Host-rotaxanes model proteins that promote ligand association through a favorable change in configurational entropy

Jing Zhu and David B. Smithrud*

Received 25th May 2007, Accepted 27th July 2007
First published as an Advance Article on the web 9th August 2007
DOI: 10.1039/b707955a

Proteins can reduce the entropic penalty for ligand association through a favorable change in configurational entropy. To investigate this process, the ΔG^{o}, ΔH^{o}, and ΔS^{o} of complexes formed between host-rotaxanes and guests were determined and compared to discover the relationship between rotaxane-structure and the energies involved in guest-association in water and DMSO. Fluorescence quenching assays provided the association constants. Van't Hoff analysis of variable temperature assays gave the enthalpies of binding. The driving force for the association of a guest and a host-rotaxane can switch from being enthalpically to entropically driven with a change in the solvent or guest. This study shows that a dramatic increase in the entropy of binding can be obtained through the addition of a rotaxane-wheel to a synthetic host. An increased motion of the wheel appears to be the source of the positive binding entropy, which would be an example of favorable configurational entropy promoting complex formation.

Introduction

According to the classic 'lock-and-key'[1] and 'induced-fit'[2] models for protein–ligand interactions, compounds in a bound state have less freedom than in the unbound state. Desolvation of the surfaces that come into contact in the complex can produce a favorable change in entropy. For complexation in water, this can be the driving force for association.[3] Recent investigation of several proteins *via* NMR relaxation experiments has revealed that some protein residues, even ones at the combining site, increase their freedom of motion when a ligand is bound.[4] The binding of a hydrophobic pheromone to the mouse major urinary protein results in an observable enhancement in the flexibility of several backbone residues near the binding site.[5] The calculated entropy of configuration ($T\Delta S_{conf}$) from this enhanced motion is large enough to give a significant contribution to the stability of the complex. Backbone residues of the dimerization/docking domain of the protein kinase holoenzyme become more flexible when bound to a hydrophobic domain of the Ht31 peptide.[6] Six residues in the active site of 4-oxalocrotonate tautomerase increase their mobility upon binding to an inhibitor.[7] In this case, however, other residues become more restricted, resulting in an overall loss in binding free energy. These findings suggest that a favorable $T\Delta S_{conf}$ is a possible energy source used by proteins to pay for some of the intrinsic loss of entropy that occurs upon complexation. This mechanism may be necessary for the binding of low molecular weight ligands, which do not undergo extensive water desolvation upon complexation.

Protein–ligand interactions have been investigated using synthetic hosts. Many of these hosts bind guests according to the lock-and-key or the induced-fit model. Generally, the binding of aromatic guests into preformed aromatic pockets is strongly enthalpically driven and entropically unfavorable. Host–guest complexes formed through electrostatic interactions are driven by a favorable change in entropy, which is obtained through desolvation and not from $T\Delta S_{conf}$. We use the rotaxane architecture to create synthetic hosts. Rotaxanes (Fig. 1) comprise a wheel threaded onto an axle with large blocking groups on the ends to keep the wheel from de-threading.[8] The conversion of rotaxanes into host-rotaxanes (HRs) involves using a synthetic host, *e.g.*, calixarene, cyclophane, or cleft as a blocking group.[9–11] Additional functional groups for guest recognition are attached to the wheel. Having the binding domain split between intracomponent pieces results in a unique relationship between guest association and the conformational changes that occur as the wheel slides along and pirouettes around the axle. Since these hosts use multiple conformations to bind guests, they are uniquely suited to be models of protein binding domains.

Cyclophanes and clefts (referred to as pockets) were used in the HRs to model the binding domains of antibodies and receptors. Rotaxanes with a cyclophane and wheel(s) represent an antibody's hydrophobic groove and hypervariable loops, respectively. To produce a rigid, deep, hydrophobic pocket, the cyclophane of cyclophane-[2]rotaxane **2** (Cy2R **2**) and cyclophane-[3]rotaxane **3** (Cy3R **3**) contains octamethoxy groups and piperidone rings on both ends.[12] These groups, however, keep the wheel and its arginines from forming a small combining site for guests.[13] Cyclophane-[2]rotaxane **1** (Cy2R **1**) lacks these groups on the wheel side of its cyclophane and thus forms tight complexes with small guests.[13] Cleft containing HRs more closely mimic receptors that contain functional and structural epitopes.[14] To determine the importance of wheel motion for the formation of tight complexes, the axle-amine of cleft-[2]rotaxane **4** (Cleft2R **4**) was acetylated to give cleft-[2]rotaxane **5** (Cleft2R **5**).[11] A restricted wheel resulted in weaker affinity for some guests and reduced intracellular transport. Calix[4]arene **6** (Calix **6**) contains arginine moieties

Department of Chemistry, University of Cincinnati, Cincinnati, OH, 45221, USA. E-mail: david.smithrud@uc.edu; Fax: +1 513 556 9239; Tel: +1 513 556 9254

