



Computational and Experimental Evidence of Emergent Equilibrium Isotope Effects in Anion Receptor Complexes

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ABSTRACT: The measurement of a deuterium equilibrium isotope effect (EIE) for the aryl CH...Cl⁻ interaction of anion receptor **1H/1D** is reported. Computations corroborate the results of solution measurements for a small, normal EIE in the full complex ($K_a^H/K_a^D = 1.019 \pm 0.010$). Interestingly, isotope effects involving fragments of the anion receptor (urea, aryl ring, etc.) are predicted to produce an inverse effect. This points to an unusual and subtle structural organization effect of the anion receptor complex that changes the nature of the combined interactions to a normal isotope effect. The reversal of EIE values suggests that overall architecture of the anion receptor can dramatically impact the nature of bonding in these complexes.

The ability of isotopes to alter the strength and selectivity of binding in noncovalent interactions is well-documented for biological and nonbiological systems.^{1,2} Numerous methods have been developed to utilize selective labeling experiments to differentiate the importance of individual interactions on the complete symphony of binding, including relative chromatographic retention times,³ binding studies by mass spectrometry and spectroscopic titrations,⁴ and enzyme kinetic studies.^{5,6} Deuterium equilibrium isotope effect (DEIE) measurements in biological systems highlight the complex effects of deuterium labeling on noncovalent interactions.⁵ Recent studies combining experiments and computations provide insight into the mechanisms of DEIEs in supramolecular systems that ultimately facilitate the development of models for *ab initio* sensor and drug design.^{4,7-13}

Two classic examples of such a combined experiment-and-computation approach are the acidities of deuterium-labeled pyridines and glucose binding to human brain hexokinase (HBH). Lewis and Schramm have extensively studied the EIE of tritiated glucose binding to HBH.^{14,15} Tritium EIEs for binding to HBH range from 0.927 to 1.027 depending on the site of CH substitution. Proximal hydrogen bonding groups in the binding site consistently explained normal EIEs, and steric interactions caused the large inverse isotope effect. In an approach that inspires our current experiments, competitive pH titrations by ¹³C NMR spectroscopy of 1:1 mixed H/D-pyridines allowed for the direct measurement of K_a^H/K_a^D as small as 10⁻⁴.¹¹ DEIEs favored *d_x*-pyridine protonation and

were also dependent on the location of the deuteration (K_a^H/K_a^D range from 1.0139 to 1.0828). This example highlights the hypothesis that aggregate zero-point energy (ZPE) vibration effects are responsible for changes in DEIE rather than inductive effects. These two studies highlight the power of EIE studies in fundamentally understanding reversible interactions.

The Perrin method for competitive titration requires only a fast exchanging system with resolved resonances for the precise measurement of relative association constants by NMR spectroscopy. This method has previously been applied to guest binding in supramolecular cage complexes.^{7,8} We have adapted this method to the study of DEIEs on CH...Cl⁻ hydrogen bonding by both solution measurements and computations. The synthesis of a selectively deuterated arylethynyl bis-urea anion receptor **1D** enables competitive titration DEIE measurements in DMSO-*d*₆. The observed DEIE of the singly deuterated compound **1D** and quantum mechanical computations corroborate the importance of vibrational changes on the observed DEIE. We elucidate specific structural origins for DEIEs in anion receptor complexes and have discovered an emergent behavior: the DEIE of the receptor complex is uniquely different than DEIEs of various fragments of the complex. In other words, the nature of multiple hydrogen bonding in the anion receptor is uniquely different from simpler hydrogen bonding complexes.

1H has been previously shown to act as a strong CH donor with log K_a for Cl⁻ of 4.39 ± 0.62 in CHCl₃.¹⁶ Isotopologue **1D** is prepared by methods similar to those reported in previous papers (Scheme 1).¹⁶⁻¹⁸ Visible-light catalyzed deamination in DMF-*d*₇ allows for the selective synthesis of monodeuterated intermediate **2**.¹⁹ The final receptor **1D** and key intermediates were characterized by ¹H, ²H, and ¹³C NMR spectroscopy, and high-resolution mass spectrometry (see Supporting Information).

