The Anatomy of the Energetics of Molecular Recognition by Calorimetry: Chiral Discrimination of Camphor by α-Cyclodextrin

Franz P. Schmidtchen*[a]

Dedicated to Professor Helmut Simon on the occasion of his 75th birthday

Abstract: The molecular recognition of both camphor enantiomers **2** with the chiral α-cyclodextrin (α-CD) **1** in water and D_2O was examined by calorimetry. On the basis of statistically supported determinations the thermodynamics of 2:1 host–guest binding and chiral discrimination was evaluated. The energetic signature strongly supports hydrophobic interaction as the dominant driving force for camphor encapsulation by α-CD in water. The solvent isotope effect on the binding equilibrium served to dissect the experimental enthalpy ΔH_{ass} into direct interaction (ΔH_{intr}) and solvent reorganization (ΔH_{solv}) terms. From this analysis the mutual interaction of two cyclodextrin and one camphor molecules contributes only 25% to the observed enthalpy of binding ΔH_{ass}, all the rest is attributed to solvent restructuring. Furthermore, the dramatic change in the pattern of thermodynamic state functions on solvent transfer from water to D_2O is taken as compeling evidence for the involvement of water as a structural tectone in the supramolecular architecture of the 2:1 complex. As a corollary, bilateral host–guest interactions as conveyed by the lock-and-key metaphor of molecular recognition provide an inadequate description of this seemingly simple artificial host–guest system.

Keywords: calorimetry · cyclodextrins · enantioselectivity · isotope effects · molecular recognition

Introduction

Finding a correlation between structure and energetics is at the heart of molecular recognition and preceeds any attempt at design. Two issues form the basis of the major problems here:

1) Which complex structure (i.e. the arrangement (topology) of *all* atoms involved in the binding process) best represents the ensemble of bound species (on the time-averaged basis relevant to its observation or use)?
2) How can we relate the experimental global thermodynamics of complexation to unique molecular binding events?

Nowadays we have the tools available to study and eventually answer either of the above questions. Spectroscopic methods, for example NMR spectroscopy, generate information about spatial relations and interacting groups. This may provide positive proof; however, the lack of observation of an effect does not guarantee the absence of an interaction (unfortunate time regimes or adventitious cancellations may be in play). Furthermore, there may be an intrinsic methodological bias for sensing certain types of interaction more readily and intensely than others, leading to a distorted view of the true ensemble. Even more severe is the incapability of most methods to detect and quantify solvent participation which is undoubtedly present in all associations in condensed phases and may constitute the lion's share to the total energetics.[1]

The overall energetics in turn can be readily measured with great precision by microcalorimetry, which provides quick-and-easy access to the thermodynamic state functions.[2] A grave problem arises, however, from the necessity to assign the experimental energetics to the one chemical transformation under study. Since in most recognition reactions several chemical processes run in parallel, the measured effects represent a truly global response that needs to be deconvoluted. Naturally, resolving the pattern of simultaneous reactions is easier the simpler the entire system. Thus, when delineating the basic understanding of molecular recognition the inherently simpler artificial host–guest systems may provide a more lucid evaluation of the structure–energy relationship.

A particularly good and attractive starting point is the investigation of chiral discrimination in molecular recognition. The differential interaction of a pair of enantiomers, such

[a] Prof. Dr. F. P. Schmidtchen
Institute of Organic Chemistry and Biochemistry
Technical University of Munich
Lichtenbergstrasse 4
85747 Garching (Germany)
Fax: (+49) 89-289-14698
E-mail: schmidtchen@ch.tum.de

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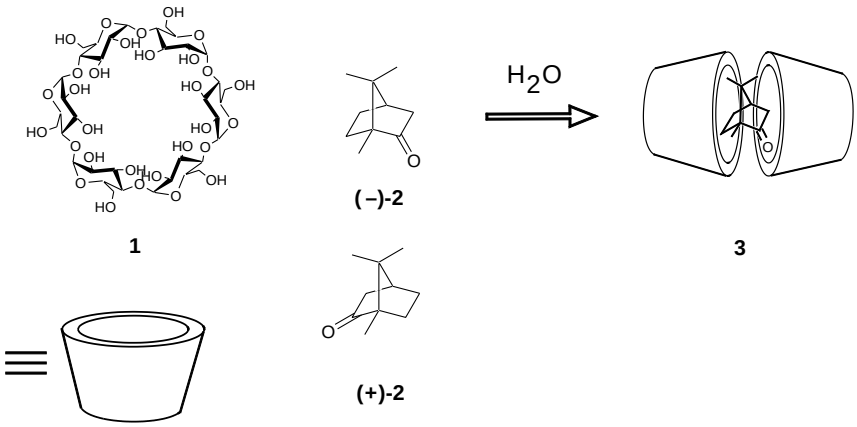

Here we report on an extension of their approach in which we analyze the complexation of both enantiomers of camphor to α-cyclodextrin in a two-step process by using calorimetry. This provides insights into the binding energetics in a prototypical host–guest system by monitoring temperature dependence and solvent isotope effects.

