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Thermodynamics of Xenon Binding to Cryptophane in Water and Human Plasma

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Noble gas spectroscopies present exciting opportunities for biomolecular imaging. Xenon-129 is nontoxic, has a spin-1/2 nucleus, and can be laser-polarized to increase nuclear magnetic resonance (NMR) signals more than 10 000-fold. ^{129}Xe polarizability contributes to a 300 ppm NMR chemical shift window,[1] which facilitates the simultaneous detection of ^{129}Xe in different chemical environments. Target-specific ^{129}Xe biosensors have been generated by attaching a xenon-binding cryptophane to various bioactive moieties.[2,3] Furthermore, ^{129}Xe bound to cryptophane can be spectrally and spatially resolved in MR imaging.[4] We sought to investigate whether xenon binds cryptophane in biological media, as xenon is an anesthetic[5] and associates weakly with many proteins.[6] Cryptophane **1** (Scheme 1) was synthesized and determined by two complementary techniques to have unprecedented affinity for xenon, in water and human plasma.

Previous determinations of aqueous xenon binding constants for small molecules have relied on NMR measurements either through the analysis of ^{1}H chemical shifts in the presence of xenon or the direct integration of free and bound ^{129}Xe resonances. The binding affinities for xenon of α-cyclodextrin,[7] cucurbitril,[8] and Huber's series of water-soluble hexa-acid cryptophanes[9] were measured in this manner. Herein, we describe more sensitive fluorescence quenching and isothermal calorimetry (ITC) methods for studying xenon binding to **1** that involved titration of aqueous xenon solutions.

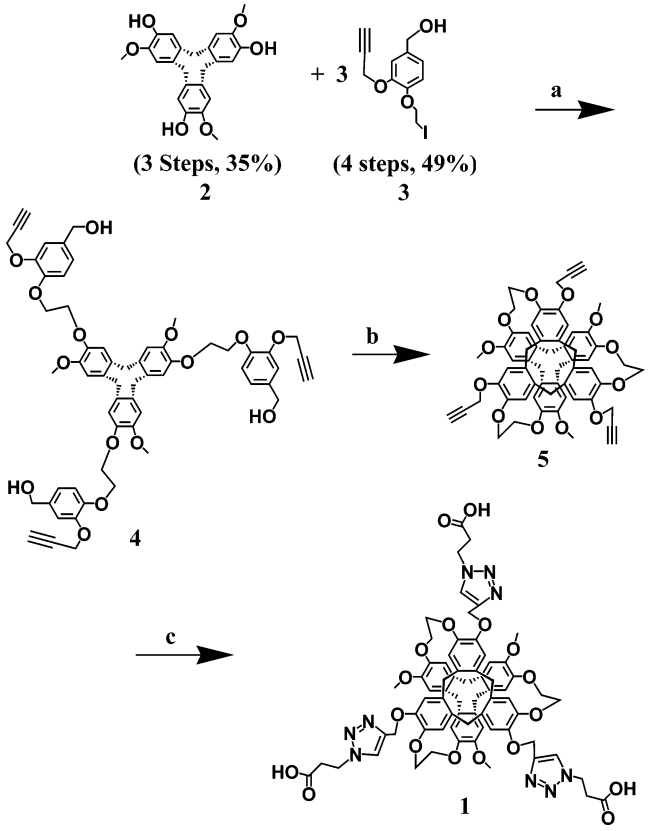
We developed a facile synthesis for water-soluble cryptophane based on the copper(I)-mediated [3 + 2] azide-alkyne Huisgen cycloaddition.[10] The 10-step synthesis, shown in Scheme 1, yielded triacid-functionalized cryptophane **1** in 4% overall yield. **1** (2 mM) was readily dissolved in aqueous base and remained soluble in 100 mM NaCl to pH ∼ 5.5. A solution of **1** (60 μM) in 1 mM, pH 7.2 phosphate buffer at 300 K had a hyperpolarized ^{129}Xe NMR chemical shift of 64.6 ppm relative to xenon gas. ^{1}H NMR measurements of **1** in D_2O showed only the crown−crown structural isomer at temperatures as high as 333 K. No evidence for a crown-saddle isomer was detected, unlike the water-soluble cryptophanes reported by Huber et al.[9]

