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COMMUNICATIONS

Published on Web 02/02/2006

A Designed Receptor for pH-Switchable Ion Binding in Water

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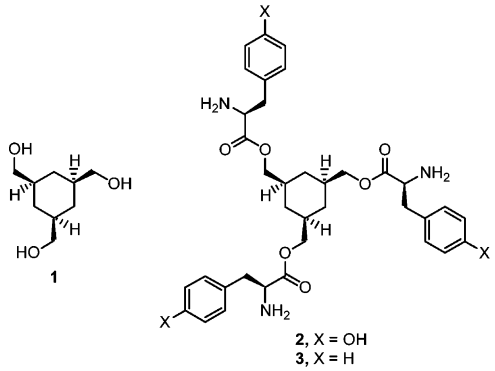
The design and synthesis of molecules with conditional or "switchable" properties is of considerable practical and fundamental interest. Fundamentally, such systems serve as models of Nature's strategies for regulating cellular processes.[1] Potential applications of "molecular switches" include drug delivery systems, optical devices, and sensors. While the majority of studies on switchable receptors have focused on photochemical switching, changes in other environmental properties, including temperature and pH, are also known. These include cyclodextrin-conjugated poly(ϵ-lysine) inclusion complexes,[2] ion recognition-mediated rotaxane switching,[3] pH-switchable polymeric capsules derived from calixarenes,[4] and pH-switchable ligands for transition metal complexation.[5]



In the context of our work on cycloalkane-based receptors for carbohydrates and other metabolites,[6] we began exploring the structures and binding properties of derivatives of cyclohexane 1,3,5-*cis*-trimethanol (**1**). In particular, we were intrigued by molecular mechanics conformational searches[7] of trityrosine derivative **2**, which suggested that this receptor could access a low-energy "closed" conformation in its neutral state, mediated by hydrogen bonding between tyrosine −OH and amino groups (Figure 1). In contrast, **2** would be expected to exist as a complex mixture of "open" conformers as either a cation or an anion. Literature values[8] for the tyrosine amino and phenolic −OH pK_a's are 9.21 and 10.46, respectively. Therefore, we anticipated that **2** might serve as a pH-switchable receptor, adopting the closed conformation only in the range of 9.2 < pH < 10.5. While phenol−amine H-bonded complexes are well-known,[9] they have been characterized almost exclusively in the solid state. Therefore, we set out to test whether the predicted conformation−switching behavior of **2** could indeed be observed in solution, and if it translated to pH-dependent differences in ion binding ability.

Synthesis of **2** was accomplished in a straightforward three-step reduction/esterification/deprotection sequence from cyclohexane 1,3,5-tricarboxylic acid, with final purification by preparative reverse-phase HPLC. Since triphenylalanine derivative **3** would not be capable of achieving the same H-bonded closed structure proposed for **2**, it was also synthesized as a control. With **2** and **3** in hand, we next set out to examine their solution conformational behavior.

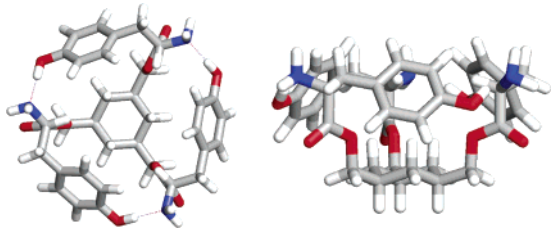


Figure 1. H-bonded closed conformation of **2** predicted by molecular mechanics.

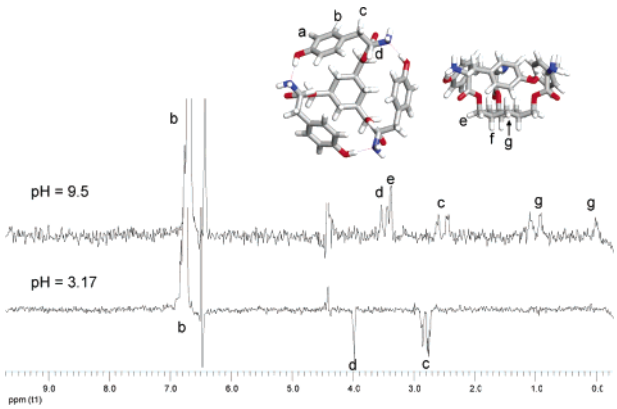


Figure 2. Extracted rows ($\delta t2 = 6.8$ ppm) from 2D ^{1}H−^{1}H NOESY spectra of **2** at pH* 9.50 (100 mM bicarbonate/carbonate buffer) and **2** at pH* 3.17 in D_2O.

We anticipated that 2D NOE (NOESY) spectroscopy would be particularly valuable for examining pH-dependent changes in the solution conformation of **2**. Indeed, NOESY spectra of **2** in D_2O at pH* = 3.17 and in a sodium bicarbonate/carbonate buffer (100 mM in D_2O; pH* = 9.40) showed significant differences in the number, intensity, and sign of proton NOEs. In particular, NOESY cross-peaks between aromatic and cyclohexyl protons are visible in the pH* 9.4 spectrum, and not in the pH* 3.17 spectrum. The change in sign of NOESY cross-peaks is also noteworthy. The intensity and sign of the NOE is a function of $\omega\tau_c$, where ω is the magnetic field strength and τ_c is the correlation time. Since "small" organic molecules tend to have values of τ_c that place them near the zero-crossing point for the theoretical NOE curve, their enhancements can be positive, zero, or negative.[10] In this case, the sign change from negative NOE (higher τ_c) at pH* 3.17 to positive (lower τ_c) is consistent with a conformational change from extended to compact. Differences in the solution conformational behavior of compounds are also reflected in their longitudinal or spin−lattice relaxation ($T1$) times.[11] $T1$ measurement of **2** at pH* = 3.17 and 9.40 showed considerable differences in the relaxation rates of several protons. These pH-dependent differences are most pro-

