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'Induced-fit' binding of an aryl phosphate by a macrobicyclic dicationic cyclodextrin derivative

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ABSTRACT: Binding of an aryl phosphate ester with a dicationic cyclodextrin derivative was compared to that with an analogous, more conformationally restricted cyclodextrin. Binding of the latter host occurs with an unexpected increase in binding free energy, resulting from an increase in binding enthalpy. A structural basis for this difference involving 'induced-fit' binding is proposed based on NMR experiments with the free host and the complex. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: cyclodextrin; aryl phosphate; 'induced-fit' binding

INTRODUCTION

Cyclodextrins (CDs) have a prominent place as host molecules in the field of molecular recognition. These core structures allow the formation of inclusion complexes with properly sized non-polar 'guests' in aqueous solution and can be (regiospecifically) modified, allowing the introduction of appropriate functional groups to enhance both binding affinity and selectivity. We have previously described the syntheses of guanidinium-containing β-CD derivatives and characterized their binding with aryl phosphates. One of the objectives of this larger project is to identify high-affinity binders for phosphotyrosine on the surfaces of cellular proteins, as such compounds have the potential to interfere with protein-protein binding events implicated in human cancers.[1–4]

Relevant to the present work, we found that CD derivative **1** (Fig. 1) binds *N*-acetyl-phosphotyrosineamide (**2**) with moderate affinity and that complex formation is both entropically and enthalpically favorable [ΔG, ΔH, $T\Delta S$ = -4.83 kcal mol^{-1}, -4.03 kcal mol^{-1}, 0.80 kcal mol^{-1} respectively, at 298 K].[5] This binding free energy corresponds to an association constant of 3500 M^{-1}, which is significantly weaker than that required to compete with protein phosphotyrosine recognition domains, which bind with their tyrosine-phosphorylated protein targets with association constants in neighborhood of 10^9 M^{-1}.[6,7] In an attempt to increase binding affinity, CD derivative **3** was prepared; this compound is identical with **1** except that the guanidinium groups are linked by a pentamethylene bridge (Fig. 1).[8] We expected that this modification would fix the guanidinium groups over the CD cavity, providing a more preorganized host and an associated decreased loss of conformational entropy upon binding.

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RESULTS AND DISCUSSION

Using isothermal titration calorimetry (ITC), the thermodynamic parameters for binding of **2** by **3** were determined. As expected, binding is associated with a more favorable entropy change relative to that with **1** [$T\Delta S = 1.81 \pm 0.34$ kcal mol^{-1}, $\Delta(T\Delta S)(\mathbf{1} \rightarrow \mathbf{3}) = +1.0$ kcal mol^{-1}]. This result is consistent with the proposal that restricting the guanidinium groups reduces losses in conformational entropy associated with binding. Surprisingly, however, we found that the binding enthalpy for **3** is significantly less than that for **1** [$\Delta H = -2.82 \pm 0.32$ kcal mol^{-1}, $\Delta\Delta H(\mathbf{1} \rightarrow \mathbf{3}) = +1.2$ kcal mol^{-1}]. The net result of these entropic and enthalpic changes is that host **3** actually binds more weakly than its 'less preorganized' counterpart [$\Delta G = -4.63 \pm 0.04$ kcal mol^{-1}, $\Delta\Delta G(\mathbf{1} \rightarrow \mathbf{3}) = +0.2$ kcal mol^{-1}].

To explain this unexpected increase in binding enthalpy, we considered the possibility that the non-polar pentamethylene linker may occupy the CD cavity of unbound **3**, maximizing stability in aqueous solution by minimizing the solvent-exposed non-polar surface area. Displacement of the linker from the cavity would result in an unfavorable enthalpic contribution to binding (i.e. disrupting favorable van der Waals interactions between the alkyl group and the interior of the cavity). This would then decrease the net enthalpy for guest complexation compared with that for **1**. This possibility was investigated by examining the solution structures of free and bound **3** by NMR spectrometry.

Initially, correlated spectroscopy (COSY) was used to assign resonances to protons on unbound host **3**, in