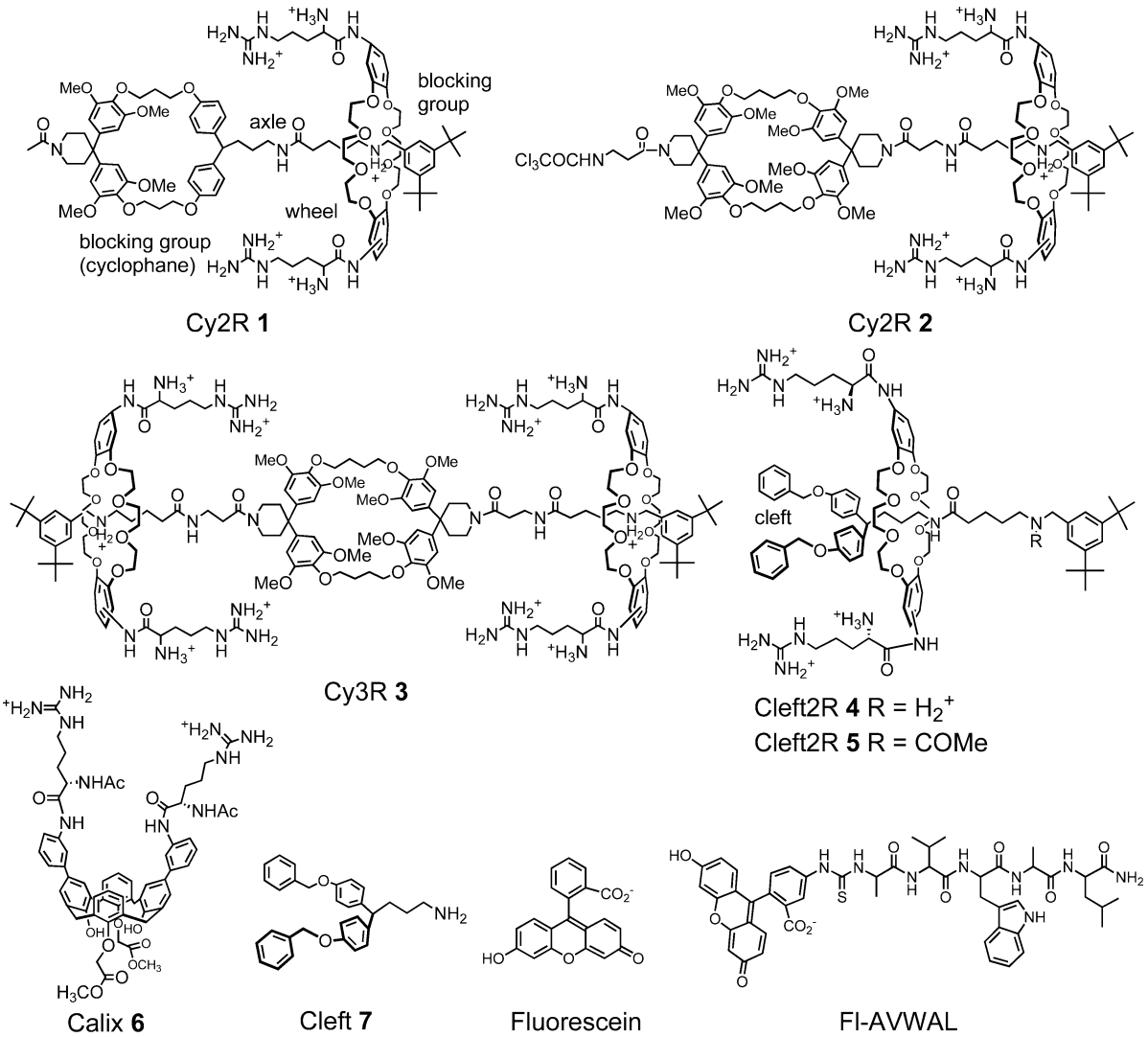


Fig. 1 Hosts and guests used in this study. The sliding motion of the wheel along the axle enables the host-rotaxanes (Cy2R **1**, Cy2R **2**, Cy3R **3**, Cleft2R **4**, and Cleft2R **5**) to form multiple conformations to bind the guests in various environments.

covalently linked to an aromatic cleft.[9] Comparing its binding affinity for guests to the HRs will demonstrate any advantage afforded the HRs by having their arginines attached to a wheel. Cleft **7** is soluble enough in water to be used as a representative aromatic pocket for association. Comparing its binding affinity for guests to the HRs will show the effect the wheel and its arginines have on complexation.

We found that only a few HRs bind with the characteristic thermodynamic energies observed for complexes that form through a lock-and-key or an induced-fit model. The unusual entropic binding energy appears to arise through a release of the wheel upon guest binding, which would be an example of a favorable $T\Delta S_{conf}$ term for association. The results show for the first time that a rotaxane-wheel can be incorporated with a host to produce a favorable entropy term for molecular association. Since traditional hosts suffer from an unfavorable $T\Delta S_{conf}$, which is correlated with favorable binding,[15] this finding is a breakthrough in the design of synthetic hosts. The results also strongly support the recent discoveries of favorable configurational entropy in protein–ligand interactions.

Results

Deriving the thermodynamic energies for association

Host-rotaxanes bind negatively charged, aromatic guests in the low micromolar range. Since fluorescence quenching assays are a convenient method to monitor tight host–guest association, fluorescein and a fluoresceinated pentapeptide (Fl-AVWAL, Fig. 1) were chosen as representative guests. Fl-AVWAL is a large guest, as compared to the hosts, and can form various conformations. Fluorescein is a smaller and rigid guest. We expected the wheel(s) of an HR to adjust its position to accommodate both the larger guest Fl-AVWAL and the smaller guest fluorescein. Repositioning of the wheel should be reflected in the thermodynamic energies. Since $T\Delta S_{conf}$ can possibly arise through a solvent effect,[5]

complexes were formed in water (phosphate buffer, pH 7.4) and DMSO. Nonlinear least squares analysis of plots that compare the fluorescence intensity of a guest to the changing concentration of a host provided the association constants (K_A's).[16] The assays were performed at five different temperatures for each complex. Slopes of the plots of $\ln K_A$ *versus* $1/T$ are equal to $\Delta H^\circ/R$, according to van't Hoff analysis. Linear plots were obtained, which shows that the change in the heat capacity is small for these complexes. Small ΔC_p° values (on average 60 cal mol^{-1} K^{-1})[17] exist for complex formation between aromatic guests and the cyclophane that is used as the pocket in Cy2R **2** and Cy3R **3**. Our studies were performed in the same temperature range as these experiments. From the free energy ($\Delta G^\circ = -RT\ln K_A$) and ΔH° values, changes in entropy were derived from the Gibbs–Helmholtz equation ($\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$). A wide range of thermodynamic energies was obtained for the various host–guest complexes (Table 1 and Fig. 2).

Investigating the lock-and-key model

Designing hosts according to the lock-and-key model is beneficial. A reorientation of the recognition elements during the binding event could cost part or all of the available binding free energy. Cyclophanes bind aromatic guests using the lock-and-key mechanism for association, showing an enthalpically driven process (highly negative ΔH° and a negative ΔS°). Even though Cy2R **1**, Cy2R **2**, and Cy3R **3** contain cyclophane-pockets, most of their complexes

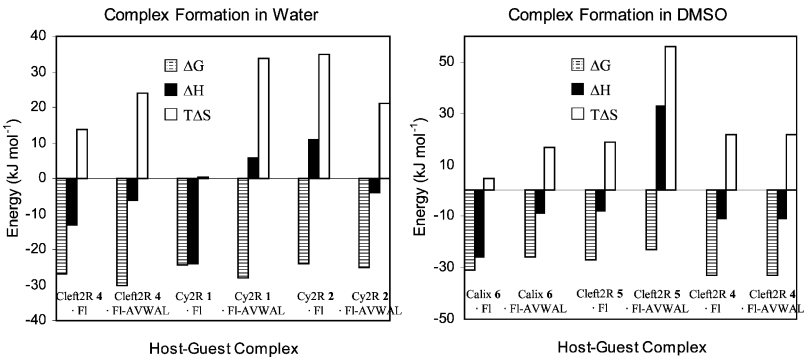


Fig. 2 Representative examples of the thermodynamic energies produced for various complexes (see Table 1).

