothermic, the complexation entropy becomes less favorable. A strong correlation is revealed in Figure 2, which shows a linear relationship ($R = 0.954$) between the complexation enthalpy and entropy.

This linear relationship commonly known as the isokinetic (isoequilibrium) relationship or the compensation effect has been observed in a variety of processes including dissolution,[38] chemical reactions[39] and exchanges,[28] and protein[40] and chemical[41] catalysis.

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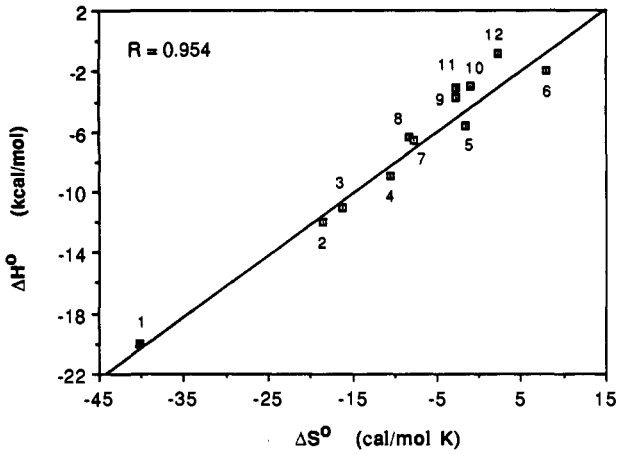

Figure 2. Isoequilibrium relationship between the enthalpy ($\Delta H°$) and the entropy ($\Delta S°$) for the formation of complex **1** at T = 303 K in various solvents. For the numbering of the solvents, see Table II.

An isoequilibrium relationship holds if the enthalpic change of a process is linearly related to the entropic change according to eq 1. The slope of the line has the unit of temperature and is defined by the parameter β (or α in eq 2, which is equal to $T\beta^{-1}$), called the isokinetic temperature T_{iso}. The subject of linear relationships between $\Delta H°$ and $\Delta S°$ has been reviewed.[40,42] There have been a variety of theories put forward to explain these relationships, but a universal concept has not been found.[43-45]

$$\partial \Delta H° = \beta \partial \Delta S° \quad (1)$$

$$\alpha \partial \Delta H° = T \partial \Delta S° \quad (2)$$

Linear relationships correlating the changes in enthalpy and entropy have also been explored in biological and chemical molecular recognition studies. Examples for biological recognition studies are the binding of nucleotides to ribonuclease A[42] and the binding of flavin analogues to the riboflavin-binding protein of eggwhite.[46] In chemical molecular recognition, linear isoequilibrium relationships have been obtained for studies in which the nature of either the host or the guest was systematically altered.[21b,c,47] Inoue et al. have correlated a large number of the complexation studies that use crown ethers, cryptands, podands, and antibiotics to bind a variety of monovalent and divalent cations.[48] Our findings of a compensation effect (α = 0.72, T_{iso} = 421 K) between complexation enthalpy and entropy across a wide range of solvents of all polarities is unprecedented.

Another compensation effect was seen in the plots of enthalpy against entropy for the complexation of the various monosubstituted benzene derivatives by cyclophane **2**. A linear correlation coefficient $R = 0.86 \pm 0.03$ was obtained in the isoequilibrium relationships at all three temperatures where enthalpic data had been measured. The correlation at 293 K yielded an α value of 1.14 and T_{iso} = 257 K.

It follows from the Gibbs free energy equation that if $\Delta H°$ and $\Delta S°$ are related, then $\Delta G°$ and $\Delta H°$ along with $\Delta G°$ and $\Delta S°$