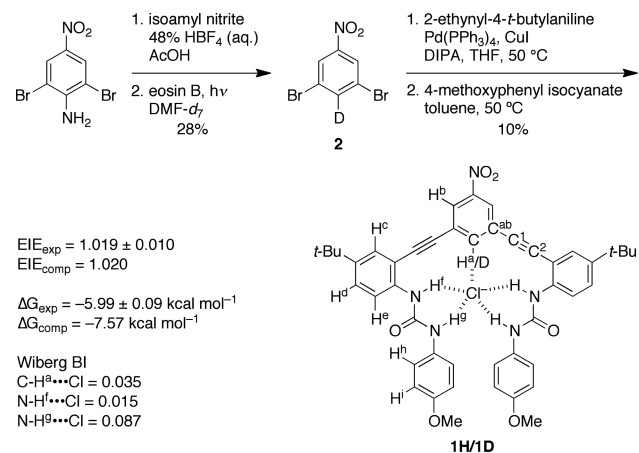
As shown by Perrin et al., ¹³C NMR spectroscopy is exceptionally sensitive to chemical shifts from isotopic labeling.^{11,20} Figure 1a illustrates the similarity between **1H/1D**. The two key differences are the loss of the H^a peak in **1D** and small shifts in ¹³C peaks. Competitive titrations were performed in DMSO-*d*₆ with ~1:1 mixture of **1H** and **1D**

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Scheme 1. Selective Deuterium Labeling of Anion Receptor 1H/1D, Summary of Equilibrium Isotope Effect, Anion Binding Energy and Wiberg Bond Index



receptors at a combined concentration of 6.1 mM, and Bu_4NCl was added in 5 μL aliquots as a 1.8 M solution.²¹ Many ^1H and ^{13}C signals shift over the course of the titration, and four ^{13}C peaks are resolved by isotope shifts between **1H** and **1D** to differentiate the two species. The labeled ^{13}C signal (C^{a}) is too weak for accurate measurement of δ during the titration, which leaves three signals (alkyne C^1 , alkyne C^2 , and C^{ab}) for possible fitting.

The binding isotherm for mixed **1H**:**1D** is plotted in Figure 1b. C^{ab} is the closest carbon to the labeled CH hydrogen bond donor and, along with the alkyne peaks, is well resolved between **1H** and **1D** with greater intensity than the labeled peak. The C^{ab} peak and alkyne C^2 are complex curves indicative of possible multistep equilibria. Alkyne C^1 presents a large downfield shift and the isotherm for this carbon approximates a binding curve for a 1:1 association. A large shift in the alkyne carbons is surprising due to the large distance from the nearest hydrogen bonding site. One explanation is the through space effect of an approaching anion that shields the alkyne. An alternative effect is rotation about the alkyne upon binding of the anion, a conformational change that has also been observed in similar alkyne receptors.^{22,23}

The remaining ^1H and ^{13}C NMR peaks produce simpler and consistent binding curves, which can be split into two categories (Figure 1b). First, several peaks reach a saturation point near 15 equiv. Cl^- and remain mostly flat for the remainder of the titration (H^{a} , C^{e} , and C^{ab}). The second category includes peaks that never appear to reach saturation in the range studied but continue to increase linearly after 15 equiv. (H^{g} , H^{f} , C^{x} , and C^{d}). The second case is consistent with a weak 1:2 complex, whereas the first case is more indicative of a 1:1 binding event. An estimated value for the overall K_{a} ($\log \beta$) is obtained from the fitting of the ^1H resonances and C^{ab} to a 1:2 $1:\text{Cl}^-$ model using the combined $[\text{1H}+\text{1D}]$. The binding in $\text{DMSO-}d_6$ is significantly weaker ($\log \beta \sim 1.8$) than in CHCl_3 ($\log K_{\text{a}} = 4.39 \pm 0.62$), which is expected for a solvent system with high polarity and strong hydrogen bond acceptors. As well, the second Cl^- association is likely very weak with $K_{\text{a}}^{1:2} < 5$. Based on the curve shape and position on **1** of atoms for the two binding isotherm categories, it is clear that the first Cl^- binds in the expected pocket to CH^{a} with both ureas, and then a second Cl^- weakly binds to one of the ureas at high $[\text{Cl}^-]$, forcing the alkyne to rotate open to accommodate it.^{22,23} The complex binding isotherm, however, does not prevent the use of a linearized plot to determine the relative K_{a} values for **1H** and **1D** with greater precision than possible by direct analytical titration and nonlinear regression.

