High Precision Measurement of Isotope Effects on Noncovalent Host-Guest Interactions

Jeffrey S. Mugridge, Robert G. Bergman,* and Kenneth N. Raymond*

Department of Chemistry, University of California, Berkeley and Chemical Science Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720-1460

RECEIVED DATE (automatically inserted by publisher); rbergman@berkeley.edu; raymond@socrates.berkeley.edu.

Isotope effects (IEs) are a powerful tool for examining the reactivity of, and interactions between, molecules.[1] Recently, secondary IEs have been used to probe the nature of noncovalent interactions between guest and host molecules in supramolecular systems.[2-6] While these studies can provide valuable insight into the specific interactions governing guest recognition and binding properties, IEs on noncovalent interactions are often very small and difficult to measure precisely.[7] The Perrin group has developed an NMR titration method capable of determining ratios of equilibrium constants with remarkable precision.[8] They have used this technique to study small, secondary equilibrium isotope effects (EIEs) on the acidity of carboxylic acids and phenols[9] and on the basicity of amines,[10] measuring differences down to thousandths of a pK_a unit. It occurred to us that this titration method can in principle measure relative equilibrium constants for any process which is fast on the NMR timescale and for which the species under comparison are distinguishable by NMR. Here we report the application of this method to measure very small EIEs on noncovalent host-guest interactions in a supramolecular system.

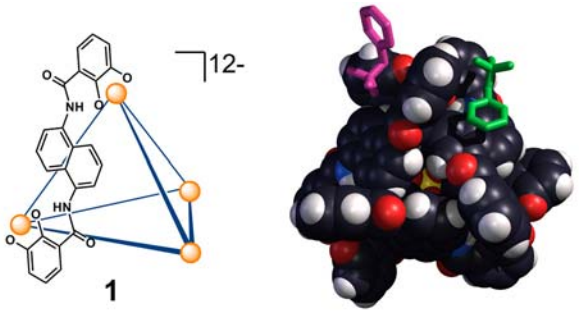
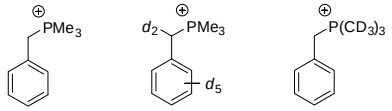


Figure 1. (Left) Schematic framework of **1**, only one ligand is shown for clarity. (Right) Spacefilling model of **1** with exteriorly bound trimethylbenzyl ammonium cations interacting through cation-π (purple) and π-π (green) interactions, adapted from the crystal structure of $(Me_3BnN)_{11}[Me_3BnN \subset \mathbf{1}]$.[11]

The Raymond group has developed a self-assembling, tetrahedral $[Ga_4L_6]^{12-}$ supramolecular assembly[12] (**1**, L = 1,5-bis(2,3-dihydroxybenzamido)naphthalene, Figure 1), which can act as a versatile host for a variety of monocationic[13] and neutral[14] guest molecules. The interior cavity of **1** is hydrophobic and has been shown to mediate[15] or catalyze[16] chemical transformations of encapsulated guests. The exterior of **1** is hydrophilic due to its 12- charge, which imparts water solubility and an affinity for ion-association of cationic molecules.[17] The external naphthalene and catecholate aromatic surfaces also give rise to noncovalent interactions, such as cation-π, CH-π and π-π, between the host and exteriorly bound guest (Figure 1).[11]

We have adapted Perrin's titration method to precisely measure very small EIEs on the binding affinity of benzyltrimethyl phosphonium isotopologues (**2-*d*$_n$**, Scheme 1) to the exterior of host **1**. This study reveals subtle differences in the noncovalent interactions between **2-*d*$_n$** isotopologues and the exterior of **1** and more generally, demonstrates the broader utility of this NMR titration method.

Scheme 1. **2-*d*$_n$** isotopologues.



2-*d*$_0$ **2-*d*$_7$** **2-*d*$_9$**

In a typical Perrin pK_a isotope effect determination, acid isotopologues are combined in solution in an NMR tube and aliquots of base are added to progressively deprotonate the acid. The resulting changes in chemical shift of a reporter nucleus (^{1}H, ^{19}F or ^{13}C) for each isotopologue are followed throughout the titration by NMR. Equation 1 describes the relationship between the chemical shifts of the isotopologues (δ^o are starting chemical shifts of the acid, δ^f are final chemical shifts of the conjugate base and δ are shifts during the titration) and acid isotopologue dissociation constants (K_H or K_D); plotting $(\delta_H{}^o\text{-}\delta_H)(\delta_D\text{-}\delta_D{}^f)$ versus $(\delta_H\text{-}\delta_H{}^f)(\delta_D{}^o\text{-}\delta_D)$ gives a line with intercept zero and slope K_H/K_D. The only requirements for applying this technique to different chemical systems are: (a) the species to be compared (in this case isotopologues) must have distinguishable chemical shifts, (b) exchange between states (i.e. protonated/deprotonated or bound/unbound) must be fast on the NMR timescale and (c) the endpoint of the titration must be measurable. Since this is a competition experiment which measures only relative changes in chemical shifts, it is insensitive to the presence of impurities and does not require knowledge of the absolute concentrations of any of the target species, making this a remarkably robust and precise method for measuring relative equilibrium constants.

$$(\delta_H{}^\circ - \delta_H)(\delta_D - \delta_D{}^f) = \frac{K_H}{K_D}(\delta_H - \delta_H{}^f)(\delta_D{}^\circ - \delta_D) \qquad (1)$$

