ORGANIC LETTERS

2000
Vol. 2, No. 23
3575−3578

Aryl Phosphate Complexation by Cationic Cyclodextrins. An Enthalpic Advantage for Guanidinium over Ammonium and Unusual Enthalpy–Entropy Compensation

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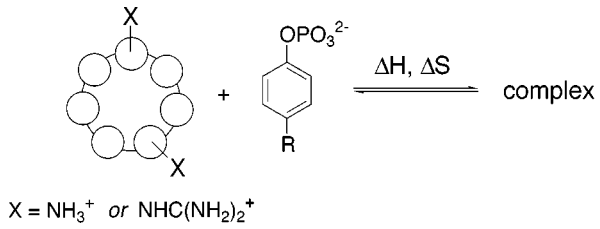
Received August 25, 2000

ABSTRACT



The enthalpies and entropies for phosphate complexation by ammonium and guanidinium groups have been compared using cationic cyclodextrins. Aryl phosphate binding by guanidinium hosts is associated with more favorable enthalpies and less favorable entropies, consistent with the idea that guanidinium–phosphate interactions are stronger but more directional than ammonium–phosphate interactions. The slope of an enthalpy–entropy plot suggests that complexation occurs with surprisingly small changes in the order of these systems.

The reversible phosphorylation of tyrosine residues on the surfaces of cellular proteins plays a key role in many signal transduction pathways. Phosphorylation serves as a switch, inducing binding of secondary cytosolic proteins at the site of modification, which in turn alters the activity and/or subcellular location of the secondary protein.[1] These secondary proteins contain domains that recognize and bind to the phosphorylated tyrosine. Two types of phosphotyrosine recognition domains have been identified and are termed SH2 and PTB domains. These share little sequence homology or structural similarity, suggesting they are products of convergent evolution. Importantly, however, both types of domains use the guanidinium group of arginine residues to form direct contacts with the phosphate group of phosphotyrosine.[2] These interactions are essential for complex formation, as proteins containing unphosphorylated tyrosine show no appreciable binding and SH2 domains possessing an arginine to lysine mutation in the phosphotyrosine binding pocket are effectively unable to bind ($\Delta\Delta G$ > 4 kcal/mol).[3] The fact that this interaction has been selected in two evolutionarily distinct protein domains suggests that guanidinium groups may be significantly more effective for complexation of aryl phosphates than ammonium groups.

An apparent inconsistency exists. For the biological systems described above, lysine appears to be inferior to arginine for phosphate complexation. In contrast, results with model systems generally suggest that ammonium groups are as or more effective than guanidinium groups for the

(1) Pawson, T. *Nature* **1995**, *373*, 573−580.

(2) (a) Waksman, G.; Shoelson, S. E.; Pant, N.; Cowburn, D.; Kuriyan, J. *Cell* **1993**, *72*, 779−790. (b) Shoelson, S. E.; Eck, M. J.; Nolte, R. T.; Dhe-Paganon, S.; Trüb, T. *Cell* **1996**, *85*, 695−705.

(3) (a) Mayer, B. J.; Jackson, P. J.; Van Etten, R. A.; Baltimore, D. *Mol. Cell. Biol.* **1992**, *12*, 609−618. (b) Marengere, L. E. M.; Pawson, T. *J. Biol. Chem.* **1992**, *267*, 22779−22786. (c) Bibbins, K. B.; Boeuf, H.; Varmus, H. E. *Mol. Cell. Biol.* **1993**, *13*, 7278−7287.

10.1021/ol006503r CCC: $19.00
Published on Web 10/27/2000

complexation of phosphates in aqueous solution.[4,5] We considered that this discrepancy could at least in part result from the more stringent geometrical requirements for guanidinium−phosphate interactions. In flexibile model systems, complexation can be expected to result in significant loss of conformational entropy; this effect is presumably more pronounced in the case of guanidinium due to the preference of this group to form two hydrogen bonds with phosphate.[6,7] The "entropic disadvantage" of guanidinium relative to ammonium in model systems is likely to be absent in protein systems, where amino acid side chains within a binding site are generally highly oriented. Comparisons with models would then be expected to underestimate the phosphate-complexing ability of arginine relative to lysine in a protein.