as (+)-**2** and (−)-**2**, with a chiral host (e.g. α-cyclodextrin **1**) offers the benefit of being exclusively due to the direct mutual interaction of host and guest. Differences, for example, in the solvation of the initial states prior to the association do not exist. As calorimetry measures the change in enthalpy in the course of a reaction, the unfolding of the binding energetics is more straightforward than in a closely homologous series of guests, for which more or less reasonable assumptions on the starting state have to be met. If the final state were of the inclusion type, that is the electroneutral enantiomeric guests are sequestered into the host structure and totally shielded from solution, these states too would be almost identical in their interaction with the solvent. Thus, the energetics measured would relate exclusively to the differences in the interaction of the enantiomers with the chiral host. Of course, this mode would comprise solvent contributions, but only those that emerge directly from the diastereotopic contacts and not from solvent–solvent interactions.

Unfortunately, chiral discrimination is at best negligible in abiotic systems in water which lend themselves to intimate investigation. Cyclodextrins, for instance, have been studied in great detail in recent years,[3–16] and yet have revealed no unifying view. A wide variety of chiral compounds has been probed by isothermal titration calorimetry by Rekharsky, Inoue et al. who reported subtle binding modes and thermodynamic driving forces in the interaction with natural and functionalized α-, β-, or γ-cyclodextrins in water.[17–20] These investigations on enantiomer binding were severely hampered by small enthalpic differences, which were compensated further by counteracting entropic contributions, leading to rather moderate differences in affinity. A tantalizing problem arose from the tendency of cyclodextrins to form complexes with higher stoichiometries. In most cases such complications lead to untractable tasks in the deconvolution of the underlying host–guest equilibria, unless experimental conditions can be found that simplify the overall scenario. Even then careful considerations are necessary to probe the significance of a binding model mapped by computer fit to the experimental observations. As Rekharsky, Inoue et al. convincingly argue a reliable procedure is to find a concentration regime for host and guest that favors 1:1 stoichiometry. Additional support can then be sought from an evaluation of statistical errors involving multiple independent determinations.

Results and Discussion

The complexation of camphor by α-cyclodextrin in water has been characterized as an enantiodifferentiating and highly cooperative process.[21, 22] On the basis of NMR titrations and corresponding Job plots, the complexation of one camphor molecule (**2**) to α-cyclodextrin (α-CD; **1**) is followed by the association of another host molecule to give a α-CD:camphor 2:1 noncovalent complex **3**.[21] Since Hill plots[23] reveal a strong positive cooperativity (Hill coefficients of 1.98 and 1.89 for the (+) and (−) enantiomers, respectively), the second binding step possesses a much greater affinity (actually by a factor of 10^4) than the initial step. Despite their respectable affinities ($K_{ass}(+) = 6.6 \times 10^5$ M^{-2}; $K_{ass}(-) = 3.6 \times 10^5$ M^{-2}) the complexes undergo rapid guest exchange at ambient temperature making reequilibration after a change in concentration a speedy process. Although the free energy difference $\Delta\Delta G^o_{ass}$, which differentiates the 2:1 binding modes of the optical antipodes to α-CD, is only 1.6 kJ mol^{-1}, this is one of the most massive effects reported to date in the series of natural cyclodextrins and their simple derivatives and suffices to facilitate chiral separations in reverse-phase chromatography.[22] This system seemed well suited to evaluate the energetic origin of the overall affinity and the comparatively minute chiral discrimination by using isothermal titration calorimetry (ITC) as a sensitive tool.

A solution of the camphor enantiomer in water was titrated with a concentrated solution of α-CD in the same solvent in a fully computer-operated calorimeter. The aliquots added were adjusted to cover a maximal complexation range, yet, not to exceed the heat susceptibility of the instrument. A typical output of the heat pulses for both enantiomers is shown in Figure 1 along with the time integrals for the titration curve. The latter represent the raw data introduced into the computer fit. A sequential two-step binding model was chosen in which camphor was taken as the host (in the terminology of the mathematical treatment implemented in the calorimeter software). Inspection of the fit functions generated clearly shows a very satisfactory approximation of the experimental data points over the whole titration range. We conclude that the 2:1 sequential binding model is an appropriate representation of all significantly populated states participating in the host–guest binding equilibria. The fit algorithm itself, however, only furnished well con-

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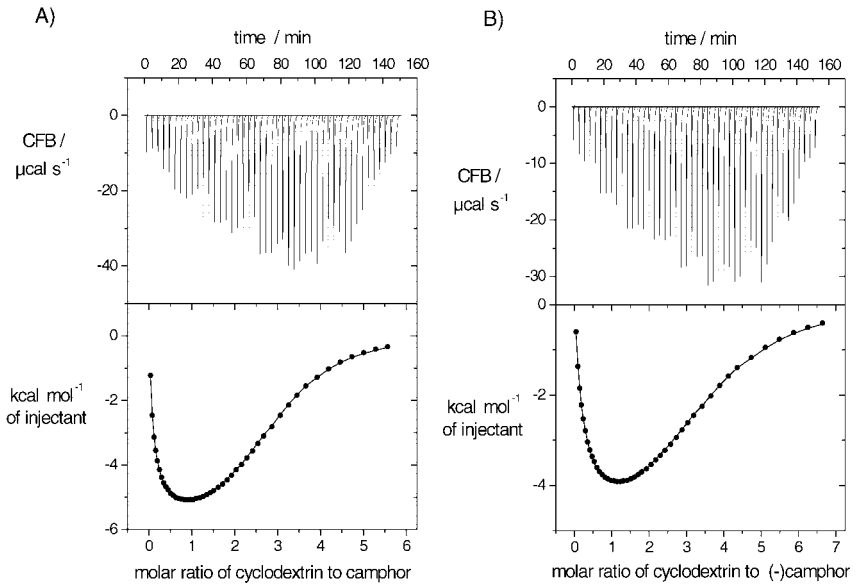