Isotopologues of benzyltrimethyl phosphonium (**2-*d*$_n$**) are readily distinguished by ^{31}P{^{1}H} NMR and exchange between **2-*d*$_n$** cations bound to the exterior of host **1** and **2-*d*$_n$** in bulk solution is rapid on the NMR timescale. This rapid exchange makes measurement of equilibrium constants for external association impossible using conventional NMR integration methods, but well-suited to the above titration method. In the competition titration, two isotopologues are combined in an NMR tube in D_2O

solution and the $^{31}P\{^{1}H\}$ chemical shifts are measured relative to a trimethyl phosphate internal standard. A D_2O solution of $[NEt_4 \subset \mathbf{1}]^{11-}$ (where $\subset$ denotes encapsulation) with an excess of additional NEt_4^+ (ranging from 1-5 equiv) is added in small aliquots to the solution of isotopologues. The strongly binding guest NEt_4^+ prevents encapsulation of **2-d_n** isotopologues and differing amounts of exterior NEt_4^+ do not affect the EIE measurement (see supporting information). As the host complex $[NEt_4 \subset \mathbf{1}]^{11-}$ is added to the NMR tube, **2-d_n** isotopologues associate to the host exterior which induces an upfield shift of the $^{31}P\{^{1}H\}$ NMR resonances. The endpoint of the titration is reached when $^{31}P\{^{1}H\}$ chemical shifts stop changing, indicating that all of cation **2-d_n** is bound to the exterior of **1** (Figure 2).[18] Linearized plots of the chemical shift data are constructed in accordance with Equation 1 (where δ^o are chemical shifts of **2-d_n** in bulk solution, δ^f are chemical shifts of exteriorly bound **2-d_n** and δ are shifts during the titration) to extract the EIEs for exterior association (Figure 2).

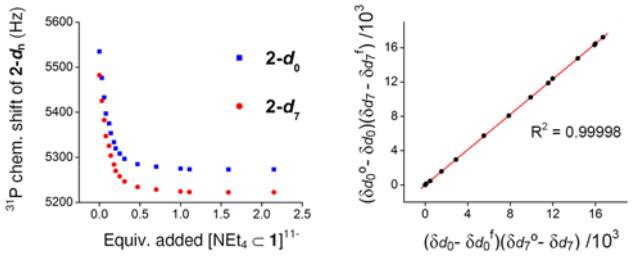


Figure 2. (Left) $^{31}P\{^{1}H\}$ NMR chemical shifts of **2-d_n** move upfield upon addition of $[NEt_4 \subset \mathbf{1}]^{11-}$ and reach an endpoint soon after 1 equiv. added host. (Right) Linearized plot of the chemical shift data for a titration with **2-d_0** and **2-d_7**, linear fit shown in red.

Very small EIEs are observed on the binding affinity of **2-d_n** to the exterior of **1** upon deuteration of either the ring and benzyl positions or the phosphonium methyl groups (Table 1). Titrations comparing **2-d_0** with **2-d_7** or **2-d_9** show that in both cases, the protiated **2-d_0** binds more strongly to the exterior of **1** than does either of its deuterated counterparts. To further confirm the precision and accuracy of these measurements, a titration was carried out comparing **2-d_7** to **2-d_9**. The EIE measured in this titration (Table 1) perfectly matches the EIE obtained by taking the ratio of the previous titrations: $(Kd_0 / Kd_9) / (Kd_0 / Kd_7) = Kd_7 / Kd_9 = 1.016(2)$. Finally, the EIE per deuterium atom (EIE/D; treating each deuterium as contributing equally to the EIE) can be calculated by normalizing the experimentally determined EIEs for the number of deuterium substitutions. Although the EIE/D values differ by less then 0.001, the precision of this NMR titration method allows for discrimination of the noncovalent interactions between the exterior of host **1** and different guest C-H/D bonds. The origin of these and other IEs in this system are currently under investigation and will be reported in a later article.

Table 1. Measured EIEs and EIEs/D for the association of **2-d_n** to the exterior of host **1**.

Ratio	EIE	EIE/D[b]
$\boldsymbol{Kd_0 / Kd_7}$	1.0302(4)[a]	1.00426(6)
$\boldsymbol{Kd_0 / Kd_9}$	1.047(2)[a]	1.0051(2)
$\boldsymbol{Kd_7 / Kd_9}$	1.017(3)	–

[a] EIEs reported as a weighted average of several titrations, see supporting information for individual titration data. [b] $EIE/D = (Kd_0/Kd_n)^{1/n}$.

In conclusion, we have shown that the NMR titration method originally developed by the Perrin group for measuring small pK_a differences can be used to measure secondary EIEs on the binding affinity of cationic guest **2-d_n** to the exterior of supramolecular host **1**. The exquisite precision obtained using this NMR technique enables differentiation of very small EIEs and the EIE per deuterium atom for different isotopologues in this equilibrium. Deuteration of the guest methyl groups is found to have a larger EIE and EIE/D than deuteration at the ring and benzyl positions, suggesting subtle differences in the interactions of these C-H/D bonds with the exterior of **1**. The measurement of EIEs on the noncovalent interactions in our supramolecular host-guest system nicely demonstrates the versatility and generality of Perrin's titration method for the high precision measurement of relative equilibrium constants by NMR. We look forward to the application of this technique by other researchers to precisely measure relative equilibrium constants in a wide range of chemical systems.

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Supporting Information Available: Experimental procedures, sample titration spectra, linearized titration plots, individual EIE titration data and error analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) As can be inferred from the left side of Figure 2, multiple **2-d_n** cations can bind to the exterior of **1**. Our analysis assumes changes in **2-d_n** ^{31}P chemical shifts are independent of the number of other **2-d_n** bound to **1**. This assumption is justified by the excellent linearity seen in the linearized plot on the right side of Figure 2.