To determine if there is in fact an entropic disadvantage for phosphate complexation by guanidinium groups in relatively flexible host−guest systems, we have compared the binding of bis-ammonium and bis-guanidinium β-cyclodextrin (β-CD) derivatives with a series of aryl phosphate monoesters.[8] Isothermal titration calorimetry was employed for analysis, a technique that provides entropies and enthalpies for complexation.[9]

The hosts and guests included are shown in Figure 1, and the results are given in Table 1.[10,11]

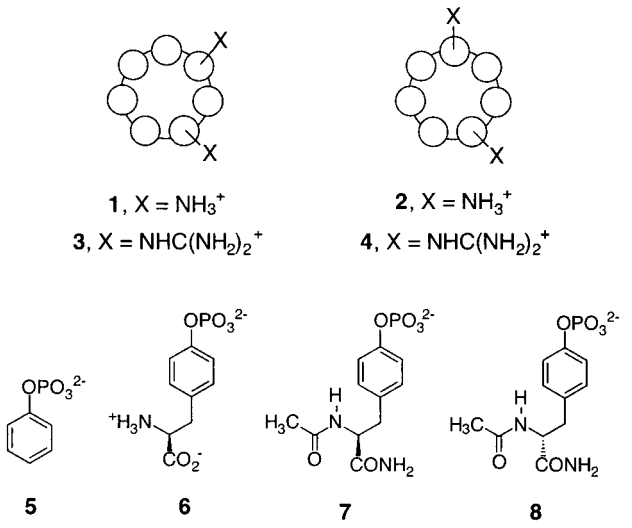


Figure 1. Hosts and guests employed for the present study. For compounds **1**−**4**, each small circle represents a glucose unit of β-cyclodextrin and X is a group covalently bonded to C-6 of the respective monomer.

A number of general observations can be made from these data. In all cases, binding is both enthalpically and entropically favorable, and while the binding free energies displayed by the four hosts with a given guest are very similar ($\Delta\Delta G \leq 0.4$ kcal/mol), there are significant differences in the respective enthalpic and entropic contributions. Relative to phenyl phosphate, each host exhibits slightly weaker binding with phosphotyrosine, which is primarily associated with less negative enthalpies. In contrast, binding with diamides **7** and **8** is significantly stronger, which results from much more negative enthalpies despite decreases in the associated entropies.[12] With regard to relative orientation of the two cationic groups, for the guanidinium derivatives, the A,D

Table 1. Thermodynamic Parameters for Host−Guest Complex Formation[a−c]

		host			
guest		**1**	**2**	**3**	**4**
5	$-\Delta G$	3.94 ± 0.07	3.84 ± 0.08	3.74 ± 0.06	3.98 ± 0.06
	$-\Delta H$	1.12 ± 0.15	1.24 ± 0.13	1.64 ± 0.24	2.32 ± 0.46
	$T\Delta S$	2.82 ± 0.21	2.60 ± 0.17	2.10 ± 0.29	1.66 ± 0.51
6	$-\Delta G$	3.73 ± 0.23	3.58 ± 0.37	3.35 ± 0.39	3.36 ± 0.12
	$-\Delta H$	0.76 ± 0.16	0.87 ± 0.56	0.54 ± 0.30	1.32 ± 0.25
	$T\Delta S$	2.96 ± 0.40	2.71 ± 0.91	2.81 ± 0.68	2.05 ± 0.35
7	$-\Delta G$	4.50 ± 0.02	4.44 ± 0.05	4.57 ± 0.04	4.83 ± 0.05
	$-\Delta H$	2.84 ± 0.28	2.48 ± 0.54	3.17 ± 0.68	4.03 ± 0.55
	$T\Delta S$	1.66 ± 0.29	1.97 ± 0.58	1.40 ± 0.72	0.80 ± 0.60
8	$-\Delta G$	4.47 ± 0.01	4.35 ± 0.02	4.49 ± 0.01	4.71 ± 0.01
	$-\Delta H$	2.66 ± 0.39	2.88 ± 0.11	3.42 ± 0.10	3.98 ± 0.34
	$T\Delta S$	1.81 ± 0.40	1.47 ± 0.12	1.07 ± 0.09	0.72 ± 0.33

[a] Values in kcal/mol. [b] Experiments were carried out at 25.0 °C at 100 mM total phosphate concentration, pH 7.00. [c] Results represent the averages from three to five replicate measurements and uncertainties are the corresponding standard deviations.