Figure 1. Thermogramms (CFB = cell feedback current) and thermometric titration curves (solid line is obtained by nonlinear regression of the entire data set) for representative titrations of (+)-camphor (A) and (−)-camphor (B) with α-cyclodextrin in H_2O at 20 °C.

vergent results if the titration curve contained a clearly discernable inflection point (cf. ref. [17]), that is if the complexation progressed to >90% completion.

Even in this case, finding the global error minimum on what appeared to be a rather flat hypersurface required changing the starting parameters 20–30 times. The most reliable approach involved the systematic variation of the initial set of the four fit parameters (the stepwise binding constants K_1 and K_2 and their corresponding enthalpies ΔH_1° and ΔH_2°) to yield a series of solutions that could be ranked with respect to the goodness of fit. The parameters obtained in the top five of this list were further evaluated in subsequent fit cycles as input sets which finally converged to stable error minima. The parameters derived in this way showed excellent reproducibility for the overall 2:1 binding process ($K_1 \times K_2$; $\Delta H_1^\circ + \Delta H_2^\circ$), but not with respect to the stepwise constants because of extensive parameter correlation (correlation coefficients of K_1,K_2 and ΔH_1°, $\Delta H_2^\circ > 0.9$). On this basis the error limits of the stepwise parameters are of similar size as the absolute values thus preventing meaningful interpretations besides the notion that the Gibbs enthalpies and enthalpies in the first binding event are always very much less negative than in the subsequent association of the second cyclodextrin molecule.

In view of the documented complications in data analysis associated with two-step binding,[20] confirmation of the reliability and precision of the calorimetric measurements appeared mandatory. Thus, all experiments were repeated twice and in two selected cases an analysis of the true statistical errors was undertaken which included the preparation of the host–guest solutions. From six independent determinations in each case we calculate a statistical error of the mean of 0.6% (i.e. 0.4 kJ mol^{-1}), which is significantly smaller than the enthalpy differences between the camphor enantiomers and justifies a detailed anatomy of the discrimination energetics.

The thermodynamic parameters for the 2:1 association of α-cyclodextrin and the camphor enantiomers in H_2O in the temperature range from 5–40 °C are given in Table 1. The heat capacities ΔCp_{ass} which appeared temperature independent were obtained from a linear regression of the enthalpy data depicted in Figure 2. As is true for almost all other compounds that bind to α-cyclodextrin the association is driven by a favorable enthalpy that is compensated in part by an unfavorable entropy contribution. Though this very general result is in line with expectation it is the quantitative energetic signature that stands out among comparable guests.

While binding of α-CD to guest analogues, such as 2-methylcyclohexanone ($\Delta H^o_{ass} = -8.9$ kJ mol^{-1}, $T\Delta S = -0.6$ kJ mol^{-1} [26]), 2-norbornylacetate ($\Delta H^o_{ass} = -14.2$ kJ mol^{-1}, $T\Delta S^o_{ass} = -2.9$ kJ mol^{-1}[27]), or decanediol as a compound with the same number of C atoms ($\Delta H^o_{ass} = -24.8$ kJ mol^{-1}, $T\Delta S^o_{ass} = -2.7$ kJ mol^{-1}[28]), show moderate exothermicities and weakly counteracting entropic terms, the magnitude of enthalpy and entropy (as $T\Delta S$) is much more profound in the case of camphor complexation. Of course, a 2:1 binding process may be conceived to involve more intense

Table 1. Energetics of the 2:1 binding of camphor **2** to α-cyclodextrin in H_2O.

Temperature [K]	ΔH_{ass} [kJ mol^{-1}]	$100 \times \Delta H_{ass}$ $(\Delta H_{ass} + T\Delta S_{ass})^{-1}$	ΔS_{ass} [J K^{-1} mol^{-1}]	$T\Delta S_{ass}$ [kJ mol^{-1}]	ΔG_{ass} [kJ mol^{-1}]	K_{ass}(2:1) [M^{-2}]
			(+)-camphor			
278.44	−57.24	72.2	−79.1	−22.05	−35.20	40.5×10^5
286.42	−62.08	69.2	−96.6	−27.68	−34.40	18.9×10^5
293.99	−67.92	66.4	−116.7	−34.32	−33.60	9.5×10^5
303.43	−73.22	64.2	−133.9	−40.63	−32.59	4.1×10^5
313.29	−78.83	62.2	−153.1	−47.98	−30.85	1.4×10^5
298 from fitting	−69.73	65.5	−123.1	−3669	−33.03	6.16×10^5
			(−)-camphor			
280.94	−57.50	70.3	−86.7	−24.37	−33.12	14.47×10^5
288.03	−61.32	68.0	−100.5	−28.95	−32.34	7.38×10^5
293.57	−65.56	66.0	−115.1	−33.79	−31.75	4.49×10^5
303.27	−71.98	63.6	−135.4	−41.07	−30.91	2.12×10^5
313.29	−78.33	61.7	−155.0	−48.57	−29.76	0.92×10^5
298 from fitting	−68.39	64.9	−124.1	−37.00	−31.39	3.18×10^5