Table 1 Association constants (M^{-1}) and thermodynamic energies[a]

Host	Guest	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C	$\Delta G^{\circ b}$	$\Delta H^{\circ c}$	$\Delta S^{\circ d}$
DMSO										
Cy2R **1**	Fl		37	35	24	21	14	−31	−36	−17
	Fl-AVWAL		40	44	46	49	50	−32	8	130
Cy2R **2**	Fl		2.7	3.0	3.2	3.4	3.5	−26	10	120
	Fl-AVWAL		13	14	15	16	17	−30	10	130
Cy3R **3**	Fl		5.5	5.1	4.9	4.2	4.0	−27	−12	50
	Fl-AVWAL		50	48	47	45	43	−33	−7	87
Cleft2R **4**	Fl		56	50	47	45	40	−33	−11	74
	Fl-AVWAL		62	55	52	49	45	−33	−11	74
Cleft2R **5**	Fl		5.0	4.8	4.6	4.4	4.0	−27	−8	64
	Fl-AVWAL		0.9	1.2	1.8	1.9	2.1	−23	33	190
Calix **6**	Fl		34	30	22	20	18	−31	−26	17
	Fl-AVWAL		5.3	4.8	4.7	4.2	4.1	−26	−9	57
Cleft **7**	Fl		1.4	1.2	0.87	0.81	0.73	−23	−26	−10
	Fl-AVWAL		2.4	2.2	1.9	1.5	1.3	−24	−22	7
98% H_2O^e–2% DMSO										
Cy2R **1**	Fl	3.6	2.4	2.2	2.0	1.7		−24	−24	0.4
	Fl-AVWAL	9.7	10	11	12	12		−28	6	110
Cy2R **2**	Fl	1.8	1.9	2.3	2.4	2.4		−24	11	120
	Fl-AVWAL	3.1	2.9	2.8	2.8	2.7		−25	−4	70
Cy3R **3**	Fl	3.2	3.1	2.8	2.7	2.4		−25	−11	47
	Fl-AVWAL	3.4	3.4	3.3	3.3	3.2		−25	−2	77
Cleft2R **4**	Fl	47	42	38	35	33		−27	−13	47
	Fl-AVWAL	19	18	17	17	16		−30	−6	81
Cleft2R **5**	Fl	3.2	2.7	2.2	2.1	1.4		−24	−27	−7
	Fl-AVWAL	2.9	2.8	2.6	2.5	2.4		−25	−6	64
Calix **6**	Fl	2.0	1.7	1.4	1.3	1.2		−24	−20	13
	Fl-AVWAL	2.6	2.4	2.3	2.1	1.8		−24	−12	44
Cleft **7**	Fl	0.59	0.50	0.39	0.33	0.27		−21	−29	−27
	Fl-AVWAL	1.3	1.2	1.1	1.1	1.0		−23	−8	50

[a] Values divided by 1×10^4, obtained from fluorescence quenching assays, uncertainty in K_A's $\leq 5\%$. [b] ΔG° calculated for 25 °C, kJ mol^{-1}. [c] kJ mol^{-1}, uncertainty in $\Delta H^\circ < 10\%$. [d] Calculated for 25 °C, J mol^{-1} K^{-1}, uncertainty in $\Delta S^\circ < 10\%$. [e] Buffered with 1 mM phosphate pH 7.4.

are entropically favorable (Table 1). Only the complex between Cy2R **1** and fluorescein in DMSO ($\Delta H^\circ = -36$ kJ mol^{-1} and $\Delta S^\circ = -17$ J mol^{-1} K^{-1}) shows the characteristic thermodynamic energies of cyclophanes. For the structurally similar HRs (Cleft2R **4**, Cy2R **1**, and Cy2R **2**), the preorganization of their binding pockets increases from Cleft2R **4**, Cy2R **1**, to Cy2R **2**. According to the lock-and-key model, Cy2R **2** should show the most and Cleft2R **4** the least favorable enthalpies of binding. Complexes containing Cy2R **2**, however, are entropically driven and the enthalpy of binding is positive, except for a small amount of heat produced for the binding of Fl-AVWAL in water. Cleft2R **4** consistently shows a favorable enthalpy and entropy of binding. Cy2R **1** binds fluorescein in a highly enthalpically driven process and Fl-AVWAL in a highly entropically driven process. Since the HRs do not show the characteristic binding energies observed with cyclophanes, *i.e.*, the lock-and-key mechanism, their wheels are involved in the binding event. The involvement of wheel(s) could result in an induced-fit mechanism.

Investigating the induced-fit model

In the induced-fit model, a binding pocket rearranges its recognition elements upon guest binding. Geometry optimization leads to a maximization of the enthalpic energy for binding with a concomitant loss of entropic energy. The wheel of an HR can slide along or rotate around the axle to adjust its recognition elements (arginines and aromatic rings) upon guest association. A loss of any rotational or translational freedom of the wheel should detract from the overall binding free energy. Comparing the series of hosts that contain an aromatic cleft and two arginine moieties, the greatest freedom of the recognition elements increases from Calix **6**, Cleft2R **5**, to Cleft2R **4**. Therefore, the greatest loss of freedom for association should be seen for Cleft2R **4**, Cleft2R **5**, and then Calix **6**. Contrary to what would be expected for an induced-fit mechanism, Cleft2R **4** demonstrates a favorable entropy of binding for the complexes investigated, and in most cases, complex formation is entropically driven. Calix **6** binds fluorescein in an enthalpically driven process, and its association of Fl-AVWAL shows a favorable entropy and enthalpy of binding. The binding energies of the complexes between Cleft2R **5** and the guests are highly varied. Interestingly, its binding of Fl-AVWAL in DMSO is highly entropically driven and the enthalpy of binding is large and positive. Apparently, the wheel cannot adjust its position to obtain stable noncovalent bonds with the guest. Even though the wheel is involved in the binding event and can change its position on the axle, the thermodynamic energies of most of the HR-complexes are not consistent with an induced-fit mechanism.