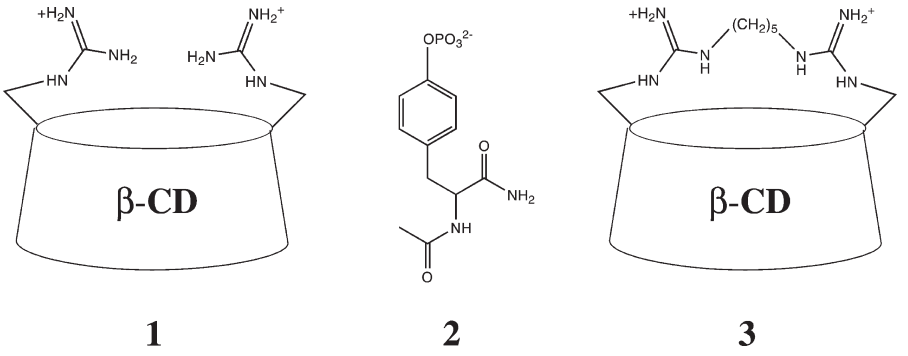


Figure 1. Representations of A,D-*bis-guanidino* β-*CD* **1**, *N*-acetyl-L-phosphotyrosineamide **2**, A,D-'pentamethylene capped'-*bis*-guanidino β-CD **3**; for the cyclodextrin derivatives **1** and **3**, the primary hydroxyl groups of the A and D sugars have been replaced with guanidinium groups

particular protons in the methylene linker (designated Hα, Hβ and Hγ, Fig. 2) and the H3 and H5 protons, which are located within the CD cavity. Based on chemical shift and integration, the most upfield signal ($\delta = 1.32$–1.45 ppm) was assigned to Hγ; Hβ and Hα were then readily assigned based on strong cross-peaks in the COSY spectrum (H$\beta = 1.56$–1.64 ppm, H$\alpha = 3.11$–3.21 ppm). The resonances of H3 protons ($\delta = 3.90$–3.99 ppm) were determined from COSY cross-peaks with H2, which exhibit well-resolved cross-peaks with H1. In this case, the H1 protons were used for initial reference, as these protons are furthest downfield and integrate to the expected relative value of 7. Resonances for the H5 protons could not be determined using the H1 protons as the initial reference point. Instead, H5 resonances on modified glucose residues were identified based on cross-peaks with H6 protons on putative modified glucoses (indicated by H6*), which were identified based on their splitting patterns and integration [Fig. 2(A)]. The assigned chemical shifts for H5 protons ($\delta = 3.77$–3.85 ppm) correspond well with the chemical shifts of H5 protons on unmodified β-CD.[9]

The spatial relationship between the pentamethylene linker and the interior of the cyclodextrin was investigated using rotating-frame Overhauser enhancement spectroscopy (ROESY) [Fig. 2(B)]. Medium intensity ROESY cross-peaks were observed between the protons on the pentamethylene linker (Hα, Hβ, Hγ) and H3, and low intensity cross-peaks were observed between linker protons (Hα, Hβ) and H5. Additionally, no cross-peaks were observed between H1, H2 and Hα, Hβ, or Hγ. Thus, the data indicate that the pentamethylene linker resides within the cyclodextrin cavity in the absence of guest [the possibility of dimerization between two **3** molecules leading to intermolecular ROEs was considered unlikely because an NMR dilution experiment (4 to 0.5 mM) failed to produce changes in chemical shifts of any proton signals].

Similar NMR experiments were carried out with the complex of **3** and phosphotyrosine diamide **2**. COSY was used to assign resonances for protons Hα, Hβ, and Hγ and H3 of host **3** and also protons Ha–He of guest **2** [Fig. 3(A)]. The resonances of linker protons are assigned as follows: Hβ–H$\gamma = 1.55$–1.71 ppm, H$\alpha = 3.12$–3.29 ppm; the resonances assigned to H3 protons are 3.91–4.01 ppm. Unlike for unbound **3**, cross-peaks with H5 protons were not sufficiently resolved in the COSY spectrum for assignment. For guest **2**, signals are assigned as follows: Ha/Hb = 7.18–7.23 ppm, Hc = 2.95–3.05 ppm and 3.15–3.28 ppm (diasteriotopic protons), Hd = 4.50–4.54 ppm, He = 1.95–2.03 ppm.

ROESY was then used to determine whether the linker is still in close proximity to the CD interior when complexed with the guest [Fig. 3(B)]. Cross-peaks previously observed between linker protons Hα, Hβ, and Hγ and cavity protons H3 are now absent whereas strong cross peaks are seen between H3 and Ha/b. These results indicate that the guest forms an inclusion complex, binding within the CD cavity and displacing the pentamethylene linker.

Our NMR results are consistent with an 'induced-fit' rather than a 'lock-and-key' binding mechanism (Scheme 1).[10] Structural modifications intended to preorganize host **1** (and thus provide our 'lock') actually produced an increase in binding free energy due to the enthalpic cost of displacing the linker from the CD cavity. The decrease in affinity is modest, however, and it is conceivable that such systems, where complex formation is accompanied by large conformational changes could be exploited in the development of nanoscale devices and sensors.[11] These experiments tend to validate to the idea that reducing the conformational freedom of the guanidinium groups can reduce losses in conformational entropy associated with complex formation. Experiments are under way to determine if incorporation of alternative, more hydrophilic linkers (that are unlikely to occupy the CD cavity) may in fact provide very high affinity hosts for aryl phosphates.