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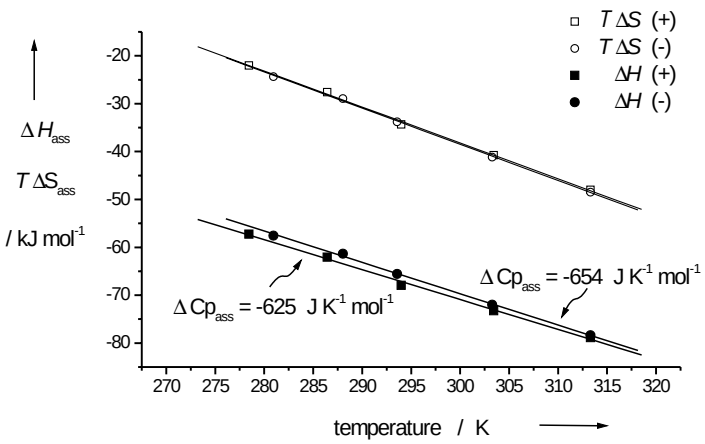


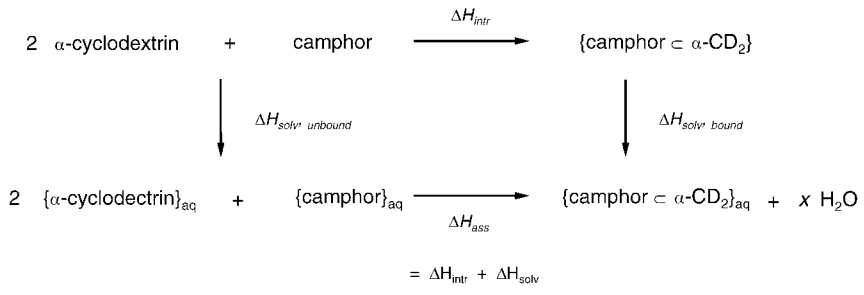
Figure 2. Temperature dependance of ΔH_{ass} and $T\Delta S_{ass}$ of (+)-camphor (squares) and (−)-camphor (circles) binding to α-cyclodextrin in H_2O

interactions that would result in augmented energetic contributions. But the more than proportional increase points to a special feature that may be related to the unusually high chiral discrimination observed. Judging from the size of the entropy, which constitutes more than one third to the resulting Gibbs enthalpy of interaction, an exclusive focus on the direct mutual interaction of host and guest is not likely to unfold the origin of the chiral discrimination. Owing to the fuzzy analysis of the stepwise binding process a critical interpretation of the successive events is not possible. However, it seems safe to state that (−)-camphor always exceeds its enantiomer in the cooperativity of binding (K_2/K_1). Cooperativity in turn increases strongly with temperature for both enantiomers, indicating a much more negative enthalpy in the second binding step than in the primary host–guest association.

Before we turn to the evaluation of the differences in enantiomer binding, it is appropriate to analyze the overall guest complexation with the help of a thermodynamic cycle (Scheme 1) that builds on earlier suggestions of Grunwald and Steel,[29] and Toone and Chevernak.[30] Here the observed reaction enthalpy ΔH_{ass} in water is broken into a term ΔH_{solv}, which refers to the heat emerging from the reorganization of the hydration shells on host–guest association, and ΔH_{intr}, which describes the direct interaction of the binding partners. Only the latter can contribute substantially to the chiral discrimination especially if the host–guest complexes are of the encapsulation type. Inclusion complexation prevents differential solvation of the diastereomeric complexes formed.