(4) (a) Dietrich, B.; Fyles, D. L.; Fyles, T. M.; Lehn, J.-M. *Helv. Chim. Acta* **1979**, *62*, 2763−2787. (b) Cotner, E. S.; Smith, P. J. *J. Org. Chem.* **1998**, *63*, 1737−1739. (c) Niikura, K.; Metzger, A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1998**, *120*, 8533−8534.

(5) One prior study found that dianionic phosphate is bound more strongly by guanidinium than *n*-butylammonium: Springs, B.; Haake, P. *Bioorg. Chem.* **1977**, *6*, 181−190.

(6) Cotton, F. A.; Day, V. W.; Hazen, E. E., Jr.; Larsen, S.; Wong, S. T. K. *J. Am. Chem. Soc.* **1974**, *96*, 4471−4478.

(7) This idea is consistent with recent experiments examining the binding of arginine and lysine by calixarenes substituted with four to eight sulfonate groups. It was found that in all cases arginine binding with a given calixarene occurred with greater loss in entropy. Douteau-Guével, N.; Coleman, A. W.; Morel, J.-P.; Morel-Desrosiers, N. *J. Chem. Soc., Perkin Trans. 2* **1999**, 629−633.

(8) Experiments with monocationic cyclodextrins would arguably provide a more direct comparison. However, calorimetric titrations of monoammonium and monoguanidinium β-CD derivatives with phenyl phosphate failed to provide heats sufficient for quantitation. We were also unable to detect binding between a monoguanidinium β-CD derivative and phosphotyrosine using competitive spectroscopy (ref 4b). These results underscore the weak nature of ionic bonds in aqueous environments, where both cations and anions are effectively solvated. The use of dicationic hosts complicates interpretation of the results somewhat in that both groups may not form energetically equivalent interactions with the phosphate moiety within a complex.

(9) Freire, E.; Mayorga, O. L.; Straume, M. *Anal. Chem.* **1990**, *62*, 950A−959A.

(10) The syntheses of **2** and **4** are reported in ref 4b. Compounds **1** and **3** were prepared by analogous chemistry, starting with a 6A,6C-disulfonate of β-CD (Cuchinotta, V.; D'Alessandro, F.; Impellizzeri, G.; Vecchio, G. *Carbohydr. Res.* **1992**, *224*, 95−102). Diamides **7** and **8** were prepared using published methods: Burke, T. R., Jr.; Barchi, J. J., Jr.; George, C.; Wolf, G.; Shoelson, S. E.; Yan, X. *J. Med. Chem.* **1995**, *38*, 1386−1396.

(11) Calorimetric measurements were made using a MicroCal VP-ITC isothermal titration calorimeter; equilibrium constants and enthalpies were obtained from the primary data using the software supplied by MicroCal. More details and representative primary data are available as Supporting Information.

(12) With unmodified β-CD, a similar increase in association constant on going from phenylalanine to an uncharged amide of phenylalanine has been observed: Rekharsky, M. V.; Schwarz, F. P.; Tewari, Y. B.; Goldberg, R. N. *J. Phys. Chem.* **1994**, *98*, 10282−10288.

substitution pattern (i.e., compound **4**) is marginally preferred in terms of free energy and highly preferred in terms of enthalpy. In fact, binding by host **4** affords the most negative enthalpy values as well as the least positive entropy values with the guests. For the ammonium compounds, A,C substitution is slightly preferred in terms of free energy, but no clear trends exist with regard to enthalpy or entropy. Finally, none of the hosts display significant enantioselection between diamides **7** and **8**.[13]