EXPERIMENTAL

Materials. CD derivatives **1** and **3** and guest **2** were synthesized, purified and analyzed as previously de-

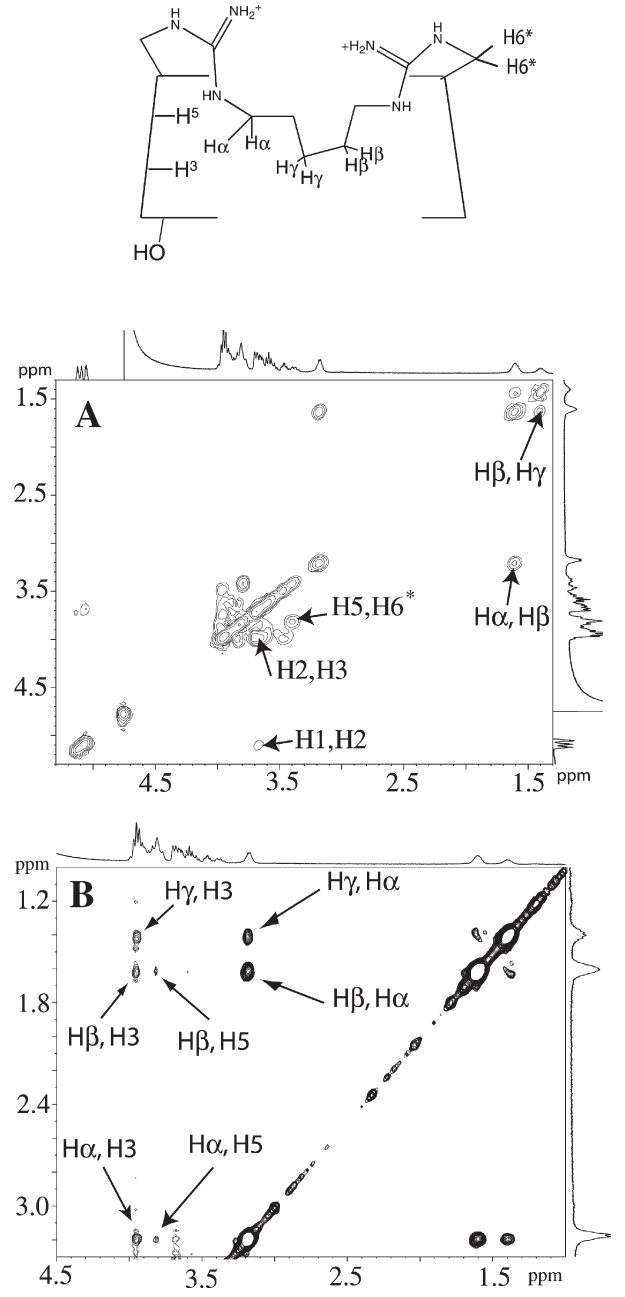


Figure 2. A representation of **3**. (A) COSY spectrum of **3** identifying important cross-peaks used to assign resonances to methlyene bridge protons Hα, Hβ and Hγ and cavity protons H3 and H5 (H6* refers to protons on modified glucose units, α to the guanidinium groups); (B) ROESY spectrum of **3**