In this scheme the solvation contribution represents the difference in hydration of the unbound and bound host–guest partners (i.e. the enthalpic difference of the vertical processes). Generally, one expects an increase in entropy if water contained in the more restricted state of a solvation shell of a solute is released to the bulk on solute–solute association irrespective of whether the aggregation follows hydrophobic or electrostatically driven binding (e.g. in the formation of ion pairs). The strongly negative entropy of association in our case does not contradict this basic inference but rather suggests an astoundingly high ordering effect that favors a well-structured complex. The concomitant negentropy of structuring the complex apparently overrides the gain attributed to solvent disordering. The potential well (negative ΔH^o_{ass}) characterizing the peculiar topology of all molecules participating in the host–guest complexation (i.e. the complex structure including the attached solvent molecules) allows very much less alternative arrangements than possible with the unbound partners. Better structural definition should help in sensing subtle differences in the stereochemical relations of host and guest and thus should create an improved basis for chiral discrimination. Nevertheless, the hydrophobic interaction constitutes the fundamental platform of host–guest binding as can be read from the negative change in the heat capacity ΔCp_{ass}.(Figure 2, $\Delta Cp_{ass}(+)$-**2** = $-625\,J\,K^{-1}\,mol^{-1}$; ΔCp_{ass} (−)-**2** = $-654\,J\,K^{-1}\,mol^{-1}$). The heat capacity is well recognized to depend on the surface area involved in hydrophobic hydration.[31–33] If this fraction is diminished on association a negative change in heat capacity will result. It is precisely this which is observed in camphor binding to α-CD, and to an extent that exceeds the effect seen with 1,10-decanediol by more than 50% (decanediol: $\Delta Cp_{ass} \approx 400\,J\,mol^{-1}\,K^{-1}$[28]). The sheer magnitude of ΔCp_{ass} suggests that large parts if not the entire surface of the guests are desolvated and in direct contact with the host interior, corroborating prior NMR evidence that demonstrated the proximity of the methyl groups of camphor with the interior lining of the cyclodextrin cavity.[21]

If a dissection of the experimental enthalpy ΔH_{ass} into ΔH_{intr} and ΔH_{solv} were possible, it would provide a better estimate of which fraction of the total mutual enthalpy change ΔH_{intr} is the stereodifferentiating factor. A reasonable way to separate the enthalpic components into direct interaction ΔH_{intr} and solvent reorganization ΔH_{solv} is based on the measurement of a solvent isotope effect.[30] With respect to H_2O, D_2O is considered on theoretical[34, 35] and experimental grounds[36] to be a stronger hydrogen-bonded solvent owing to its lower zero point vibrational energy. Based on enthalpies of solvent transfer the



Scheme 1. Thermodynamic cycle relating the solvation independent ΔH_{intr} and solvation dependent ΔH_{solv} contributions to the experimental enthalpy of host–guest binding ΔH_{ass}.

strength of hydrogen bonds is increased by 10% relative to H_2O (though the hydrogen bonding distance is unchanged[31]), while the concomitant enhanced directionality leads to a loss in entropy. In summing up the components the free energy ΔG_{trans} of solvent transfer between light and heavy water of a hydrophobic surface is very close to zero showing almost perfect enthalpy–entropy compensation. However, since the complexation of camphor by α-cyclodextrin involves the desolvation of hydrophobic surface areas as indicated by the heat capacity change ΔCp_{ass} a solvent isotope effect $\Delta\Delta H_{ass} = \Delta H_{ass}(H_2O) - \Delta H_{ass}(D_2O)$ is to be expected. Its magnitude amounts to about 10% of the change in the enthalpy of solvent reorganization ΔH_{solv}. With this crude estimate at hand one can calculate the intrinsic enthalpy change $\Delta H_{intr} = \Delta H_{ass} - \Delta H_{solv}$ and the enthalpy of chiral discrimination as a fraction of this mutual host–guest interaction. This estimate is unaffected by changes in the solvation of hydrogen bonding functional groups of host and guest as long as the number of hydrogen bonds remain the same in H_2O and D_2O.[30] In ordinary hydration this can be taken for granted. As will be shown below the tacit assumption breaks down when complexes with distinct water participation are formed.

Table 2 contains the thermodynamic state functions for the camphor–α-CD complexation in D_2O. Linear regressions of the temperature-dependent data (Figure 3) reveal considerably more negative heat capacities than found in light water (ΔCp_{ass} (+)-**2**, $D_2O = -931\ J\,K^{-1}\,mol^{-1}$; ΔCp_{ass} (−)-**2**, $D_2O = -754\ J\,K^{-1}\,mol^{-1}$), completely in line with the expectation[31] of overwhelmingly hydrophobic desolvation being the dominant cause of guest binding. At 25 °C the enthalpic solvent isotope effect $\Delta\Delta H_{ass} = \Delta H^o_{ass}(H_2O) - \Delta H^o_{ass}(D_2O)$, for example, for (−)-camphor complexation is 5.18 kJ mol^{-1}. Following the suggestion[30] that $\Delta\Delta H_{ass}$ represents about 10% of the heat change due to solvent reorganization one arrives at $\Delta H_{solv} = -52$ kJ mol^{-1} and $\Delta H_{intr} = -16$ kJ mol^{-1}. Considering the widely spread idea that inclusion complexation by cyclodextrins can be described by the lock-and-key metaphor of Emil Fischer[25, 37] we find it quite instructive that more than 75% of the total experimental enthalpy of interaction arises from solvent restructuring and not from the mutual host–guest fit. The strong focus on the host–guest duality expressed in Fischer's model appears to severely underestimate the role of solvation even on the observable enthalpy of a binding process in the aqueous environment let alone on the free energy.