To compare the entropies and enthalpies of phosphate complexation by ammonium and guanidinium groups, we have compared these quantities for the binding of structurally analogous ammonium and guanidinium hosts (**1** and **3**, **2** and **4**) with each guest (Figure 2). As shown in panel A,

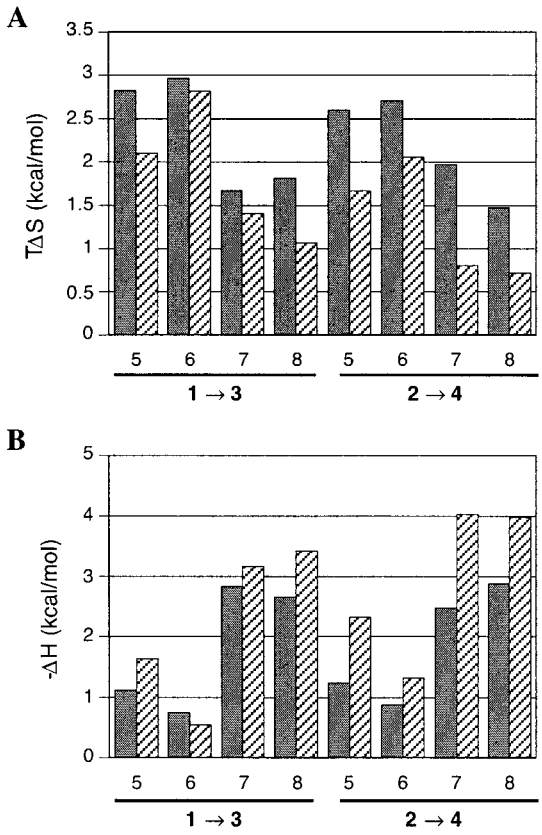


Figure 2. Comparison of the entropies (panel A) and enthalpies (panel B) of binding by structurally analogous ammonium and guanidinium cyclodextrins with each guest. The solid and striped bars depict data for the ammonium and guanidinium hosts, respectively.

binding by the ammonium compound is more entropically favorable in every case. The average difference in $T\Delta S$ at 25.0 °C is 0.67 kcal/mol (or 0.33 kcal/mol per cationic group). As shown in panel B, binding by the guanidinium compound is more enthalpically favorable in all but one case, with an average difference in ΔH of 0.70 kcal/mol (or 0.35 kcal/mol per cationic group). These results are consistent with the idea that guanidinium−phosphate interactions have more stringent geometrical requirements but, if properly oriented, lead to stronger association (as indicated by the enthalpies reported here). The structure of an energy-minimized complex between **3** and **7** is shown in Figure 3. It is important

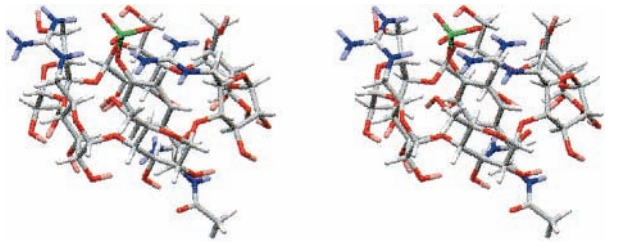


Figure 3. Structure of energy-minimized complex between **3** and **7**.[14]

to point out, however, that these effects are small relative to the large free energy changes observed in the mutagenesis experiments described above ($\Delta\Delta G_{R\rightarrow K} \geq 4$ kcal/mol), suggesting that other factors contribute to the observed preference in the protein systems.

