Enthalpic Domination of the Chelate Effect in Cyclodextrin Dimers

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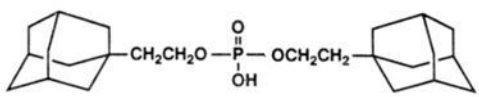
We have described the strong binding of ditopic substrates, in water and other highly polar solvents, into cyclodextrins dimerized with various linkers.[1-4] The free energies of binding were often almost double those for binding involving corresponding monomers, and sometimes even higher. This is an example of the chelate effect.[5-7] We have now examined the thermodynamics of binding to some of our cyclodextrin dimers using direct titration calorimetry. Strikingly, the chelate effect here—which is classically explained[7] in entropy terms—is entirely due to an improved *enthalpy* of binding that overcomes an unfavorable entropy change.

The binding of substrates **1–4** into hosts **5–8** was examined in 20 mM HEPES aqueous buffer at 25.0 °C. Titration microcalorimetry was performed using an OMEGA calorimeter[8] by titrating a 2–10 mM solution of **5** into a 0.07–0.5 mM solution of guests **1–4** or a 0.3 mM solution of hosts **6–8** into a 0.01 mM solution of guests **2–4**. The data reported in Table I are the average of three or more independent runs. The binding constants determined by this method were also compared with those from fluorimetric measurements. As the data in entries 1, 4, 5, and 8 of Table I show, the agreement was excellent.



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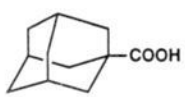

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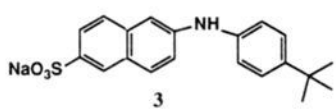

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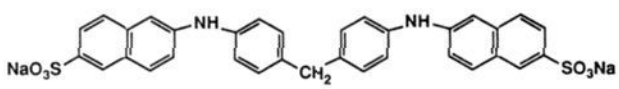

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Titrating β-cyclodextrin **5** into ditopic substrates **2** or **4** elicited two well-separated binding curves for the formation of a one-to-one and a two-to-one complex. As entries 2 and 3 show, the second binding constant for the **5/2** complex was 50 times smaller than the first, while for **5/4** the ratio is 34. Since statistics would have led to a ratio of only 4, these results show that binding of the first cyclodextrin interferes geometrically to some extent with the binding of the second cyclodextrin. This also shows up in the $\delta\Delta H°$ for **5/2**, but less so for **5/4**.

Table I. Complex Stability Constants (K_f) and Thermodynamic Parameters for Monotopic and Ditopic Binding to β-Cyclodextrin and β-Cyclodextrin Dimers in Water at 25 °C[a]

no.	host	guest	K_f (M^{-1})	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$T\Delta S°$ (kcal/mol)
1	**5**	**1**	$(3.95 \pm 0.15) \times 10^4$ [i]	−6.27	−5.21 ± 0.05	1.06
2	**5**[b]	**2**[c]	$(2.26 \pm 0.52) \times 10^5$	−7.30	−7.00 ± 0.09	0.30
3	**5**[d]	**2**	$(4.39 \pm 0.37) \times 10^3$	−4.97	−3.84 ± 0.11	1.13
4	**6**[e]	**2**	$(1.79 \pm 0.25) \times 10^7$ [j]	−9.89	−16.15 ± 1.05	−6.26
5	**7**[f]	**2**	$(1.13 \pm 0.11) \times 10^7$ [k]	−9.62	−14.45 ± 0.84	−4.83
6	**8**[g]	**2**	$(2.14 \pm 0.13) \times 10^6$	−8.63	−14.89 ± 1.30	−6.26
7	**5**	**3**[e]	$(5.57 \pm 0.30) \times 10^4$	−6.47	−6.04 ± 0.07	0.42
8	**6**	**3**	$(3.67 \pm 0.50) \times 10^6$ [l]	−8.95	−15.66 ± 0.50	−6.71
9	**5**[b]	**4**[h]	$(8.05 \pm 0.31) \times 10^4$	−6.69	−4.42 ± 0.40	2.27
10	**5**[d]	**4**	$(2.34 \pm 0.20) \times 10^3$	−4.59	−3.87 ± 0.03	0.72
11	**7**	**4**	$(3.50 \pm 0.11) \times 10^7$	−10.29	−21.40 ± 1.30	−11.11

[a] Values determined microcalorimetrically in 20 mM HEPES buffer solution (pH 7.0); average of more than three independent runs. Error ranges are shown only for the quantities directly measured, not for those calculated. [b] Binding of the first host molecule to the guest. [c] Synthesized from the corresponding alcohol and characterized by MS and ^{1}H NMR. [d] Binding of the second host molecule to the guest. [e] Reference 1. [f] Reference 3. [g] Synthesized from β-cyclodextrin-6-iodide and the corresponding dithiol; characterized by MS and ^{1}H NMR. [h] Reference 2. [i] 4.0×10^4 by fluorescence, from ref 18. [j] $(1.35 \pm 0.24) \times 10^7$, by fluorescence, from this work. [k] $(2.05 \pm 0.50) \times 10^7$, by fluorescence, from this work. [l] 5.0×10^6 by fluorescence, from ref 1.