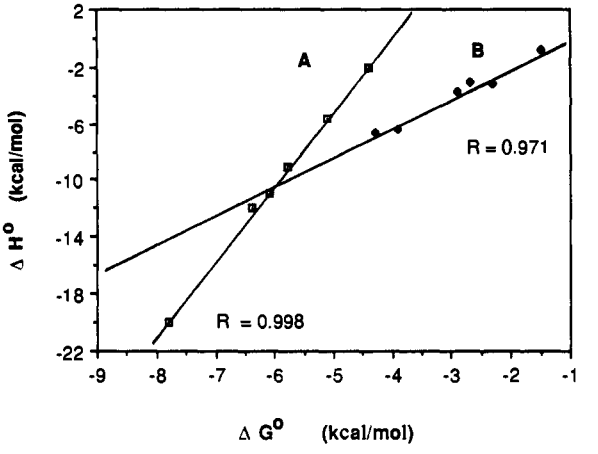

Figure 3. Dual isoequilibrium relationship between the enthalpy ($\Delta H°$) and the free energy ($\Delta G°$) for the formation of complex **1** at T = 303 K in various solvents. The solvents on line A and line B correspond to those in runs 1-6 and 7-12 in Table II, respectively.

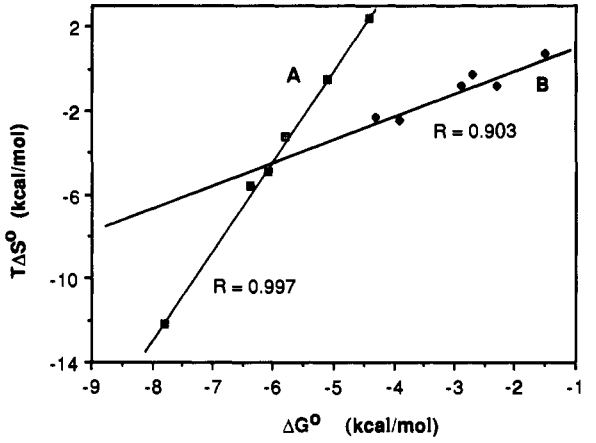

Figure 4. Dual isoequilibrium relationship between the entropy ($T\Delta S°$) and the free energy ($\Delta G°$) for the formation of complex **1** at T = 303 K in various solvents. The solvents on line A and line B correspond to those in runs 1-6 and 7-12 in Table II, respectively.

should also be related. When these plots are examined for the solvent-dependent formation of **1** (Figures 3 and 4), there appears to exist two rather than one linear correlation between the thermodynamic parameters. The linear correlation coefficients are more significant using a dual relationship compared to single linear relationships, which have values of R = 0.812 for the correlation in Figure 3 and R = 0.619 for that in Figure 4.

The six solvents that give the most favorable free energies for the formation of **1** ($-\Delta G° > 4.4$ kcal mol^{-1}, runs 1-6 in Table II) fall on one line (line A in Figures 3 and 4), and the other line B is composed of the other six solvents. In their studies of α-cyclodextrin and β-cyclodextrin binding of alcohols, Takagi et al. had previously obtained dual isoequilibrium relationships dependent upon the free energies.[49] The separation of solvents in our case appears to be caused by the ability of the solvent to serve as a H donor in a hydrogen bond formation. Except for DMA, all solvents that give the most favorable $\Delta G°$ values (line A) are protic solvents (Table II). A plot of the acceptor number AN of these solvents[50] versus the thermodynamic quantities for formation of **1** shows strong correlations: AN plotted versus $\Delta G°$ gives R = 0.922, AN plotted versus $\Delta H°$ gives R = 0.914, and AN versus $T\Delta S°$ gives R = 0.912.

The dual isoequilibrium relationship seen in the plots correlating the free energy (Figures 3 and 4) must also be expressed in the plot of the enthalpy against entropy shown in Figure 2. Indeed,

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upon reinvestigation, the two sets of solvents are revealed, giving $R = 1.00$ for the solvents having the larger free energies (runs 1–6) and $R = 0.979$ for the other six solvents. The origin of these strong dual isoequilibrium relationships is now the subject of further investigations.

Conclusions

More than 10 years after the first unambiguous demonstration of apolar inclusion complexation by cyclophanes in the laboratory of K. Koga,[51] we describe here the first calorimetric investigation of the thermodynamic characteristics of such association processes. With their well-defined binding sites of pronounced apolar character, the cyclophanes used in this study are particularly suitable for a meaningful analysis of the thermodynamic quantities characterizing tight apolar complexation processes. A variety of novel results have been obtained.

