Quantitative effects of antihydrophobic agents on binding constants and solubilities in water

(cyclodextrins/urea/guanidinium/chaotrope)

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ABSTRACT The effects of urea and of guanidinium chloride on binding constants in water for 6-(4-tert-butylandilino)naphthalene-2-sulfonate and of bis(p-tert-butylphenyl) phosphate binding to β-cyclodextrin and to N,N'-bis(6-β-cyclodextrinyl)mimidazolium ion have been determined. Their effects on the water solubility of p-tert-butylbenzyl alcohol and p-methylbenzyl alcohol have also been examined. Quantitative correlations show that the effects of these additives, which diminish hydrophobic effects, are similar for release of a tert-butylphenyl group from a cyclodextrin cavity into water or for solubilizing such a group from a second phase. The effects of these agents on the binding constants for double-ended substrates binding to the bis(cyclodextrin) host are much larger than for a simple substrate binding to monomeric cyclodextrin, consistent with additivity of free-energy perturbations. Ethanol also decreases binding in these systems, and increases solubilities, but the quantitative correlations are less straightforward.

Substances such as urea and guanidinium ion diminish the hydrophobic effect (for general references, see refs. 1 and 2). With proteins or nucleic acids this change leads to unfolding, so such substances are sometimes called denaturants or chaotropes; they also lead to the increased solubility of hydrophobic molecules in water, so they may be called “salting in” agents when this property is being addressed. However, they are not all salts. They have sometimes been called “water structure breakers,” but previous evidence indicates that they do not work primarily by breaking up water structure. Since the above properties reflect a decrease of the hydrophobic effect, we propose that such substances be called simply antihydrophobic agents.

We have used such materials to diagnose the presence of hydrophobic effects in the transition states of simple organic reactions such as Diels–Alder additions and benzoin condensations (4). However, it seemed likely that more would be learned if these effects were made quantitative. That is, in any given case, one would like to know not just that a hydrophobic effect was present but also how much hydrophobic surface was involved in the effect. We have now examined the quantitative effects of antihydrophobic agents on (a) the binding constant for a tert-butylphenyl group binding in a cyclodextrin cavity, (b) the binding constant for two tert-butylphenyl groups of a double-ended substrate binding in the two cavities of a cyclodextrin dimer, and (c) the water solubility of p-tert-butylbenzyl alcohol (1) and p-methylbenzyl alcohol (2). Quantitative correlations prove to exist and furnish insight into the nature of the interactions in some strongly bound host–guest systems.

MATERIALS AND METHODS

Urea and guanidinium chloride were Aldrich 99+% pure and were used as supplied. Water was deionized by a commercial unit, p-tert-Butylbenzyl alcohol (Scheme 1, compound 1) and p-methylbenzyl alcohol (2) were Aldrich 98% pure, confirmed by m.p. (for 2) and 1H NMR. β-Cyclodextrin was obtained from American Maize (Hammond, IN) and recrystallized once from water. 6-(4-tert-Butylanilino)naphthalene-2-sulfonate (BNS) sodium salt (3) was prepared as described briefly elsewhere (5). It was isolated as its triethylamine salt and characterized by 1H NMR.

Bis(tert-butylphenyl) phosphate (4) was prepared as follows. Methyl dichlorophosphate was added slowly to 2 equivalents of sodium tert-butylphenolate in methylene chloride solution to form the crude phosphate triester. This was then demethylated with KI in acetone under reflux for 18 hr. The product was isolated in the acid form and recrystallized from chloroform/pentane. It was obtained in 44% yield, had the expected mass spectrum and 1H NMR spectrum, and was pure by 1H NMR and by TLC.

The dimeric cyclodextrin derivative 5 was prepared by heating β-cyclodextrin-6-imidazolide with an excess of β-cyclodextrin-6-iodide in minimal 1,3-dimethyl-2-imidazolidinone at 120°C for 3 days under an argon atmosphere. The product was dissolved in water, filtered, and chromatographed on a Sephadex C-25 column with NH4HCO3 gradient elution. The product was isolated in 38% yield and was characterized by 1H NMR, fast atom bombardment MS, and TLC.

Binding constants were determined in water at ~25°C by fluorescence methods (5, 6). For the binding of BNS (3) to β-cyclodextrin or to the dimer 5, binding leads to a strong increase in fluorescence of BNS; the binding constant was directly determined by adding 5 to a BNS solution at a concentration low enough that an excess of 5 was required for saturation of the signal. The binding constant for binding of BNS to tetramer 6 was determined by competition of 4 with BNS, with monitoring of the BNS fluorescence.