1 exhibited similar fluorescence in water (λ_{em} = 313 nm) to the six 1,2-dialkoxybenzenes that form its cage. This led us to study the xenon binding equilibrium by heavy-atom quenching:

$$\mathrm{Xe}_{(aq)} + \mathbf{1}_{(aq)} \rightleftharpoons \mathrm{Xe@}\mathbf{1}_{(aq)} \qquad (1)$$

Xenon was previously shown to quench 2-phenanthrene sulfonate and pyrene bound to apomyoglobin,[11] but this required a large overpressure of xenon due to its low affinity for the protein. In the current study, fluorescence quenching by xenon provided a sensitive method of measuring Xe binding, even at substoichiometric Xe concentrations.

Scheme 1. Ten-Step Synthesis of Cryptophane **1**[a]



[a] Conditions: (a) Cs_2CO_3, DMF, 55 °C, 8 h, 60%; (b) $HClO_4$, MeOH, room temperature, 14 h, 48%; (c) 3-bromopropionic acid, NaN_3, $CuSO_4$, 2,6-lutidine, Na-ascorbate, K_2CO_3, room temperature, 12 h, 92%.

Fluorescence quenching experiments were conducted at 293 K with 1.5 × 10^{-5} M solutions of **1** in 1 mM, pH 7.2 phosphate buffer. A saturated xenon solution at 310 K ([Xe] = 3.3 mM)[12] was titrated into the septum-sealed cuvette by gastight syringe. To obtain a saturated xenon measurement, xenon gas was bubbled directly into the cuvette. In all cases, fluorescence spectra were collected after thermal equilibration at 293 K (Figure 1).

The fluorescence maxima were fitted to a single-site binding model using the following relationship:

$$\frac{[\mathrm{Xe@}\mathbf{1}]}{[\mathrm{Xe}] + [\mathbf{1}]} = \frac{[\mathrm{Xe}]}{[\mathrm{Xe}] + K_D} \qquad (2)$$

where K_D is the Xe dissociation constant for cryptophane **1**. At xenon saturation, [Xe] = 5.05 mM at 293 K and 1 atm,[12] the cryptophane fluorescence was half quenched (F_o/F = 2). This may be explained by preferential Xe interaction, on the subnanosecond time scale of fluorescence, with one of the two cyclotriveratrylene

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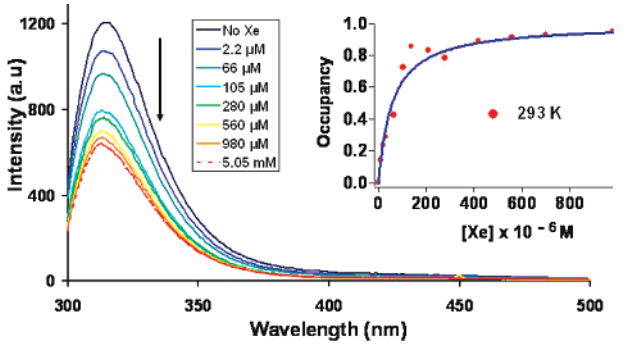

Figure 1. Fluorescence quenching of **1** (15 μM) by Xe in 1 mM, pH 7.2 phosphate buffer, 293 K. Inset: Curve fits for a single-site binding model.