(1) If absolute enthalpic values are large, the van't Hoff analysis of variable-temperature ^{1}H NMR or optical binding titrations provides changes in enthalpy that are in good qualitative agreement with the direct $\Delta H°$ values determined by calorimetric measurements. The increased uncertainty of the van't Hoff data for the complexation of benzene derivatives by cyclophane **2** must be assigned to the significant changes in the heat capacity $\Delta C_p°$ that accompany apolar inclusion complexation.

(2) In water, the inclusion complexation of benzene derivatives by cyclophane **2** is strongly exothermic and accompanied by an unfavorable entropic term. An enthalpic driving force had also previously been measured for the binding of these substrates by proteins, DNA, and cyclodextrins. The thermodynamic characteristics for tight apolar inclusion complexation ($\Delta H° \ll 0$, $T\Delta S° < 0$) of small solutes in water, therefore, differ dramatically from those established for the formation of looser associations such as micelles and membranes. Therefore, the terms "hydrophobic effect" or "hydrophobic bonding" should not be used to imply a specific thermodynamic driving force for association.

(3) The accurate thermodynamic quantities $\Delta H°$ and $T\Delta S°$ obtained in calorimetric studies reveal specific molecular interactions that are not expressed in the free energy term. Large enthalpic driving forces for the complexation of *p*-cresol and hydroquinone by cyclophane **2** provide strong evidence for intermolecular hydrogen bonding between the hydroxyl groups of the guests and the methoxy groups of the host. However, these additional oriented interactions impose large geometric constraints on the complexes, resulting in a strong unfavorable entropy change, which compensates for the additional gain in enthalpy.

(4) Negative heat capacity changes $\Delta C_p°$ have been measured for all apolar inclusion processes in water and in methanol performed in this study. In the inclusion of benzene derivatives, a significant temperature dependence of $\Delta C_p°$ is evident from the nonlinearity of the plots of $\Delta H°$ as a function of temperature. The largest negative $\Delta C_p°$ values are measured for guests with a molecular dipolar moment and hydroxy residues, e.g., *p*-nitrophenol and *p*-cresol. This result suggests that changes in the strong interactions between these guests and their solvent cage dominate the changes in complexation enthalpies that occur by altering the temperature.

(5) Calorimetric measurements of the enthalpies for formation of complex **1** in 12 solvents of all polarities show that binding in all environments is enthalpy-driven. The exothermicity generally increases from apolar solvents, to dipolar aprotic solvents, to protic solvents. The study also shows that binding in two solvents exhibiting a very similar change in free energy, e.g., acetone and DMA, can differ dramatically in their enthalpic and entropic terms. When the thermodynamic characteristics of micelle formation and tight apolar complexation are compared, it appears that the enthalpic driving force seen in the latter association originates from strong host–guest interactions. The question remains open whether desolvation prior to host–guest complexation is exothermic in some solvents and endothermic in others or whether solvent effects are always endothermic with the least unfavorable enthalpic solvent effects occurring in water and other polar protic solvents. The answer to this question lies in the determination of the enthalpy of inclusion of the guest into the host, which could potentially be found by either studying gas-phase complexation or performing computational modeling.

(6) A strong unprecedented isoequilibrium relationship has been obtained by correlating the enthalpy and entropy for formation of complex **1** in the different solvents. However, this plot also expresses a strong dual isoequilibrium relationship that is also obtained when the free energy is correlated with either the enthalpic or the entropic term. Correlations between thermodynamic quantities in the polar protic solvents and, surprisingly, in DMA differ substantially from similar correlations existing between the residual aprotic solvents that were considered. Further calorimetric studies are now being pursued to determine the origin of these interesting compensation effects.

Acknowledgment. We thank the National Science Foundation and the Office of Naval Research for their support of this work. The calorimeter was purchased with funds provided by the National Science Foundation. We would like to thank Dr. P. Brown, Tronac, UT, for many valuable discussions concerning the operation of the calorimeter.

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