The wheel can produce a favorable entropy of binding

To determine the nature of the binding energy provided by the wheel, a systematic study was performed on a series of hosts: Cleft **7** does not have a wheel, Cleft2R **5** has a restricted wheel, and Cleft2R **4** has a wheel that can slide further along the axle. Cleft **7** binds the guests in an enthalpically driven process except for the association of Fl-AVWAL in water, which occurs through a favorable change in enthalpy and entropy. The existence of a wheel in Cleft2R **4** and Cleft2R **5** results in more entropically favorable complexes. In water, Cleft2R **4**, with a freer wheel, gives a more favorable entropy of binding as compared to Cleft2R **5** (for Cleft **7**, Cleft2R **5**, and Cleft2R **4** bound to Fl-AVWAL $\Delta S^\circ = 50$, 64, 81 J mol^{-1} K^{-1}, respectively and bound to fluorescein $\Delta S^\circ = -27$, -7, 47 J mol^{-1} K^{-1}, respectively). A more dramatic increase in the entropy of binding for complexes formed by Cleft2R **5** and Cleft2R **4**, as compared to Cleft **7**, is seen in DMSO (for the binding of fluorescein $\delta\Delta S^\circ \approx 80$ J mol^{-1} K^{-1} and Fl-AVWAL $\delta\Delta S^\circ \approx 70$–180 J mol^{-1} K^{-1}). Cleft2R **4** and Cleft2R **5** bind both guests with a more favorable entropy of binding than Calix **6** as well, except for the Calix **6**–fluorescein complex as compared to the Cleft2R **5**–fluorescein complex. These results show that the wheel is responsible for the favorable entropic energy, and its role goes beyond just providing arginine moieties for guest recognition.

The existence of a second wheel on an HR, however, can decrease the magnitude of the favorable entropy of binding. Cy3R **3** and Cy2R **2** have the same cyclophane pocket, but Cy3R **3** has an additional wheel. The association of Fl-AVWAL by Cy3R **3** and Cy2R **2** in water shows nearly identical energy values. For the other complexes, Cy2R **2** binds the guests with a large, positive entropy of binding and a positive enthalpy of binding. Cy3R **3** binds these guests with a smaller positive entropy of binding and a negative enthalpy of binding. Most likely, the second wheel of Cy3R **3** interacts with the guests (negative ΔH°), which restricts its motion (negative ΔS°). These results are consistent with the induced-fit model.

Solvent dependency for association

Water is a strong H-bond donor and acceptor, and it forms rigid structures around apolar groups. Breaking these structures upon the extrusion of apolar compounds results in a large favorable change in entropy, which is called the "hydrophobic effect".[3] DMSO, on the other hand, is a moderate H-bond acceptor and does not produce a similar hydrophobic effect upon the release of apolar compounds. Therefore, we were surprised to find that similar thermodynamic energies occur for complex formation in water and DMSO. An examination of the plots of $\Delta H^\circ_{\mathrm{DMSO}}$ *vs.* $\Delta H^\circ_{\mathrm{water}}$ and $\Delta S^\circ_{\mathrm{DMSO}}$ *vs.* $\Delta S^\circ_{\mathrm{water}}$ shows a rough correlation ($R = 0.84$), excluding two complexes of Cleft2R **5** (Fig. 3). Approximately the same slope of 0.7 is observed in both plots. Interestingly, complexation is more entropically driven in DMSO and more enthalpically driven in water. These results indicate that the hydrophobic effect does not drive association in water. Cleft2R **5**'s binding of fluorescein in water and Fl-AVWAL in DMSO do not correlate with the other complexes. Cleft2R **5** is the same host as Cleft2R **4** except that its axle's amine is acetylated. The acetyl group keeps the wheel near to the pocket and reduces its sliding motion. The formation of the Cleft2R **5**–fluorescein complex in water is enthalpically driven and entropically unfavorable. Keeping its wheel next to the cleft results in a tight complex with a guest and the formation of van der Waals interactions. The formation of the Cleft2R **4**–fluorescein complex in water, on the other hand, is entropically driven and shows a favorable binding enthalpy. To form a tight complex, Cleft2R **4** would be entropically unfavorable because the wheel slides on the axle. This could be considered as an example of an "anti-induced-fit" mechanism. Cleft2R **4** binds fluorescein and Fl-AVWAL more favorably than Cleft2R **5** in both solvents. The greater ability of Cleft2R **4** to adjust its binding mode with a change in solvent makes it a better host.

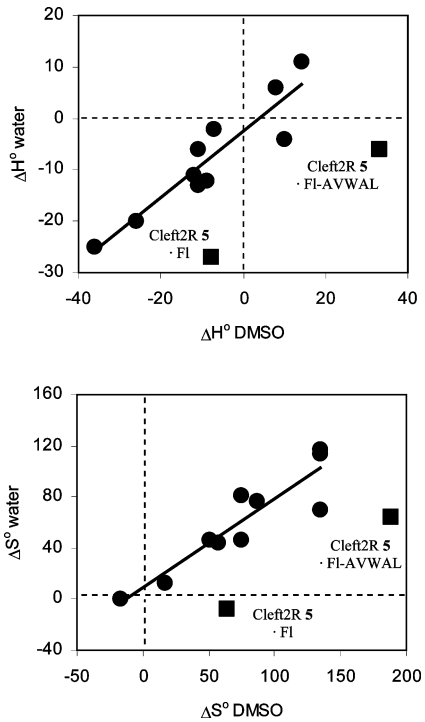


Fig. 3 Plots of enthalpy of binding and entropy of binding for the complexes given in Table 1.