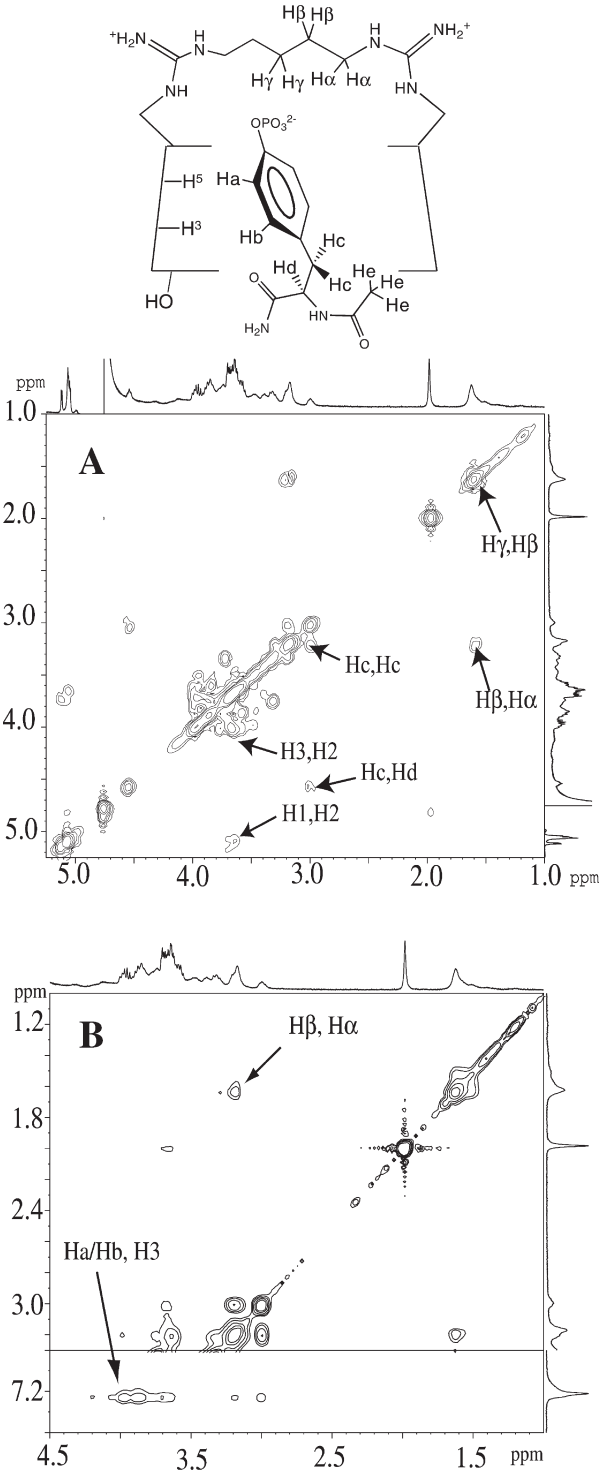
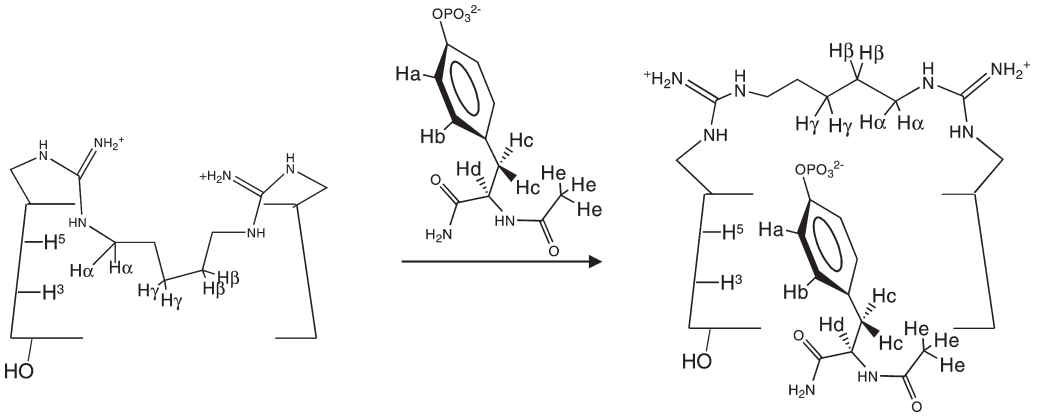


Figure 3. A representation of the complex of **3** and **2**. (A) COSY spectrum of the **3/2** complex identifying cross-peaks used to assign resonances to methlyene bridge protons Hα, Hβ and Hγ and cavity protons H3 on **3**, and protons Hc and Hd on guest **2**. (B) ROESY of the **3/2** complex

scribed.[8,12,13] The bicarbonate salts of **1** and **3** and the ammonium salt of **2** were used for both ITC and NMR experiments.

Calorimetric binding experiments. Binding experiments were conducted using a MicroCal VP-ITC instrument and all data were analyzed using the software provided by MicroCal. For all experiments, the calorimeter cell (1.7 ml) was filled with a solution of host (**1** or **3**, $\sim$200 µM in 100 mM phosphate buffer, pH 7.00). After equilibration of the cell to 25.0 °C, 180 ml of guest (**2**, 25 mM in buffer) were added in successive 10 µL



Scheme 1. Binding by an 'induced-fit' mechanism

injections. The resulting data were used to obtain binding enthalpies and association constants after subtraction of the heat of dilution for the guest (obtained from a titration of **2** into buffer). Results represent the average of three independent experiments and the error reported is the standard deviation for the three values obtained.

NMR measurements. 2-D NMR spectra (COSY and ROESY) were acquired using a Bruker DRX-500 NMR and processed using the accompanying software. Samples contained a 4 mM concentration of host **3** with or without an equimolar concentration of guest **2** in 100 mM deuterophosphate buffer, pH 7.00. ROESY spectra were recorded with a mixing time of 450 ms.

Acknowledgements

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