The linearized plot of $K_{\text{a}}^{\text{H}}/K_{\text{a}}^{\text{D}}$, eq 1, provides an accurate measure of the DEIE from a competitive titration with errors as small as 0.0004 reported.^{8,11} The method requires free and bound species to be in fast equilibrium with resolved peaks but is independent of receptor concentration. Typical methods of K_{a} determination rely on nonlinear regression, and errors are restricted to ~ 5 – 10% by the accuracy in concentration. Least squares linear regression of the ^{13}C NMR chemical shifts of **1H** and **1D**, eq 1, provides the relative $K_{\text{a}}^{\text{H}}/K_{\text{a}}^{\text{D}}$ with greater precision.²⁰ The chemical shifts for alkyne C^1 ^{13}C peaks were fit with an $R^2 = 0.996$ shown in Figure 1c. The weighted average $K_{\text{a}}^{\text{H}}/K_{\text{a}}^{\text{D}}$ was 1.019 ± 0.010 for two independent titrations.

$$(\delta_{\text{H}}^{\circ} - \delta_{\text{H}})(\delta_{\text{D}} - \delta_{\text{D}}^{\text{f}}) = (K_{\text{a}}^{\text{H}}/K_{\text{a}}^{\text{D}})(\delta_{\text{H}} - \delta_{\text{H}}^{\text{f}})(\delta_{\text{D}}^{\circ} - \delta_{\text{D}}) \quad (1)$$

Quantum mechanical computations are effective at predicting kinetic and equilibrium isotope effects.^{10,11,24} Structures were optimized using PBE/6-31G(d) level of theory with the