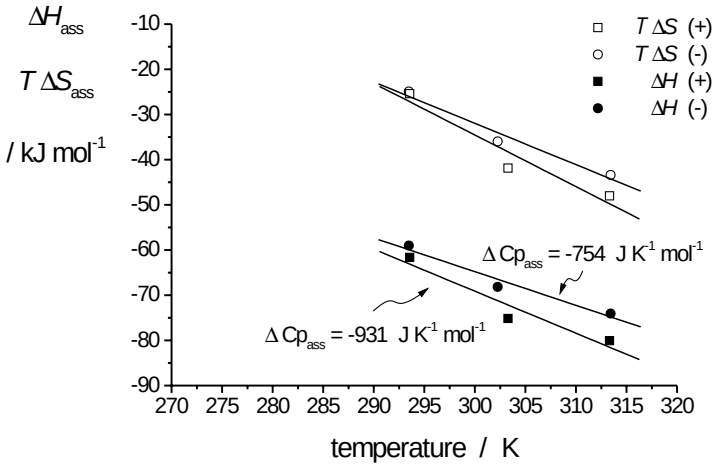


Figure 3. Temperature dependence of ΔH_{ass} and $T\Delta S_{ass}$ of (+)-camphor (squares) and (−)-camphor (circles) binding to α-cyclodextrin in D_2O

Another consequence of our analysis is the reasonable and intuitively anticipated result that the differential enthalpy of camphor enantiomer binding $\Delta\Delta H_{ass}(+/-) = \Delta H_{ass}(+) - \Delta H_{ass}(-) = 1.35$ kJ mol^{-1} constitutes about 8% of the *intrinsic* association enthalpy ΔH_{intr}. The inspection of Figure 2 reveals that the enantiomeric discrimination in light water is almost exclusively due to the difference in association enthalpy. Since the starting situation before the association events are identical by definition the congruent entropy contributions of (−)- and (+)-camphor binding indicate that the host–guest complexes must also be very much alike in structural terms. This is compatible with the assumption of guest encapsulation into the cavity formed by face-to-face interaction of two cyclodextrin molecules. The diastereomeric 2:1 complexes would present identical skins to the solvent causing the main source of entropy changes to vanish. The marginally more negative heat capacity seen in (−)-camphor binding (Figure 2) leads to a considerable improvement in chiral differentiation with decreasing temperature. Within the temperature range explored the ratio of binding constants $K_{ass}(+)/K_{ass}(-)$ increases from 1.51 at 40 °C to 2.48 at 5 °C. Enantiomer separation would thus be favored on lowering the temperature as was found in chiral separations by HPLC using, however, water/methanol mixtures for elution.[22]

A rather different picture is seen in the enantioselective complexation of camphor in heavy water. At 25 °C the enthalpic difference in binding the camphor antipodes is dramatically enhanced by threefold to $\Delta\Delta H_{ass}$ (+/−) = 4.17 kJ mol^{-1} (see Figure 3). Moreover, the association entropies of both enantiomers are more positive than in water and in addition are no longer identical but show differences ($\Delta\Delta S_{ass}(+/-) =$

Table 2. Energetics of the 2:1 binding of camphor **2** to α-cyclodextrin in D_2O.

Temperature [K]	ΔH_{ass} [kJ mol^{-1}]	$100 \times \Delta H_{ass}(\Delta H_{ass} + T\Delta S_{ass})^{-1}$	ΔS_{ass} [J K^{-1} mol^{-1}]	$T\Delta S_{ass}$ [kJ mol^{-1}]	ΔG_{ass} [kJ mol^{-1}]	K_{ass}(2:1) [M^{-2}]
			(+)-camphor			
293.55	−61.60	71.3	−86.4	−25.36	−36.22	28.13×10^5
303.28	−75.14	64.2	−138.0	−41.86	−33.28	5.44×10^5
313.32	−80.03	62.5	153.2	−47.99	−32.02	2.19×10^5
298 from fitting	−67.38	67.8	−107.1	−31.93	−35.44	16.3×10^5
			(−)-camphor			
293.47	−59.00	70.0	84.8	−24.89	−34.09	11.78×10^5
303.25	−68.13	65.5	118.6	−35.96	−32.15	3.47×10^5
313.42	−74.05	63.1	138.4	−43.37	−30.67	1.30×10^5
298 from fitting	−63.21	68.0	−99.9	−29.79	−33.42	7.31×10^5

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$-7.2\,J\,mol^{-1}\,K^{-1}$) that account for about 50% of the total discriminatory free energy in D_2O $\Delta\Delta G^o_{ass}(+/-)$ at 25 °C. Unexpectedly, the enantioselectivity as conveyed by the ratio of association constants is significantly higher in heavy water (Figure 4) supporting the involvement of water molecules in

