Water soluble cucurbit[6]uril derivative as a potential Xe carrier for ^{129}Xe NMR-based biosensors†

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The water soluble cucurbit[6]uril derivative CB*[6] forms a thermodynamically and kinetically stable host–guest complex with xenon in water, the binding affinity of which is about 3 × 10^3 M^{-1}, comparable to those of cryptophanes, suggesting that it may serve as an effective molecular "carrier" for ^{129}Xe NMR-based biosensors.

Hyperpolarized (HP) ^{129}Xe NMR[1] has drawn much attention as it provides a useful tool for molecular imaging *in vivo*.[2] Detection of specific biomolecules in solution is achieved by Xe biosensors, which trap Xe atoms in molecular cages that have been functionalized to bind the specific target. Wemmer and Pines *et al*. recently reported a new approach to MR imaging of Xe biosensors based on chemical exchange between the biosensor encapsulated and free Xe atoms, which increases the sensitivity substantially.[2a] Cryptophanes have been exclusively used as molecular cages in the Xe NMR-based biosensors because of their strong affinity for xenon ($K \approx 10^3$–10^4 M^{-1} in aqueous solution), resulting in a large separation in chemical shifts between free and encapsulated Xe atoms.[3,4] Other host molecules such as cyclodextrins and calixarenes do not match cryptophanes in terms of binding constants for xenon and exchange rates.[5] Nevertheless, cryptophanes suffer some drawbacks including tedious multi-step synthesis and separation of enantiomers. Thus, developing new molecular cages for Xe biosensors is important for practical applications of Xe biosensors. Here we report a new potential Xe carrier for NMR-based biosensors, which forms a stable complex with xenon thermodynamically as well as kinetically.

Cucurbit[*n*]uril (CB[*n*], n = 5–10), a family of macrocyclic compounds comprising *n* glycoluril units,[6] have a hydrophobic cavity accessible through two identical portals surrounded by polar ureido carbonyl groups, which allow them to bind a wide range of guest molecules.[7] In particular, CB[6], with a cavity of 5.5 Å in diameter, is suitable for complexation with xenon.[8,9] The binding affinity of CB[6] for xenon was reported to be ~200 M^{-1}, which was indirectly measured using ^{1}H NMR spectroscopy in 0.2 M aqueous Na_2SO_4 solution.[8] However, the poor solubility of CB[6] ($<10^{-5}$ M^{-1}) in pure water[7,10] makes it very difficult to investigate ^{129}Xe NMR properties such as the kinetics of binding and the nuclear polarization lifetime of xenon in CB[6]. The recently synthesized cucurbituril derivative CB*[6] (Fig. 1),[11] whose cavity dimensions are essentially the same as those of CB[6], is soluble (2×10^{-1} M) in pure water. This remarkable solubility of CB*[6] led us to examine the thermodynamics and ^{129}Xe NMR properties associated with its complexation with xenon in water (Scheme 1) by isothermal titration calorimetry (ITC) and HP ^{129}Xe NMR methods, respectively, to evaluate the potential of CB[6] as a Xe carrier for NMR-based biosensors. We also investigated the effect of a cation on inclusion of xenon inside CB*[6] using 2D ^{129}Xe exchange NMR spectroscopy.

To obtain the binding affinity and thermodynamic parameters associated with the complexation of xenon with CB*[6], we carried out ITC experiments in pure water at 295 K. A typical ITC titration curve is shown in Fig. 2. The binding constant of xenon to CB*[6] was measured to be $(3.4 \pm 0.1) \times 10^3$ M^{-1}, which is comparable to those of water soluble cryptophanes[4a] and one or two orders of magnitude larger than those of other host molecules such as cyclodextrins, calixarenes and proteins.[5,12] The complexation of xenon with CB*[6] is driven to a similar extent by both enthalpy and entropy ($\Delta H^\circ = -2.3 \pm 0.3$ kcal mol^{-1}, $T\Delta S^\circ = 2.4 \pm 0.3$ kcal mol^{-1}). The enthalpic gain for the complex is presumably due to the van der Waals interaction between xenon and the inner wall of the host cavity as well as the removal of high energy waters in the cavity.[13] The large positive entropy value may result from extensive dehydration of the water molecules surrounding xenon and encapsulated in the cavity of CB*[6] upon complexation. The entropy value, 2.4 kcal mol^{-1}, is similar to that (2.55 kcal mol^{-1}) of the cryptophane synthesized *via* click chemistry by Hill *et al*.[4b]

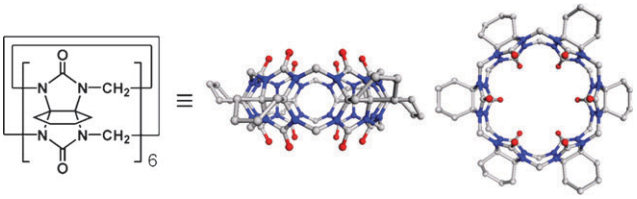

Fig. 1 Structural formula and X-ray crystal structure of CB*[6].