The compensatory enthalpic and entropic contributions to binding for each host with a given guest prompted us to make a plot of ΔH vs $T\Delta S$ for these data (Figure 4). All 16 data

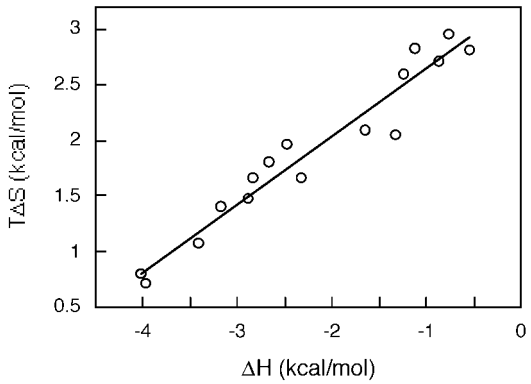


Figure 4. An enthalpy−entropy compensation plot obtained from the data in Table 1.

points give a good fit to a straight line with a slope of 0.61 and an intercept of 3.26 ($r = 0.97$).[15] Inoue has suggested

(13) For a recent comprehensive study on enantioselectivity by unmodified cyclodextrins, see: Rekharsky, M. V.; Inoue, Y. *J. Am. Chem. Soc.* **2000**, *122*, 4418−4435.

(14) Generated with MacroModel version 5.0 using the AMBER* force field and water solvation parameters contained within MacroModel. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.

(15) We have recently carried out binding experiments with **1**−**4** and 4-*tert*-butylphenyl phosphate. The resulting data do not fit this line, but rather define a second line which has a similar slope but a different intercept.

that the slope of such a plot indicates the extent of conformational change associated with binding in a particular system.[16] The slope of 0.61 for the present system is significantly smaller that that observed for binding by unmodified β-CD (0.80)[16c] and is effectively equal to that observed for two highly rigid porphyrin-based systems (slopes = 0.60 and 0.61).[16a] On the basis of the hosts surveyed by Inoue, this result would indicate that introduction of two cationic groups in β-CD significantly increases host preorganization. We considered that this in turn could result from hydrogen bonding of the cationic groups with hydroxyl groups on neighboring glucose residues, thereby rigidifying the macrocycle; minimal evidence for such hydrogen bonding was found using molecular mechanics or dynamics simulations, however. As an alternate explanation, we propose that, rather than being more preorganized, the present hosts may *retain a greater degree of conformational flexibility* upon complexation than does β-CD. Assuming a significant Coulombic contribution to binding, **1**−**4** would be able to take advantage of the energy from ionic interactions while forming looser or less ordered complexes than would be associated with purely dipole−dipole or dispersion interactions.[17] Further experiments are currently underway to explore the validity of this hypothesis.

These experiments provide the first comparison of phosphate complexation by ammonium and guanidinium groups that partitions binding free energy into its entropic and enthalpic components. The differences observed for these thermodynamic quantities suggest that orientational preferences can create biases in model studies that seek to compare discrete binding interactions in proteins and argue for the use of binding enthalpies rather than free energies in such investigations. In contrast to results from prior studies, the data indicate that arginine may in fact be more effective than lysine as a phosphate-complexing moiety in protein systems.

Acknowledgment. This work was supported by the National Science Foundation under Grant CHE-9985300 and by the National Institutes of Health, National Research Award GM 08663 from the Minority Access to Research Careers Undergraduate Student Training in Academic Research (MARC U*STAR) Program. We thank Mr. Patrick Pohlhaus and Mr. Greg Foti for assistance in preparing some of the host molecules and Professor Bradley Arnold of this department for useful discussions regarding this work.

Supporting Information Available: Details for binding experiments and representative primary data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) (a) Inoue, Y.; Liu, Y.; Tong, L.-H.; Shen, B.-J.; Jin, D.-S. *J. Am. Chem. Soc.* **1993**, *115*, 10637−10644. (b) Inoue, Y.; Hakushi, T.; Liu, Y.; Tong, L.-H.; Shen, B.-J.; Jin, D.-S. *J. Am. Chem. Soc.* **1993**, *115*, 475−481. (c) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875−1917.

(17) The distance dependence for formation of an ion pair is inversely related to *r*, the inter-ion separation, while dipole−dipole and dispersion interactions vary inversely with r^3 and r^6, respectively (Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*, 2nd ed.; VCH: Weinheim, 1990; pp 9−13). The shapes of the corresponding potential functions indicate that changes in *r*, such as those resulting from dymanic motion within a complex, will have a smaller effect on the interaction energy of two ionic groups than it will on two dipoles or nonpolar groups.