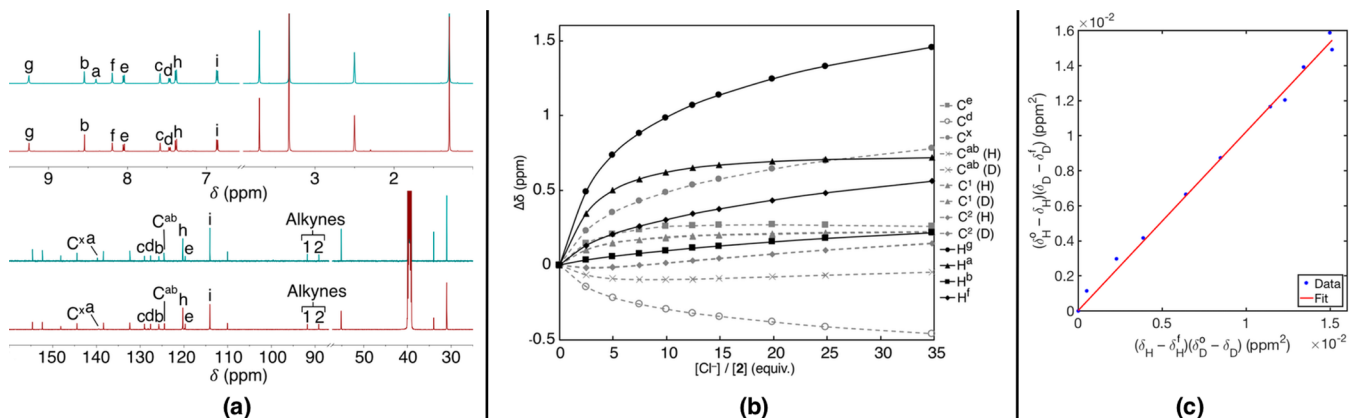


Figure 1. (a) ^1H (top) and ^{13}C (bottom) NMR spectra of **1H** (blue) and **1D** (red) in $\text{DMSO-}d_6$. C^{ab} is the aryl carbon between H^{a} and H^{b} . (b) Binding isotherm with Cl^- for 1:1 **1H**:**1D** in $\text{DMSO-}d_6$ by ^1H (solid lines) and ^{13}C (dashed lines) NMR spectroscopy; lines added as a guide for the eye. Isotherm shape is consistent with weak 1:2 binding where $K_{\text{a}}^{1:1} \gg K_{\text{a}}^{1:2}$. ^{13}C NMR peaks are used for linear EIE fitting; the isotherms overlap, although the peaks are resolved. (c) Linearized plot of $K_{\text{a}}^{\text{H}}/K_{\text{a}}^{\text{D}}$ (eq 1) for **1H**:**1D** ^{13}C NMR peak corresponding to alkyne C^1 .

polarizable continuum model (PCM) for DMSO as implemented in Gaussian 09.^{25–27} Exhaustive conformational searches led to structures of anion receptor complexes and free receptors that corroborate known crystal structures of related compounds (see SI). The computed EIE for Cl[−] binding (complex [1H/1D⋯Cl[−]]) was 1.020, in good agreement with experimental values (1.019 ± 0.010). Moreover, the binding energy between the receptor and Cl[−] was computed to be −7.57 kcal mol^{−1}, in good agreement with experiments (−5.99 kcal mol^{−1}).^{16,28}

The computed 1H/1D structure is shown in Figure 2. The chloride anion is quintuply hydrogen bonded by the central aryl

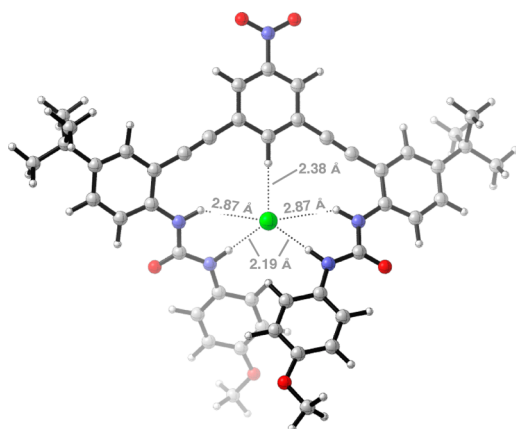


Figure 2. Quantum mechanically optimized structure of anion receptor 1H/1D with chloride anion (PBE/6-31G*/PCM(DMSO)).

CH and the four urea protons. The two substituted urea arms of the anion receptor are not planar in the presence of the anion, and the terminal urea aryl rings rest on top of each other. This structure forces an asymmetric hydrogen bond between the chloride anion and the two protons of each urea, the proton that is distal from the CH site is closer (2.19 Å) than the proton proximal to the CH (2.87 Å).

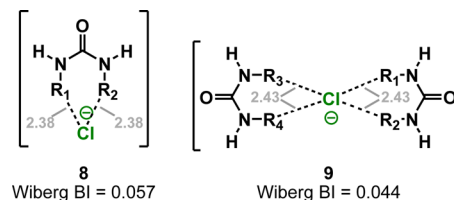
The normal EIE favors association of Cl[−] with the unlabeled receptor 1H. We fragmented the receptor into key components to explore the isotope effects of the basic receptor segments (Figure 3, S20). All fragments led to more inverse EIE values. For example, the addition of an alkyne to 4 (i.e., 6) lowered the DEIE from 1.000 to 0.989. Removing half of the receptor (5) also led to an inverse DEIE (0.993), and the addition of another alkyne (7) results in an even more inverse DEIE of

0.979. These results are surprising because no data from the fragments of the anion receptor would suggest that a normal EIE is possible (nonadditive), yet the experimentally measured and computed EIE is 1.020. This indicates that the isotope effect for [1H/D⋯Cl[−]] is more than the sum of its parts.