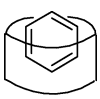
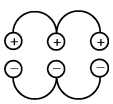
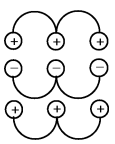
Discussion

Changing the functional groups of synthetic hosts and their guests can result in a wide range of enthalpic and entropic energies. For example, a dimeric calixarene binds a dimeric diaryl sulfonate in a highly enthalpically driven process ($\Delta H^\circ = -85$ kJ mol^{-1} and $\Delta S^\circ = -252$ J mol^{-1} K^{-1}),[18] whereas a pyridine-capped calixarene binds D-valine through a large, positive entropy of binding ($\Delta H^\circ =$ 40 kJ mol^{-1} and $\Delta S^\circ = 200$ J mol^{-1} K^{-1}).[19] The large range in values observed for the complexes with the HRs ($\delta\Delta H^\circ =$ 69 kJ mol^{-1} and $\delta\Delta S^\circ = 205$ J mol^{-1} K^{-1}, Table 1), however, are obtained for hosts that have similar structures and recognition elements. Large differences in the enthalpy and entropy of binding are observed for each guest, and only two HR complexes show an unfavorable entropy of binding. Fl-AVWAL tends to bind through a more entropically favorable process. This is most likely not caused by the peptide portion of Fl-AVWAL. It would have to have more freedom in the bound state than in the unbound state. Furthermore, complexation of peptides by cyclodextrins and modified cyclodextrins are enthalpically driven and the entropic energies are large and negative.[20]

Sources of entropic energy

Tight complexation of a guest within a host results in the loss of rotational and translational degrees of freedom. If the guest forms a 'lid conformation' over the pocket, the loss of entropic energy will not be as large.[24] Other sources of entropic energy are the release of solvent molecules as salt bridges are formed between a host and guest[25] or from the hydrophobic effect.[3] Both processes are likely to occur for complexes between the HRs and the guests. Table 2 gives representative examples from the literature that show the thermodynamic energies obtained for these types of complexes. The hosts investigated in this study do not show the enthalpically driven, tight complexes of cyclophanes (entry 1 or 2 of Table 2). The ΔH° and ΔS° values (Table 1) match more closely to the values observed for complexes that form through salt bridges in DMSO (entry 6 and 7 of Table 2) or multiple salt bridges in water (entries 3 and 4 of Table 2). Because of the geometric constraints imposed by the wheel, a single salt bridge is most likely formed between the HRs and the guests. Calix **6** could

Table 2 Thermodynamics of representative host–guest complexes[a]

Entry	Water	ΔG°	ΔH°	ΔS°	(Ref)	Entry	DMSO	ΔG°	ΔH°	ΔS°	(Ref)
1		−36	−112	−255	(21)	5		−17	−27	−35	(17*a*)
2		−21	−50	−84	(17*a*)						
3		−18	−5	+44	(22)	6		−22	−14	+26	(23)
4		−16	+13	+94	(22)	7		−13	+11	+76	(23)

[a] Cartoon depiction of hosts and guests, showing key features: complete burial (entries 1 and 5), partial burial (entry 2), and ionic interactions (entries 3, 4, 6, 7). ΔG° and ΔH° kJ mol^{-1} and ΔS° J mol^{-1} K^{-1}.

form two salt bridges between its arginine moieties and fluorescein or the fluorescein moiety of Fl-AVWAL. The enthalpy of binding for the Calix **6**–guest complexes, especially for fluorescein, is more favorable than would be expected for the formation of salt bridges only. For hosts that bind the guests with favorable changes in enthalpy and entropy, they most likely bind through a combination of salt bridge formation and aromatic–aromatic interactions. H-bonds are also possible between the guests and the hosts. The greater entropy of binding generally observed for the association of Fl-AVWAL is most likely a result of its larger size than fluorescein. It cannot be buried as deeply inside a pocket as fluorescein, and thus, it forms a more lid-like binding conformation. Furthermore, one of the many peptidic side chains of Fl-AVWAL can reside in a pocket, giving it multiple binding conformations.

Maximization of the free energy of binding

The induced-fit and lock-and-key models are based on a maximization of the favorable enthalpy of binding with a concomitant loss of entropy. The HRs bind guests through a maximization of the binding free energy, whether it comes from favorable change in enthalpy, entropy, or a combination of both terms. They can optimize their conformations for the binding of guests of different sizes, *e.g.*, fluorescein and Fl-AVWAL. In principle, for larger guests, the wheel(s) move away from the pocket, and for smaller guests, the wheel(s) move towards the pockets. Cy2R **1** and Calix **6** bind fluorescein in water ($\Delta G^\circ = -24$ kJ mol^{-1}) and in DMSO ($\Delta G^\circ = -31$ kJ mol^{-1}) with the same free energy of binding (experimental uncertainty of <5%). To obtain these free energies, Cy2R **1** demonstrates a greater enthalpic driving force than does Calix **6** in water and especially in DMSO. Cy2R **1** can adjust its functional groups to form a tight complex with fluorescein (negative ΔH° and negative ΔS°). Fl-AVWAL, on the other hand, is bound more favorably by Cy2R **1** than by Calix **6** in water ($\Delta G^\circ = -28$ and -24 kJ mol^{-1}, respectively) and DMSO ($\Delta G^\circ = -32$ and -26 kJ mol^{-1}, respectively). Calix **6** binds Fl-AVWAL in both solvents with a similar contribution from the entropies and enthalpies of binding. A striking difference in the enthalpies and entropies of binding are seen for the association of Fl-AVWAL by Cy2R **1** as compared to Calix **6**. The Cy2R **1**–Fl-AVWAL complex is entropically driven and enthalpically unfavorable in both solvents. Cy2R **1**'s dramatic ΔH° to ΔS° switch in the driving force with a change in guest does not occur for Calix **6**. The existence of a wheel, however, does not guarantee that a host will have higher affinities for guests. For example, Cy2R **2** binds fluorescein weaker than Cy2R **1** and Calix **6** in DMSO. The pyridinyl ring of Cy2R **2** separates the wheel from the pocket, which prevents the tight, enthalpically driven complex from forming as seen with Cy2R **1**. These results demonstrate that HRs can operate as planned by switching their binding modes to obtain the most stable complex. However, to maximize the binding free energy for some guests, both the wheel and the pocket have to be involved in the complex.