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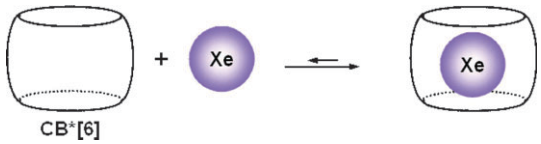

Scheme 1

The NMR properties of xenon bound to CB*[6] were also examined using the HP ^{129}Xe NMR technique. All ^{129}Xe NMR experiments were performed at 295 K on a Bruker Avance 300 spectrometer (83.02 MHz frequency for ^{129}Xe) equipped with a super-widebore magnet and a 10 mm broad-band probe, and chemical shifts were referenced to free xenon gas at 0 ppm. The xenon polarization was 4 orders of magnitude higher than the thermal one. Fig. 3a shows the HP ^{129}Xe NMR spectrum of xenon in the presence of 3 equiv. CB*[6] in pure water. The signal of the encapsulated xenon was observed at 97 ppm, remarkably shifted to higher field (by 93 ppm) compared with that of free xenon at 190 ppm in water. The two well-separated signals for free and bound xenon indicate that the complexation and decomplexation of xenon is sufficiently slow on the NMR time-scale. From the integration of the signals for free and bound xenon (total [Xe] = 0.83 mM) in the presence of 3 equiv. CB*[6], the binding constants for the complexation of xenon with CB*[6] were estimated to be around 1300 M^{-1}, which is the same order of magnitude as that measured by ITC. In this case, an accurate estimation was difficult because of the line broadening due to the exchange of xenon in and out the host.

To examine the effects of cations on inclusion of xenon, a ^{129}Xe NMR spectrum was also obtained for the complex in 0.2 M aqueous Na_2SO_4 solution (Fig. 3b). The NMR signal of the bound Xe in the salt solution is shifted downfield compared with that in pure water, which is presumably caused by the interactions between the encapsulated Xe and the cations on the portals.[14] In this case, the population of the bound xenon is smaller than that of free xenon, opposite to the situation in pure water, indicating that the binding affinity of CB*[6] for xenon in the salt solution is smaller than that in pure water. The binding constant for the complexation of xenon with CB*[6] in aqueous Na_2SO_4 solution was determined to be 180 M^{-1} by integrating the signals of the free and bound species (total [Xe] = 0.76 mM). This value is consistent with the one reported for CB[6] ($\sim$200 M^{-1}) under the same conditions, which was measured by changes in the chemical shifts of the CB[6] protons or competition experiments with another guest, tetrahydrofuran.[8] Despite the smaller peak separation between the free and bound signals, the narrower line-widths of the ^{129}Xe NMR signals in aqueous Na_2SO_4 solution compared to those in pure water suggested that the Xe exchange rate in aqueous Na_2SO_4 solution is slower than that in pure water.

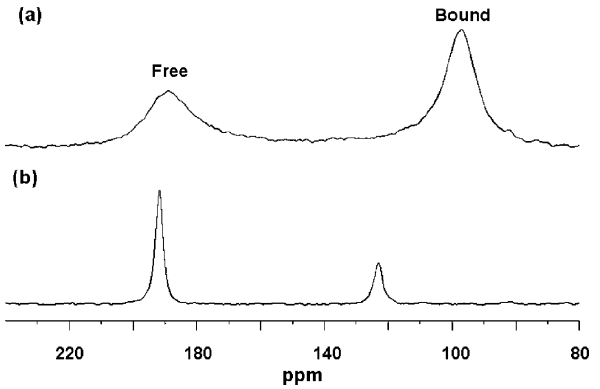

Fig. 3 Hyperpolarized ^{129}Xe NMR spectra of xenon in the presence of 3 equiv. CB*[6] (a) in pure water and (b) in 0.2 M aqueous Na_2SO_4 solution at 295 K.