The computation of the fragment complexes revealed structural details relevant to elucidating the origin of this unexpected EIE of 1H/1D. In 3, the CH⋯Cl[−] geometry is bent (161°) with Cl[−] still within the plane of the aryl group. In 4 and 6, this geometry is completely linear, just as in 1H/1D. Although repulsions between the chloride anion and the alkyne groups may be responsible for this geometric change, the CH⋯Cl[−] distance in the monoalkynylated complex 4 is shorter, whereas the distance in the bis-alkynylated complex 6 is even shorter (2.66 Å vs 2.57 Å vs 2.54 Å, respectively). These data are consistent with an inductive enhancement of hydrogen bonding by the electronegative alkynes. Similar trends can be observed in complexes 5 and 7. The computed natural bond orbital (NBO) analysis revealed that the Wiberg bond index of the hydrogen bond in 3 is 0.029, whereas it is 0.036 in 4 and 0.039 in 6.²⁹ Similarly, the bond index for complex 5 and 7 are 0.025 and 0.028, respectively. The bond index for the corresponding interaction in 1H/1D is 0.035, matching closely the alkynylated complexes 4 and 6. This would suggest the EIE does not originate from the central aryl CH hydrogen bonding.

Complexes 8 and 9 reveal that the geometry of hydrogen bonding in 1H/1D is significantly distorted (Chart 1). The

Chart 1



ideal urea NH⋯Cl[−] distance is 2.38–2.43 Å, which deviates significantly from 1H/1D. NBO analysis also shows that the bond indices for hydrogen bonds in 8 and 9 are 0.057 and 0.044, respectively; significantly different from those of 1H/1D (0.015 and 0.087). These observations are consistent with the hypothesis that the unusual arrangement of urea NH and aryl-CH hydrogen bonds is responsible for the unexpected EIE of 1H/1D.

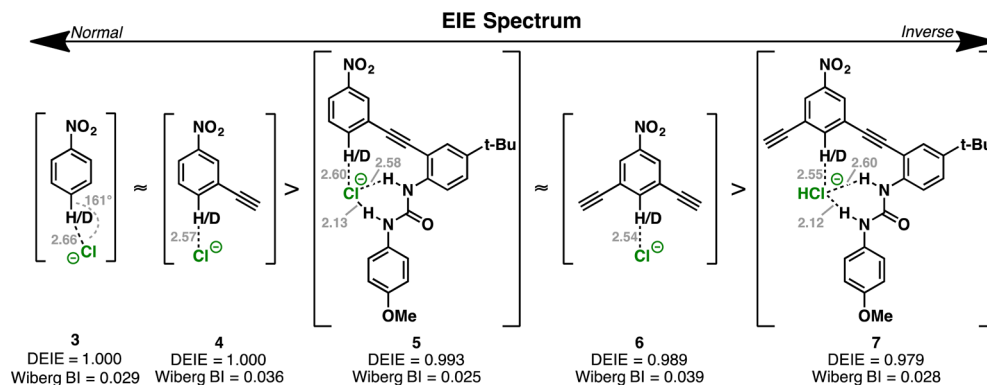


Figure 3. Computed EIE values involving chloride complexes with fragments of the anion receptor 1H/D.

In summary, the synthesis of a selectively labeled receptor has allowed for the first accurate measurement of an EIE in an aryl CH...X⁻ hydrogen bond. The combined evidence presented here is consistent with the categorization of certain aryl CH donors as hydrogen bond donors. The DEIE measurements from competitive titrations and computations agree on a small, normal DEIE that favors the formation of a CH hydrogen bond over a CD hydrogen bond. This study, more importantly, shows that supramolecular binding is a complex phenomenon that cannot be reduced to the sum of its parts. The reversal of EIE values between the complete anion receptor complex versus the fragments suggest that isotope effects are extremely sensitive to the overall architecture of the anion receptor. This discovery is highly relevant to recent applications of isotope labeling in pharmaceuticals, where the introduction of isotopes may improve the strength and selectivity of protein-drug interactions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.7b00612.

Complete synthesis and characterization for all compounds, titration data, coordinates, energies, and frequencies for all computed structures (PDF)

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Notes

The authors declare the following competing financial interest(s): NIH grant R01-GM087398 funded early-stage intellectual property that was licensed by SupraSensor Technologies, a company co-founded by M.M.H. and D.W.J.

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