Compensation between ΔH° and ΔS°

Another factor controlling the magnitude of the binding free energy is the compensation between the changes in enthalpy and entropy, which is usually observed in host–guest chemistry.[20,26] A plot of the enthalpy of binding against the entropy of binding for our hosts is linear ($R = 0.94$, Fig. 4). A plot of ΔH° *versus* ΔS° of the complexes for the representative hosts given in Table 2 is also linear ($R = 0.99$). Because the slopes and intercepts of these lines are similar for our and the representative hosts ($\Delta S^\circ = 3.3\ (\Delta H^\circ) + 93$ and $\Delta S^\circ = 2.8\ (\Delta H^\circ) + 54$, respectively), salt bridge and aromatic interactions most likely dominate complex formation for the hosts and guests used in this study. The points for our hosts are shifted slightly upward and into the right quadrant, which demonstrates the importance of positive entropic energies for complex stability. The same ΔH°–ΔS° correlation is seen for the host–guest complexes in DMSO and water. This result was unexpected because water and DMSO have different solvating properties. Furthermore, the unique H-bonding properties of water makes compensation a natural phenomenon.[27] The same compensation across solvents has been observed in previous studies. Complexes of calixarenes and negatively charged aromatic guests showed a similar ΔH°–ΔS° compensation in water and in DMF.[28] The very tight complex of pyrene into the cyclophane pocket shown in entries 1 and 5 of Table 2 demonstrates a ΔH°–ΔS° compensation in eight different solvents.[17a]

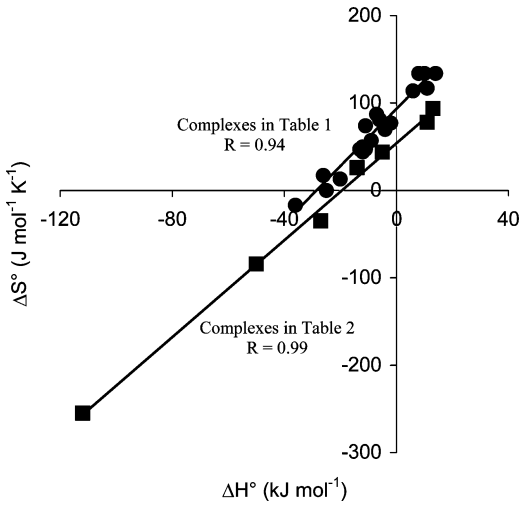


Fig. 4 Enthalpy–entropy compensation plots for the complexes given in Table 1 (circles) and Table 2 (squares).

The slope (α) and intercept ($T\Delta S^\circ$) of plots of ΔH° *versus* $T\Delta S^\circ$ are used as a quantitative measure of the conformational changes and the extent of desolvation, respectively, that occur during complexation.[26c] A ΔH° *versus* $T\Delta S^\circ$ plot for the complexes presented in Table 1 gives $\alpha = 0.97$ and $T\Delta S^\circ = 27$ kJ mol^{-1}. A slope of $\alpha = 0.97$ shows that the hosts, guests, or solvent undergo substantial changes in conformation during the binding events. The large, positive $T\Delta S^\circ$ value of 27 kJ mol^{-1} suggests that extensive desolvation occurs upon complexation. Similar α and $T\Delta S^\circ$ values occur for the association of *p*-sulfonatocalixarenes with metal cations and alkylammonium ions ($\alpha = 1.1$ and $T\Delta S^\circ = 20$ kJ mol^{-1}).[20,25] In this system, however, the large entropy of binding stems from the release of a large number of water molecules as the lanthanide metals (Ln^{3+}) bind to four SO_3^- groups, which are linked to the calixarene. Multiple ionic bonds are highly unlikely to occur for the complexes discussed here. Furthermore, the largest entropic gains for the HR-complexes

occur in DMSO. As compared to water, DMSO weakly associates with anionic groups, and fewer solvent molecules will be released upon binding. Antibiotics also bind metals with a similar ΔH°–$T\Delta S^{\circ}$ correlation ($a = 0.95$ and $T\Delta S^{\circ} = 23$ kJ mol^{-1}),[26d] as seen for the HRs. For these complexes to form, large conformational changes are required. An extensive conformational change is a more likely mechanism for our host-rotaxanes.

The origin of $T\Delta S_{conf}$

The most surprising results are the complexes that are highly entropically driven and produce an unfavorable enthalpy of binding. Only the HRs form these complexes, and they occur with both guests in both solvents. So what is the source of the unusually favorable entropy of binding observed with most HR-complexes? One intriguing possibility is that the wheel increases its motion when these complexes form, which pays for some or all of the intrinsic loss of entropy that occurs upon complexation. The entropic and enthalpic terms for this proposed process are given in Fig. 5. Accordingly, arginine or the aromatic moieties of the wheel interact favorably with the pocket in the unbound state. Guest binding (negative $\Delta H^{\circ}_{host-guest}$ and negative $\Delta S^{\circ}_{host-guest}$) breaks the favorable interactions between the wheel and the pocket (positive $\Delta H^{\circ}_{wheel-pocket}$ and positive $\Delta S^{\circ}_{wheel-pocket}$). Favorable interactions between the wheel and the guest diminish the wheels motion (negative $\Delta H^{\circ}_{wheel-guest}$ and negative $\Delta S^{\circ}_{wheel-guest}$). Complexes that are likely to gain binding energy from a release of the wheel occur between hosts that are not highly preorganized for the guest. These complexes experience small, negative values for $\Delta H^{\circ}_{host-guest}$ and $\Delta S^{\circ}_{host-guest}$. If $\Delta H^{\circ}_{host-guest} < \Delta H^{\circ}_{wheel-pocket}$ and $\Delta S^{\circ}_{wheel-pocket} + \Delta S^{\circ}_{wheel-guest} > 0$, the wheel is released upon guest binding, and the resulting entropy pays for some of the entropic penalty for host–guest association $\Delta S^{\circ}_{host-guest}$. For example, Cy2R **2** is not designed to recognize small guests. The piperidinyl ring keeps the wheel away from its cyclophane pocket. Cy2R **2** binds both guests in both solvents through a favorable entropy of binding. Only the association of the large Fl-AVWAL in water produces heat, albeit only a small amount. Cy2R **1** is designed to form a tight complex