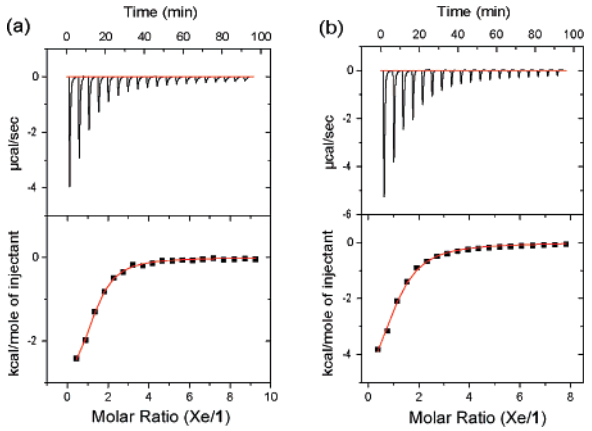

Figure 2. Enthalpograms of (a) **1** (88 μM) in phosphate buffer at 310 K titrated with saturated aqueous xenon (3.3 mM); and (b) **1** (112 μM) in human plasma at 310 K titrated with xenon-saturated plasma (4.0 mM).

units that make up the cryptophane. No cryptophane impurities or concentration-dependent phenomena were observed that might also contribute to partial fluorescence quenching. An association constant of $(1.7 \pm 0.2) \times 10^4$ M^{-1} (1 standard deviation) was obtained at 293 K. This value is roughly twice the best reported K_A values for cryptophane-A derivatives in water at room temperature.[9]

Xenon binding was also determined by isothermal titration calorimetry (ITC),[13] which directly measured the heat released during Xe association. ITC measurements were undertaken in 20 mM, pH 7.5 phosphate buffer at 293 and 310 K as well as in human plasma at 310 K (Figure 2).

The association constants obtained from fits of ITC data at 293 K ($K_A = 1.73 \times 10^4$ M^{-1}, Table 1) and fluorescence quenching were in excellent agreement. A higher binding affinity ($K_A = 3.01 \times 10^4$ M^{-1}) was observed in buffer at physiological temperature. It is apparent from the relative magnitudes of ΔH and $-T\Delta S$ at 293 and 310 K in phosphate buffer that entropy was a major contributor to xenon binding. This was likely a consequence of the 20 water molecules that make up the first solvation sphere of the Xe atom in aqueous solution.[14]

Xenon binding in plasma, while comparable to buffer at 310 K, showed significant differences in measured enthalpy and entropy. From Table 1 and the known thermodynamics of xenon partitioning into water,[12] an upper limit of $\Delta H = -7.5$ kcal/mol was calculated for the enthalpy of xenon binding to **1**. (See Supporting Information for details.) Thus, the measured ΔH of -6.04 kcal/mol, although larger than in buffer, appeared reasonable. Furthermore, the average literature value for xenon solubility in plasma at 310 K is 3.6 mM, which is ~10% higher than in buffer.[12] These results suggest that

Table 1. Thermodynamic Binding Parameters of Xe@**1** Obtained by ITC

	K_A ($M^{-1} \times 10^4$)	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	$T\Delta S$ (kcal mol^{-1})
buffer 293 K	1.73 ± 0.17	-5.69	-3.14 ± 0.20	2.55
buffer 310 K	3.01 ± 0.26	-6.36	-3.56 ± 0.13	2.80
plasma 310 K	2.19 ± 0.22	-6.16	-6.04 ± 0.33	0.12

xenon interacts with proteins and lipids in plasma, which reduces the contribution of entropy ($T\Delta S = 0.12$, Table 1) relative to Xe binding **1** in buffer. More details are provided in the Supporting Information.

In summary, we synthesized a water-soluble cryptophane **1** that exhibited better Xe binding, $K_A \approx 3.0 \times 10^4$ M^{-1} at 310 K, than any previously described compound. Fluorescence and ITC methods offered distinct advantages in sensitivity, speed, and accuracy for measuring Xe binding parameters at micromolar cryptophane and xenon concentrations. This study illustrates the important role of entropy for the encapsulation of a hydrophobic guest (xenon) by an organic host molecule (cryptophane) in water. The demonstration of xenon binding to cryptophane **1** in human plasma strongly supports the development of ^{129}Xe biosensors for in vivo studies.

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Supporting Information Available: Preparation of saturated xenon solutions, fluorescence, ITC controls, thermodynamic calculations, synthetic procedures, compound characterization, and hyperpolarized ^{129}Xe NMR spectrum of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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