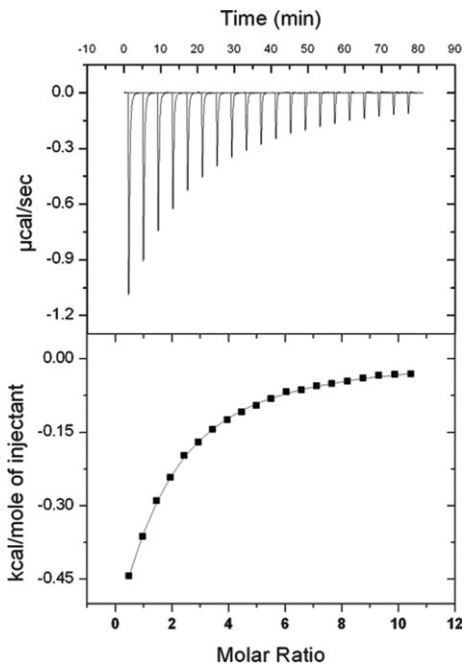

Fig. 2 ITC titration curves, thermogram (top) and isotherm (bottom), of CB*[6] (0.10 mM) titrated with saturated aqueous xenon (4.74 mM) in water at 295 K.

To quantify the kinetic behavior of xenon, the rate constants were obtained using two-dimensional (2D) HP ^{129}Xe exchange spectroscopy (EXSY).[15] The rate constants for ingression and egression, $k_{ingress}$ and k_{egress}, were extracted from the mole fractions of the two exchanging species and the integrated volumes of diagonal and cross-peaks in the 2D EXSY spectra (see ESI†) at various mixing times,[16] and the values in both pure water and aqueous Na_2SO_4 solution are listed in Table 1. The rate constants, $k_{ingress}$ and k_{egress}, in aqueous Na_2SO_4 solution are about 50 times and 8 times smaller than those in pure water, respectively, indicating that the motion of xenon to enter or leave the cavity of CB*[6] is slower in aqueous Na_2SO_4 solution, which should be attributed to the presence of Na^+ cations at the portals.[17]

Table 1 Rate constants and binding constants for inclusion of xenon in CB*[6] in pure water and 0.2 M aqueous Na_2SO_4 solution determined by HP ^{129}Xe NMR spectroscopy

	H_2O	Na_2SO_4–H_2O
$k_{ingress}/M^{-1}\ s^{-1}$	$(3.0 \pm 0.2) \times 10^6$	$(5.4 \pm 0.1) \times 10^4$
k_{egress}/s^{-1}	$(2.3 \pm 0.1) \times 10^3$	$(3.1 \pm 0.1) \times 10^2$
K/M^{-1}	$(1.3 \pm 0.1) \times 10^3$	$(1.8 \pm 0.1) \times 10^2$

To evaluate the nuclear polarization life time of the HP xenon encapsulated inside the cavity of CB*[6], the spin–lattice relaxation time (T_1) was measured with a succession of small angle read pulses (20°) at constant time intervals (17 s). The relaxation time of xenon in CB*[6] was calculated to be approximately 40 s, which should be sufficient for the transfer, mixing and detection of the polarized xenon. The relaxation time of xenon in CB*[6] is much larger than those in water soluble cryptophanes.[4a] The ^{129}Xe–^{1}H dipole–dipole interactions with the methylene protons at the portals of CB*[6] may play a major role in relaxing xenon in CB*[6]. A further increase in the xenon relaxation time would be achieved by deuterium labeling of the methylene units, which can be readily realized by use of deuterated formaldehyde in the CB synthesis.[6,11]

In summary, we have demonstrated the potential utility of the synthetic host molecule CB*[6] as a xenon carrier for NMR-based biosensors, as it forms a stable complex with Xe with a high binding affinity comparable to those of water soluble cryptophanes. Furthermore, the T_1 relaxation time measured for xenon in CB*[6] is long enough to maintain the polarization for the purpose of obtaining highly enhanced NMR/MRI signals for biological and medical applications. The kinetic stability can be further improved by addition of Na^+ ions as demonstrated by the slower exchange rate in the presence of the ions. The direct functionalization method of CB[*n*] developed recently in our laboratory[7c,18] will allow us to synthesize CB[6] derivatives that can be attached to desired targeting moieties such as a specific ligand or antibody for biosensor applications. Taken together, the remarkable Xe NMR properties and synthetic perspectives make CB[6] derivatives promising candidates as molecular cages for Xe biosensors.

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