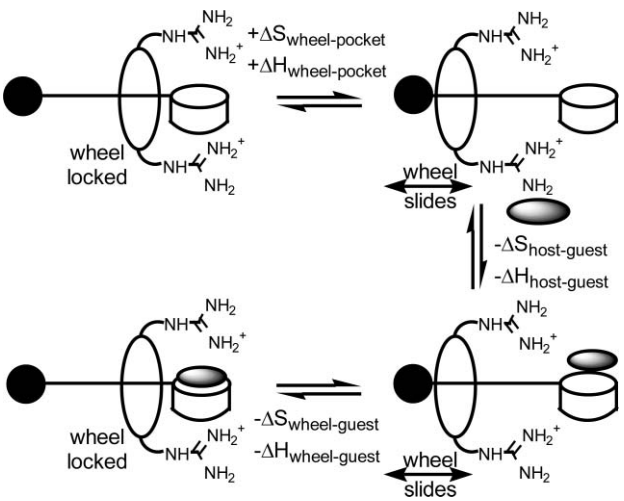


Fig. 5 A possible mechanism used by the HRs to bind guests. The wheel could provide a favorable entropic energy for the complexes in the case that $\Delta S^{\circ}_{wheel-pocket} + \Delta S^{\circ}_{wheel-guest} > 0$, which would be an example of favorable ΔS°_{conf}.

with the smaller fluorescein, but not with the larger guest Fl-AVWAL. In both solvents, the association of fluorescein is highly enthalpically driven, whereas Fl-AVWAL is entropically driven.

The largest $T\Delta S_{conf}$ produced should occur for cases in which the wheel gains the greatest amount of freedom after complexation. Cleft2R **4** appears to have a less restricted wheel than Cleft2R **5**. Contrary to our prediction, the largest positive entropy and enthalpy of binding are observed for the binding of Fl-AVWAL by Cleft2R **5** in DMSO ($\Delta H^{\circ} = 33$ kJ mol^{-1} and $\Delta S^{\circ} = 190$ J mol^{-1} K^{-1}) and not by Cleft2R **4** ($\Delta H^{\circ} = -11$ kJ mol^{-1} and $\Delta S^{\circ} = 74$ J mol^{-1} K^{-1}). On the other hand, in water, the entropy of binding is greater for the Cleft2R **4**–Fl-AVWAL than the Cleft2R **5**–Fl-AVWAL complex ($\Delta S^{\circ} = 81$ and 64 J mol^{-1} K^{-1}, respectively), which results in a more stable complex for Cleft2R **4**–Fl-AVWAL. Possibly, in DMSO, the available ammonium ion in the axle of Cleft2R **4** interacts favorably with the wheel and reduces its freedom. In water, the interaction strength between the ammonium ion of the axle and the oxygen atoms of the wheel is reduced and the wheel is freer to slide. This solvent dependency for the binding entropy is consistent with the freedom of the wheel being a major source of $T\Delta S_{conf}$. Solvent molecules could also affect the motion of the wheel more directly. Water molecules form cage like structures around the hydrophobic groups of the wheel, blocking group, and pocket. These tight H-bonded cages may need to be broken to enable the wheel to slide along the axle, which would restrict the motion of the wheel, giving a diminished $T\Delta S_{conf}$. These cages do not form in DMSO. This difference in solvent properties can explain why a more positive entropy of binding is generally observed for complexes formed in DMSO than in water.

As discussed in the Introduction section, binding of a hydrophobic pheromone to the mouse major urinary protein results in an observable enhancement in the flexibility of several backbone residues near the binding site, which provides a favorable $T\Delta S_{conf}$ term.[5] The binding site is a β-barrel, and a large number of residues showing an enhanced motion are found in an α-helix that apparently acts as a gate to control ligand access to the barrel. Stone proposes two mechanisms that would account for the increased flexibility. The gate could be tightly coordinated to the barrel until a ligand binds, which releases the gate or a tight water cage rigidifies the gate until a ligand binds, which breaks the water cage. Both mechanisms are consistent with the results obtained for the binding of guests by host-rotaxanes. Considering the results from this study, we suggest that to maximize $T\Delta S_{conf}$, a ligand needs to both release the gate from the barrel and break the water structures that surround the residues. Protein binding sites that have weak interactions between flexible residues that do not interact strongly with a ligand and are not strongly solvated with water appear to be likely candidates for a favorable entropy of configuration term.

Conclusions

The entropy of binding is favorable for most HR-complexes, and in some cases, it is the driving force. In several cases, the driving force for association changes between being entropically driven, enthalpically driven, or a combination of both terms with a change of guest or solvent. The results from this study are consistent with the wheels adjusting their binding conformations to maximize the

binding free energy of the HR complexes. For some complexes, the wheel and pocket interact strongly with a guest. These complexes are enthalpically driven and follow an induced-fit model. Other complexes are formed through a highly entropically driven process. In these complexes, the motion of the wheel appears to increase upon guest binding. The greater motion of the wheel would produce a favorable change of entropy that could pay for some of the intrinsic loss of entropy that occurs when a guest is bound. This process models proteins that obtain binding free energy through the enhanced motion of residues after a ligand binds ($T\Delta S_{conf}$). We are currently designing new HRs to more fully investigate this process and to take advantage of this binding mechanism to improve their performance as protein mimetics.

Experimental

Fl-AVWAL was purchased from EZBiolab. Anhydrous DMSO and fluorescein were purchased from Aldrich. Fluorescence quenching assays were performed to obtain the association constants. The water solution was buffered with phosphate (1 mM) at pH 7.4. One of these solutions (2.7 mL) was placed into a 3.5 mL cuvette, and in the case of DMSO, placed under a flow of Ar. A circulating bath was used to set the temperature of the solution in the cuvette. A microthermometer was placed into the solution and read to adjust the temperature to within ±0.1 degrees. Guests were added to these solutions from a DMSO stock solution, containing 1 equivalence of Me_4NOH. Multiple aliquots of a HR stock solution in DMSO were added to the cuvette to change the HR concentration. Both stock solutions contained 3 Å molecular sieves. The total change in volume caused by addition of the guest and a HR was less than 2%. The concentrations of the components were set according to the K_A of a complex: (1) K_A of 5×10^5 to 2×10^5 M^{-1}, [guest] = 1×10^{-7} M, [host] = 5×10^{-8} to 5×10^{-4} M, (2) K_A of 1×10^5 to 5×10^4 M^{-1}, [guest] = 9×10^{-7} M, [host] = 1×10^{-6} to 9×10^{-4} M, and (3) K_A of 4×10^4 to 1×10^4 M^{-1}, [guest] = 1×10^{-5} M, [host] = 5×10^{-6} to 2×10^{-3} M. A small amount of precipitation occurred when the host concentration reached 2 mM. The same K_A within experimental error was obtained when this data point was included or excluded in the binding plot. The guests are soluble in the range investigated. The fluorescence spectrum was recorded and analyzed after each addition of a host. Plots of the changes observed in the quenching assays were fitted using a nonlinear least-squares procedure to derive K_A and ΔF_{max} values.[16] The assays were duplicated, giving a standard deviation of less than 5% of the value obtained for the association constant.