Solubilities of 1 and 2 were determined at 20°C by UV absorbance measurements on the aqueous solutions. For each value at least two saturated solutions were prepared, kept at 20°C for at least 2 days, and read several times. In some cases readings were taken on different days to assure that the values were final. The (small) effects of antihydrophobic solutes on the absorption coefficients were corrected for by examination of three authentic solutions with different known concentrations.

RESULTS AND DISCUSSION

The results are listed in Table 1. As can be seen, urea, guanidinium chloride, and ethanol all increase the solubility

Abbreviation: BNS, 6-(4-tert-butylandilino)naphthalene-2-sulfonate.
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of 1 and 2 and diminish the binding of 3 and 4 to the host molecules, as expected. However, the quantitative correlations are interesting.

With respect to the solubilities, it is important to note that in the case of 1, two liquid phases are being equilibrated and so the solute phase will not be pure. That is, some water will dissolve in liquid 1, and possibly some urea or guanidinium chloride will also be extracted from the water phase into 1. Certainly considerable ethanol is likely to enter the solute phase. For this reason the thermodynamic activity of the solute phase may be considerably perturbed by ethanol, although the perturbation is probably small with urea and guanidinium chloride. Even though 2 is a solid, it is low melting and might also become contaminated by water and solutes to some extent. Thus the relationships between solubilities and binding constants must be viewed with caution. Despite this cavil, there indeed prove to be rough relationships.

Considering first the effects of 8 M urea, a common denaturing medium, it can be seen that it increases the solubility of 1 in water by a factor of 3.3, and of 2 by a factor of 2.5. The magnitude of the two effects runs parallel to the amount of hydrophobic surface each alcohol presents to the water. That is, the increase in solubility of 2 caused by urea is only 76% of that for 1, and a standard calculation (7) of the van der Waals surface area of toluene shows that it is 71% that of tert-butylbenzene. Despite the cautions about solubility measurements, the similarity of the numbers is interesting. Urea also decreases the binding of BNS to β-cyclodextrin by a factor of 2.4. We know that it is the tert-butylphenyl group rather than the naphthylene ring of BNS that binds in the cyclodextrin cavity, since BNS binds much more strongly to cyclodextrin (5) than does the analogous compound with a methyl group in place of the tert-butyl group (8). The quantitative similarity of these binding and solubility perturbation effects can be understood if binding of urea to the tert-butylphenyl group decreases the activity coefficient of that group by a similar amount in 1 and in BNS.

The perturbation of the binding constant would have been larger than the perturbation of solubility if urea had a significant stabilizing effect on the cyclodextrin cavity. It also would have been larger if the solubility effects were appreciably distorted by contamination of the solute phase, as discussed above. It was previously argued (3) that urea does not have a general effect on water structure but instead binds to hydrophobic surfaces; we conclude here that it does not bind strongly to cyclodextrin, which is only semihydrophobic. This conclusion was not obviously expected, and illustrates the information that our quantitative studies supply.

In 8 M urea the decrease in binding of BNS to the dimeric cyclodextrin 5 is 25-fold, and 20-fold for the dimeric substrate 4. This confirms the conclusion from their high binding

Table 1. Solubilities and binding constants*

<table>
<thead>
<tr>
<th>Medium</th>
<th>Solubility of 1</th>
<th>Solubility of 2</th>
<th>Binding of 3 to β-cyclodextrin</th>
<th>Binding of 3 to 5</th>
<th>Binding of 4 to 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>0.0064 (1.00)</td>
<td>0.069 (1.00)</td>
<td>3.8 ± 0.1 × 10^4 (1.00)</td>
<td>3.5 ± 1.7 × 10^4 (1.00)</td>
<td>3.3 ± 1.3 × 10^4 (1.00)</td>
</tr>
<tr>
<td>8 M urea</td>
<td>0.021 (3.3)</td>
<td>0.17 (2.5)</td>
<td>1.6 ± 0.05 × 10^4 (0.42)</td>
<td>1.4 ± 0.2 × 10^4 (0.04)</td>
<td>1.7 ± 0.1 × 10^4 (0.05)</td>
</tr>
<tr>
<td>6 M GdmCl</td>
<td>0.025 (3.9)</td>
<td>0.19 (2.8)</td>
<td>8.6 ± 0.15 × 10^3 (0.23)</td>
<td>8.7 ± 0.5 × 10^3 (0.025)</td>
<td>4.1 ± 1.2 × 10^6 (0.012)</td>
</tr>
<tr>
<td>10% EtOH</td>
<td>0.0084 (1.3)</td>
<td>0.094 (1.4)</td>
<td>1.4 × 10^4 (0.37)</td>
<td>4.7 ± 0.2 × 10^6 (0.13)</td>
<td>4.1 ± 0.4 × 10^7 (0.12)</td>
</tr>
<tr>
<td>20% EtOH</td>
<td>—</td>
<td>—</td>
<td>6.6 × 10^3 (0.17)</td>
<td>1.1 × 10^6 (0.03)</td>
<td>1.3 ± 0.1 × 10^7 (0.04)</td>
</tr>
</tbody>
</table>