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10.1021/ja058126p CCC: $33.50

Table 1. ITC Results for Ion Binding to **2** in AMPSO, pH 9.50

ion	K (M^{-1})	ΔH (cal)	ΔS (cal)
fluoride	(very weak)		
chloride	490 ± 54	870 ± 50	15.2
bromide	680 ± 92	720 ± 44	33.8
sulfate	0		
nitrate	0		
zinc(OTf)$_2$	1410 ± 170	−2230 ± 96	7.1
Cd(OTf)$_2$	1900 ± 150	−2880 ± 70	5.5

nounced for the pendant tyrosines, consistent with the majority of the change in conformational bias occurring in this portion of **2**. In contrast, neither **3** nor tyrosine methyl ester showed pH-dependent changes in $T1$ values, supporting the idea that changes in the relaxation behavior of **2** are due to a conformational shift, and not simply due to variations in dissolved ion concentrations or to the change in ionization state.

We next examined the ability of **2** to function as a receptor for ions.[12] In addition to constituting a fundamentally interesting problem in itself, we anticipated that ion binding would serve as an efficient structural reporter for the conformation (open or closed) of **2**. Molecular modeling of the closed conformer suggested that smaller ions would likely fit into the cavity formed by the cyclohexane core and tyrosine side chains, while larger ions, such as nitrate, would likely not fit as well.

An initial screen was conducted in 96-well plates, monitoring changes in UV−vis absorbance as a function of added inorganic salt. Several species (Ce^{4+}, Ag^{+}, and Sn^{2+}) caused immediate precipitation of **2** at pH 9.5. However, both Zn^{2+} and Cd^{2+} induced changes in UV−vis absorbance without causing obvious precipitation, warranting further exploration. We also examined anion binding to **2** by UV−vis titration. Chloride and bromide anions, present from the dissociation of the corresponding tetrabutylammonium salts, bind to **2** at pH 9.50 with association constants (K_a) of 680 ± 146 (bromide) and 550 ± 112 (chloride). Binding to tetrabutylammonium nitrate or fluoride was not observed. In addition, there was no binding observed to **2** at pH 7.0, to **3** at pH 9.50, or to tyrosine methyl ester at pH 9.50.

Isothermal titration calorimetry (ITC) experiments confirmed that both chloride and bromide bind to **2** at pH 9.5 (Table 1), with association constants consistent with those determined by UV−vis titration. While ITC showed some interaction between **2** and fluoride, the small amount of heat evolved precluded extraction of thermodynamic data. No interaction between **2** and nitrate or sulfate was detectable at pH 9.5. As in the UV−vis experiments, no binding of fluoride, chloride, or bromide was observed to **2** at pH < 9.5. As the putative binding cavity of **2** is electron-rich, the observation that Cl^{-} and Br^{-} bind was somewhat surprising. ITC thermograms for bromide and chloride at pH 9.50 were endothermic, characterized by an unfavorable enthalpic term (ΔH) and balanced by positive entropy (ΔS). This suggests that anion binding may be driven not by specific interactions within the receptor cavity, but rather by the disruption of solvent within the highly hydrophobic structure. This is consistent with other authors' analysis of entropy-driven binding to hydrophobic receptors.[13]

In contrast, ITC measurements of cation binding to **2** at pH 9.5 showed exothermic binding, driven by favorable enthalpy. As with anion binding, no cation binding was observed for **2** at pH < 9.5. Confirming that none of our NMR or ion-binding results were affected by potential receptor aggregation, the ITC of **2** into buffer alone (both at pH 5.27 and 9.5) showed essentially that no heat evolved. Job plot analyses of **2** with Zn^{2+} confirmed the 1:1 binding model indicated by ITC data. Finally, an NMR titration of zinc triflate into **2** at pH 9.5 showed Zn^{2+}-dependent changes in chemical shift and line broadening of cyclohexyl protons, consistent with ion binding occurring inside the cavity formed by the closed conformation of **2**.

In conclusion, we have employed NMR spectroscopy to demonstrate that the cyclohexane-based receptor **2** undergoes a pH-dependent conformational change, consistent with molecular mechanics-based predictions. UV−vis and calorimetric titrations confirm that **2** can act as a receptor for ions at high pH, but not at low pH. In combination, these data suggest that the tyrosine OH−amine H-bond is a useful structural element for the design of pH-switchable receptors. Compounds such as **2** may also serve as useful models for naturally occurring phenol−amine H-bonds. Hydrogen bonds to tyrosine hydroxyl protons are common structural motifs in proteins and often manifest as changes in pK_a. RNAse A is a prototypical example, with several buried/H-bonded tyrosine residues exhibiting pK_a's > 11.5.[14] Since new methods for characterizing such interactions will be particularly useful, an immediate goal will be to extend our recently reported method for pK_a determination by ITC to tyrosines.[15]

Acknowledgment. The authors thank the NIH-NIGMS for support of this work (5-R01-GM-062825). S.G.T. was supported via an NIH training grant in dermatology (T32AR007472).

Supporting Information Available: Synthetic procedures and characterization of compounds **2** and **3**, ITC, UV and NMR titration experiments, Job plots, NOESY spectra, and ^{1}H $T1$ measurements (29 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA058126P