Acknowledgements

This material is based upon work supported by the National Science Foundation under grant no. CHE-0400539.

Notes and references

1 E. Fischer, *Ber. Dtsch. Chem. Ges.*, 1894, **27**, 2985.
2 D. Koshland, *Proc. Natl. Acad. Sci. U. S. A.*, 1958, **44**, 98.
3 (*a*) J. Kyte, *Biophys. Chem.*, 2003, **100**, 193; (*b*) H. F. Xu and K. A. Dill, *J. Phys. Chem. B*, 2005, **109**, 23611; (*c*) K. Dill, *Biochemistry*, 1990, **29**, 7133; (*d*) C. Tanford, *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, Wiley, New York, 2nd edn, 1980.
4 (*a*) P. J. Finerty, Jr., A. K. Mittermaier, R. Muhandiram, L. E. Kay and J. D. Forman-Kay, *Biochemistry*, 2005, **44**, 694; (*b*) M. J. Stone, *Acc. Chem. Res.*, 2001, **34**, 379.
5 L. Zídek, M. V. Novotny and M. J. Stone, *Nat. Struct. Biol.*, 1999, **6**, 1118.
6 J. D. Scott and P. A. Jennings, *J. Biol. Chem.*, 2003, **278**, 18581.
7 J. T. Stivers, C. Abeygunawardana, A. S. Mildvan and C. P. Whitman, *Biochemistry*, 1996, **35**, 16036.
8 (*a*) B. Balzani, M. Gomex-Lopez and J. F. Stoddart, *Acc. Chem. Res.*, 1998, **31**, 405; (*b*) *Molecular catenanes, rotaxanes and knots: a journey through the world of molecular topology*, ed. J.-P. Sauvage and C. O. Dietrich-Buchecker, Wiley-VCH, Weinheim, 1999.
9 I. Smukste, B. E. House and D. B. Smithrud, *J. Org. Chem.*, 2003, **68**, 2559.
10 V. Dvornikovs, B. E. House, M. Kaetzel, J. R. Dedman and D. B. Smithrud, *J. Am. Chem. Soc.*, 2003, **125**, 8290.
11 X. Bao, I. Isaacsohn, A. F. Drew and D. B. Smithrud, *J. Am. Chem. Soc.*, 2006, **128**, 12229.
12 F. Diederich, in Cyclophanes, *Monographs in Supramolecular Chemistry*, ed. J. F. Stoddart, The Royal Society of Chemistry, Cambridge, UK, 1991.
13 X. Bao, I. Isaacsohn, A. F. Drew and D. B. Smithrud, *J. Org. Chem.*, 2007, **72**, 3988.
14 (*a*) W. L. DeLano, *Curr. Opin. Struct. Biol.*, 2002, **12**, 14; (*b*) T. Clackson and J. A. Wells, *Science*, 1995, **267**, 383.
15 C. E. Chang and M. K. Gilson, *J. Am. Chem. Soc.*, 2004, **126**, 13156.
16 K. A. Conners, *Binding Constants, The Measurement of Molecular Complex Stability*, Wiley, New York, 1987.
17 (*a*) D. B. Smithrud, T. B. Wyman and F. Diederich, *J. Am. Chem. Soc.*, 1991, **113**, 5420; (*b*) D. B. Smithrud and F. Diederich, *J. Am. Chem. Soc.*, 1990, **112**, 339.
18 B. Zhang and R. Breslow, *J. Am. Chem. Soc.*, 1993, **115**, 9353.
19 Y. Liu, Y.-M. Zhang, S.-X. Sun, Y.-M. Li and R.-T. Chen, *J. Chem. Soc., Perkin Trans. 2*, 1997, 1609.
20 N. Douteau-Guevel, F. Perret, A. W. Coleman, J.-P. Morel and N. Morel-Desrosiers, *J. Chem. Soc., Perkin Trans. 2*, 2002, 524.
21 The values for water were estimated by plugging the free energy for the complex in water (see ref. 17*b*) into the extrathermodynamic equations given in ref. 17*a*. The derived ΔH° and ΔS° are reasonable considering values obtained by Breslow and Zhang, ref. 18.
22 M. Rekharsky, Y. Inoue, S. Tobey, A. Metzger and E. Anslyn, *J. Am. Chem. Soc.*, 2002, **124**, 14959.
23 M. Berger and F. P. Schmidtchen, *J. Am. Chem. Soc.*, 1999, **121**, 9986.
24 E. Quinones and C. E. Feliciano, *J. Photochem. Photobiol., A*, 1999, **120**, 23.
25 C. Bonal, Y. Israëli, J.-P. Morel and N. Morel-Desrosiers, *J. Chem. Soc., Perkin Trans. 2*, 2001, 1075.
26 (*a*) M. V. Rekharsky and Y. Inoue, *Chem. Rev.*, 1998, 1875; (*b*) Y. Inoue, Y. Liu, L. H. Tong, B. J. Shen and D. S. Jin, *J. Am. Chem. Soc.*, 1993, **115**, 10637; (*c*) Y. Inoue, T. Hakushi, Y. Liu, L. H. Tong, B. J. Shen and D. S. Jin, *J. Am. Chem. Soc.*, 1993, **115**, 475; (*d*) Y. Inoue and T. Hakushi, *J. Chem. Soc., Perkin Trans. 2*, 1985, 935.
27 (*a*) E. A. Lewis and L. D. Hansen, *J. Chem. Soc., Perkin Trans. 2*, 1973, 2081; (*b*) R. Lumry and S. J. Rajender, *Biopolymers*, 1970, **9**, 1125.
28 A. F. Danil de Namor, R. Traboulssi and D. F. V. Lewis, *J. Am. Chem. Soc.*, 1990, **112**, 8442.