*Given as determined values of solubilities (M) and binding constants (M^-1); values relative to those in pure water are in parentheses.
1GdmCl, guanidinium chloride. Ethanol concentrations are percent by volume.
2At 20°C, determined by UV absorbance.
3At ambient temperature (25°C) by measurement of the fluorescence of 3.
4At ambient temperature (25°C) by competition of 4 with 3.
constants that both the tert-butylphenyl group and the naphthalene group of BNS are binding to cyclodextrin units in 5 and that both of the tert-butylphenyl groups of 4 also bind. Double binding of 4 should lead to a square of the single binding antihydrophobic effect if the perturbations of free energy are additive, so an effect of 6-fold (=2.4^2) might be expected; that the effect is even larger than this for the dimeric binding suggests that urea disrupts some additional interaction of substrate segments with the linker group in 5.

Of course, binding constants in doubly interacting systems do not reflect simple doubling of the free energies of single binding, because of entropy considerations. The binding energies can be more than additive because translational entropy need not be paid for twice (9). However, no such special situation obtains to invalidate an expected additivity of free-energy perturbations by antihydrophobic agents. Thus when the effect is more than additive, as above, it indicates an additional interaction.

Very similar results are seen with 6 M guanidinium chloride. Again the solubility effect on compound 2 is only 72% of that on 1, consistent with the 76% effect with urea and the 71% surface area relationship. Again the solubility effects correlate well with the data for BNS binding to β-cyclodextrin, with a 3.9-fold increase in the solubility of 1 and a 4.3-fold decrease in the binding of BNS. Thus we conclude that guanidinium ion also stabilizes the tert-butylphenyl group in water, probably by binding to it, but has little effect on the cyclodextrin cavity. Again the effect on the binding of BNS to the dimer 5 is more than the square, 40-fold instead of 18-fold, while for the phosphate substrate 4 the binding decreases by 83-fold. The extra effect with 4 probably again reflects interaction with the linker in 5; ionic-strength increases with guanidinium chloride will also diminish any ion-pairing interactions of the linking groups in the 4-5 complex.

In contrast to urea and guanidinium ion, ethanol has a larger effect on binding than on solubility. For example, 10% ethanol increases the water solubility of 1 and of 2 only 1.3-fold, but binding of BNS to β-cyclodextrin decreases by a factor of 2.7. Ethanol might well bind both to the hydrophobic substrates and to the cyclodextrin cavities. More seriously, the solubility effects of ethanol are surely strongly altered by extraction of ethanol into the second solute phase. This will lower the thermodynamic activity of that phase relative to that of a pure substance and thus diminish water solubility compared with that for the ideal case.

However, considering only the binding data, where no partitioning problem of this kind exists, rough additivity of the free-energy perturbations is still seen with ethanol. With 10% ethanol, binding of BNS to 5 decreases by a factor of 7.7 and binding of 4 to 5 by a factor of 8.3 (expected, 2.7^2 = 7.3). With 20% ethanol, BNS binding to β-cyclodextrin decreases by a factor of 6, while BNS binding to 5 decreases by a factor of 32 and binding of 4 to 5, by a factor of 25 (expected, \(2^5 = 36\)).

These data show that the general magnitudes of the effects of antihydrophobic agents on solubilities and on binding constants can be interpreted in structural terms, in spite of the ambiguities about solubility effects when the solute phase can become contaminated. In particular, there are sensible quantitative additivity effects on binding constants of dimeric versus monomeric systems. Quantitative studies of this kind may help reveal the extent of the hydrophobic interactions in other complexes and in transition states of reactions whose rates are affected by hydrophobic packing (4).

The data also clearly support the importance of hydrophobic interactions in the host-guest systems examined here. Further, the data are consistent with the previous conclusion (3) that antihydrophobic agents produce their effect by binding directly to the hydrophobic surfaces rather than by perturbing water structure, although the present study does not furnish strong extra evidence on this matter.

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