From Table I, the binding of all guests **1–4** into monomeric cyclodextrin **5** is largely driven by enthalpy, with a small entropy contribution. This is true for the binding of an adamantane group (entries 1–3), for a *tert*-butylphenyl group (entry 7), and for a naphthalene ring (entries 9 and 10). All show similar values which are consistent with previous work. It has been noted before[9-12] that hydrophobic binding of hydrocarbon residues into cyclodextrin cavities is usually driven by *enthalpy*, even though classical hydrophobic binding is often[13] entropy driven.

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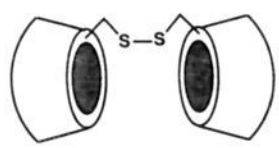

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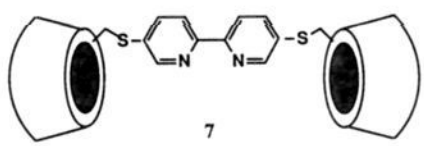

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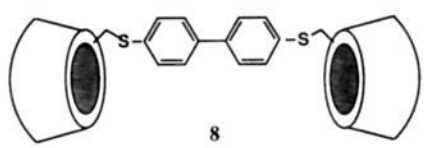

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When **4** binds to the dimeric host **7**, the $\Delta G°$ of binding (entry 11) is 1.0 kcal/mol less than the sum of the $\Delta G°$s for the first

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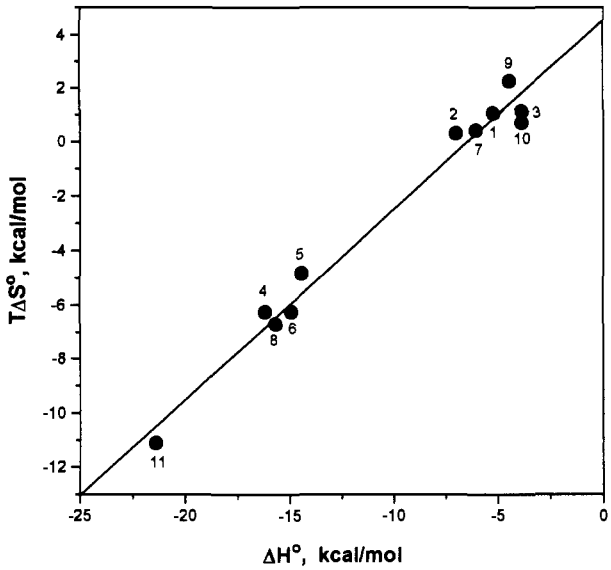

Figure 1. Enthalpy–entropy compensation plot for the binding of β-cyclodextrin and its dimers with various guests. The numbers in the graph refer to entries in Table I.

and second binding (entries 9 and 10) of **4** to **5**; the $\Delta H°$ of binding is 2.5 times as large as the sum for entries 9 and 10, more than compensated by a large decrease in $\Delta S°$. The same trend is seen with other systems. The $\Delta H°$s for binding of the dimeric phosphate **2** into disulfide **6**, into bipyridyl-linked dimer **7**, and into biphenyl-linked dimer **8** are all -15 ± 1 kcal/mol, essentially 3 times the value for the monomeric **1**/**5** case and 4 kcal/mol more favorable than the sum of the values (entries 2 and 3) for **2**/**5**. Thus all these cases with chelate binding show a significant (over 20 eu) *decrease* in $\Delta S°$. Of course, in the comparison of monotopic with ditopic binding one must worry about the interactions of the linking groups or altered geometries of binding. However, the pattern here is so general that it probably does not simply reflect such factors.

By measuring $\Delta H°$ at 7–8 temperatures between 20 and 80 °C, we have determined the $\Delta C_p°$ for **5**/**1** binding to be −96 cal/mol deg (lit.[11] $\Delta C_p°$ −95 cal/mol deg) and that for **6**/**2** binding to be −157 cal/mol deg. Negative values of this sort are typical for hydrophobic binding.[5,13-16]

Enthalpy/entropy compensation[12,17] is operating, as Figure 1 shows. As in other systems in solution, the free energy can be minimized by a tradeoff between improved solvation—which lowers enthalpy at a price in entropy—and diminished solvation, with its entropy advantages. Solvation changes apparently dominate the thermodynamics and account for our observations. Thus statistical ideas about the entropy advantages of chelate systems are an unreliable guide to the actual thermodynamics of chelate effects in solution, at least in this case.

Acknowledgment. This work has been supported by the National Institutes of Health and the Office of Naval Research.

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