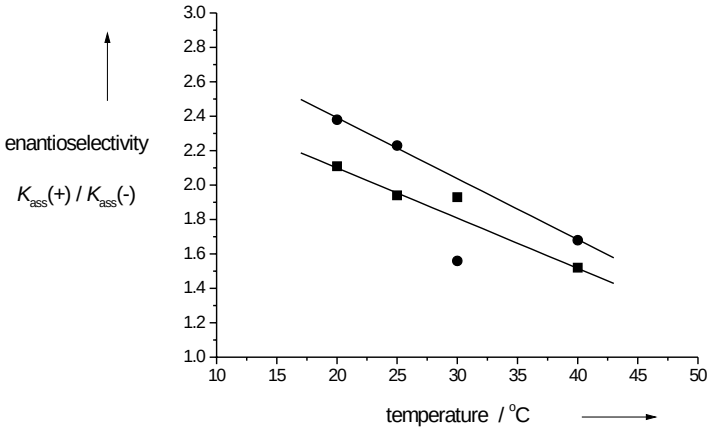

Figure 4. Temperature dependencies of the enantioselectivities $K_{ass}(+)/K_{ass}(-)$ in H_2O (squares) versus D_2O (circles).

the enantiodiscrimatory process. Evidently, the solvent isotope effect not only changes the solvation in a general physical sense (i.e. ΔH_{solv}) but also influences the specific host–guest interaction pattern by altering ΔH_{intr} as well.

We see two conceivable options that would explain the boost in chiral discrimination on switching from light to heavy water. The first explanation attributes the enhancement in differentiation between the enantiomers to the presence of dedicated hydrogen bonds donated by the cyclodextrin host to the carbonyl group of the guest. The replacement of OH- by the stronger OD-hydrogen bond donors must qualitatively exert a differential effect depending on the different orientations of the carbonyl groups in the diastereomeric complexes. This effect is recognizable only in comparing $\Delta\Delta H_{ass}(+/-)$ in H_2O and D_2O, because there is no intervention from the solvent in the inclusion complex and all other hydrogen bonding contributions are already accounted for by the general solvent isotope effect $\Delta\Delta H_{solv}$. We note serious caveats to this rationalization. Enforced specific enthalpic interactions of host and guest as inferred from stronger dedicated hydrogen bonding must make ΔH_{intr} more negative. Though it is only the minor part of the observed enthalpy change (see above) one then should also expect ΔH_{ass} to decrease. On the contrary, the experimental enthalpy ΔH_{ass} is more positive in D_2O than in H_2O and this also applies for the association entropy, suggesting less structured complexes of both enantiomers in heavy water.

The alternative rational for explaining the solvent isotope effect on ΔH_{intr} calls for the direct and specific involvement of water in complex formation. Similar to "structural" water molecules found by X-ray crystallography in protein–ligand complexes the assembly of two cyclodextrin and one camphor molecules require additional water molecules to arrive at a thermodynamically stable structure. In the literal sense we would not be dealing with a 2:1 complex but with a higher order aggregate that includes a stoichiometric amount of water molecules as binding partners. The involvement of specific water participation can also be deduced from the change in heat capacities on going from light to heavy water. For both enantiomers $\Delta\Delta Cp_{ass}$ is negative but distinctly different with respect to each other (see Figure 3). While the former result leaves no doubt that hydrophobic hydration is the dominating driving force in association,[31] the latter observation provides a strong argument in favor of an extensive reconstruction of the diastereomeric complexes on solvent transfer. In light water the identical entropies and very similar heat capacities of (+)- and (−)-camphor complexation led to the conclusion that they must be structurally very similar. In D_2O, in turn, these state functions differ grossly between the enantiomers. As a corollary one is forced to assume extensive variation in the complex structures which seem hardly likely to emerge from the rather restricted capacity of hydrogen bond formation of the camphor guest alone. The complete change in the energetic signature of camphor binding on solvent transfer rather points to the constitutional embedding of water molecules during the construction of the complex. The structural water molecules involved in this process are highly restricted in their motions and thereby add to the unusually negative entropy of association. Their replacement by D_2O will alter the relative balance of all binding contributions comprising not only the direct hydrogen bonding but also van der Waals and hydrophobic components in a profound manner that ultimately surfaces in the dramatic change of the thermodynamic pattern.

In this interpretation the chiral discrimination is not the result of host and guest interaction alone. Dedicated third party participation—in the present case of solvent molecules—play decisive roles as well.

The meticulous energetic analysis of enantioselectivity in what appears to be an easy to grasp artificial host–guest system unfolds subtle conclusions that are incompatible with the crude bilateral lock-and-key concept of cyclodextrin binding. Understanding the basics of molecular recognition mandates appreciation of the influence of the solvent not only as a medium but as an active player in complex construction. We suspect that many of these interactions go unrecognized by the traditional tools of chemical structure elucidation, making methods like calorimetry that address the unfocused change of the entire system an indispensible adjuvant.

Conclusion

In our strive to unravel the fundamental correlation between structure and energetics in molecular recognition we found an artificial host–guest system that in spite of a higher order stoichiometry proved simple enough to enable its overall energetic analysis and interpretion of the results in terms of the direct (intrinsic) mutual interactions of the binding partners in comparison to the contributions arising from solvent reorganization. The complexation of two molecules of α-cyclodextrin with one molecule of camphor in water had been investigated before[21] and led to the characterization of

a highly cooperative two-step inclusion complex formation. Now, high-precision microcalorimetry in combination with solvent isotope effect studies on chiral discriminations provided an excellent tool to dissect the thermodynamics of binding in this fortuitious case.

The overall association of two α-CD and one camphor molecule in water was found to be an enthalpically driven process (high exothermicity $-\Delta H_{ass}$) that is opposed by a substantial reduction in entropy. The temperature dependence of ΔH_{ass} revealed a strongly negative change in heat capacity ΔCp_{ass} (Figure 2) indicative of massive desolvation of the interacting partners, which is completely in line with the classic idea of hydrophobic binding being the main driving force in cyclodextrin complexation. The release of solvent molecules from the interacting surfaces is expected to result in a positive entropy contribution that is, however, not seen in experiment. Instead, a dramatically negative entropy of association is apparent (ΔS_{ass}, Table 1) that testifies to a pronounced stringency in complex formation overriding the entropy of solvent release. The molecular aggregate must be well-structured, that is only very few energetic states corresponding to a rather limited set of host–guest topologies are populated at ambient temperature and thus contribute to the experimental energetics. Again, this rational is fully compatible with guest encapsulation furnishing a distinct inclusion complex that does not allow many alternative structures in solution having free energies within a margin of about 20 kJ mol^{-1} above the most populated arrangement.

When probed with both camphor enantiomers α-CD showed chiral discrimination prefering the (+)- over the (−)-antipode (Table 1, Figure 2). Differentiation almost exculsively is based on enthalpy and rises as the temperature decreases due to a slightly more negative change in heat capacity of (−)-camphor complexation (Figure 2).

Chiral recognition can only derive from the difference in the intrinsic interaction of the chiral entities and should be solvent independent if the diastereomeric surfaces in the (+)-camphor-α-CD and (−)-camphor-α-CD molecular aggregates are not exposed to solvent, but hidden in the interior of an inclusion-type complex. In the concrete case just 2% of the *observed* total enthalpic change turns up as a chiral discriminative factor fostering the suspicion that the *intrinsic* enthalpic change of host and guest interaction is but a small fraction of the association enthalpy ΔH_{ass} measured the rest being due to solvent reorganization. This is corroborated by the solvent isotope effect found in heavy water. Contrary to common expectation which calls for a zero free energy change in hydrophobic complexation on solvent transfer from light to heavy water, the camphor α-CD complexes are unanimously stronger in D_2O by a factor of 2 to 3. Inspection of the energetic state functions reveals the surprising notion that the enhanced complex stabilities emerge from smaller exothermicities (less negative ΔH_{ass}, Table 2) that are outmatched by an even higher rise of the entropy towards less negative values. It is the more favored entropy component that makes the host–guest complexes more stable in heavy water. Enhanced entropy in general reflects diminished structural definition and must relax and counteract enthalpic chiral discrimination if only the host–guest partners dominate the binding process. In fact, in addition to the augmentation of complex strength it is also the enantioselectivity $K_{ass}(+)/K_{ass}(-)$ that is increased by about 12% in D_2O. Chiral discrimination is definitely not solvent independent as required by the original premisses. We are led to postulate that solvent water exerts a structural role in forming the host–guest complexes, surpassing the general physical solvation effects and surfacing as a component of the intrinsic host–guest energetics. The simple lock-and-key picture of bilateral complementarity-based molecular recognition is incompatible with the current facts and requires extension most probably by incorporation of stoichiometric water participation.

Experimental Section

Compounds: (+)- and (−)-Camphor were purchased from Aldrich in 98+% purity (see ref. [24]) and used as received. The stock solutions were prepared by dissolving weighed amounts of the compounds in warm water (80 °C) in closed volumetric flasks to avoid loss of the volatile guests, cooling down, and diluting to the required volume. From these stock solutions the actual titrand solutions were prepared by diluting corresponding aliquots and degassing the solutions in vacuo for less than five minutes at ambient temperature. α-Cyclodextrin was obtained from Fluka and was recrystallized from water and dried at 80 °C in vacuo overnight. For the measurements in D_2O the dehydrated α-cyclodextrin was crystallized twice from D_2O (99.9% D, Deutero GmbH, Germany) to replace exchangable protons by deuterium and dried as described above.

Isothermal calorimetry measurements: The calorimetric determinations were conducted by using a thermostated and fully computer-operated MCS-ITC calorimeter from MicroCal, LLC, Northampton, MA, USA, titrating aliquots of aqueous cyclodextrin solutions (ca. 40 mM) into the camphor solutions (1.5–2.5 mM) contained in the calorimetric cell. The volumes added were incrementally increased but were adjusted to maintain the heat evolved in each titration step within the allowable range at 80% offset current. Data analysis used the customized ITC module of the Origin 5.0 software package and employed a two-step sequential binding model. Since the fit algorithm failed to converge to a single set of regression parameters when different initial values were used, the starting set was varied systematically over a reasonable range (usually about 20 attempts), which produced a collection of resultant sets. These sets represent local minima on the error hypersurface and were reintroduced as the starting set into another fit cycle. Reiteration of this procedure 2–3 more times finally converged to a single stable error minimum which gave the parameters listed in the tables. Several blind titrations were performed to determine and correct for unspecific heat contributions (heats of mixing, heat of dilution etc.); however, all pertinent attempts gave insignificant heat effects.

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