Inside−Outside Isomerism of β-Cyclodextrin Covalently Linked with a Naphthyl Group

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Cyclodextrin complexes have been studied for their abilities to exert photophysical and photochemical control on reactive intermediates and on photophysical probes.[1] Enhanced luminescence yields, protective quenching, and controlled photochemical reactivity have been frequently observed.[1] Recent efforts have been directed to the preparation and study of cyclodextrins with covalently-attached groups capable of detecting the binding state of prospective hosts,[2] and several strategies that allow for the controlled switching of their binding and sensing capabilities have been proposed.[3,4] In this communication, we report the properties of a short-tethered naphthyl probe sterically restricted to reside inside the cavity of β-cyclodextrin at ambient temperatures (**2**, Scheme 1). It is shown that **2** is susceptible to temperature-dependent inside−outside isomerism that allows for temperature control of its complexation with other hydrophobic probes, such as 2-(*p*-toluidino)-6-naphthalenesulfonate.[5]

Compound **2** was obtained by irradiation of the inclusion complex of β-naphthyldiazomethane (**1**)[6,7] with β-cyclodextrin (β-CD), as recently reported by Abelt et al.[8] Irradiation in H_2O leads to formation of a transient β-naphthylcarbene complex which reacts by insertion into nearby hydroxyl groups[9] in a method reminiscent of photoaffinity labeling strategies.[10] Analysis of reversed-phase HPLC-purified **2** by ^{1}H NMR (D_2O, 400 MHz) showed aromatic and cyclodextrin signals integrating for the expected 1:1 stoichiometry assigned to the 3-*O*-(2-methylnaphthyl)-β-cyclodextrin derivative **2.**[11]

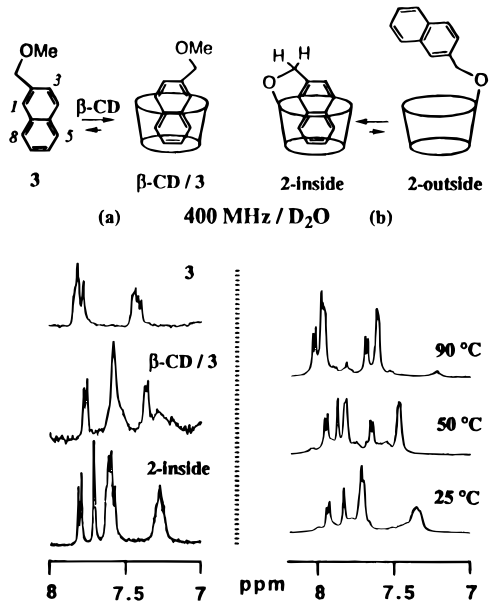
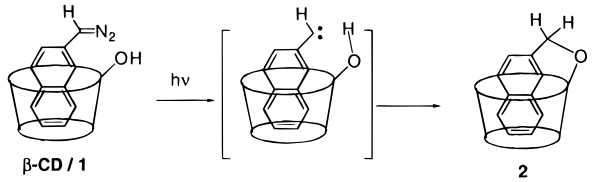


Figure 1. (a) ^{1}H NMR spectra (400 MHz, D_2O) of the aromatic region of **2** (bottom), inclusion complex β-CD/**3** (middle), and methyl ether **3** (top). (b) Aromatic region of **2** at different temperatures.

Scheme 1



Comparative ^{1}H NMR analysis of **2** with methylnaphthyl methyl ether (**3**) and its inclusion complex with β-cyclodextrin, β-CD/**3**, revealed a clear correlation between ^{1}H NMR shifts and water exposure that supports the assignment of the inside-naphthyl structure, as represented in Scheme 1 and Figure 1. Compound **3** in D_2O ($\sim 2 \times 10^{-4}$ M) shows two distinct sets of signals at 7.8 and 7.4 ppm, corresponding to α- and β-hydrogens of a freely rotating and water-exposed naphthyl group (top spectrum, Figure 1a). The spectrum of inclusion complex β-CD/**3** (prepared by addition of β-cyclodextrin to dilute aqueous solutions of **3**) displays some broadening and upfield shifts of up to ~0.2 ppm for hydrogens inside the cyclodextrin cavity.[12] The spectrum of covalently bound **2** at ambient temperatures resembles that of the inclusion complex. Relatively large upfield shifts were assigned to hydrogens inside the cavity (i.e., H5, H6, H7, and H8). A smaller upfield shift for H1 (singlet) and H4 (doublet) was taken as an indication of proximity to the water interface and partial water exposure. Small (~2%) but unambiguous NOE enhancements were observed at 30 °C in the cyclodextrin hydrogens between 3.3 and 3.7 ppm when signals at 7.86 (H4), 7.77 (H1), and 7.69 (H5 and H8) ppm were irradiated. Irradiation of the H3 doublet at 7.59 ppm gave no enhancement.

As expected for a structure possessing a short tether with restricted conformational motions, **2** presents a temperature-dependent ^{1}H NMR spectrum in pure D_2O (Figure 1b). Downfield shifts and regrouping of α- and β-naphthyl signals at high temperatures render the spectrum of **2** quite similar to that of **3**, which is itself temperature-independent. The temperature dependence of inclusion compound β-CD/**3** (not shown)

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(7) Inclusion complexes were prepared by addition of concentrated methanolic solutions of freshly prepared **1** to dilute solutions of β-cyclodextrin in a 1:1 molar ratio. Photolyses were carried out immediately with a 400 W Hanovia lamp ($\lambda > 300$ nm, Pyrex filter).

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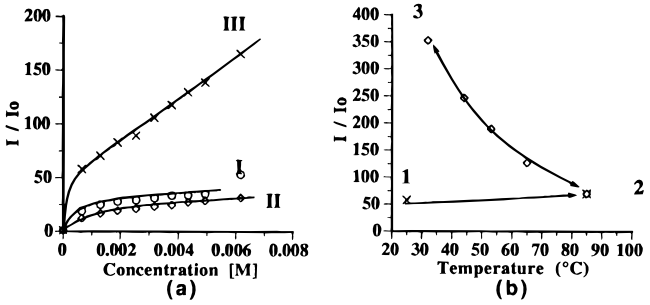


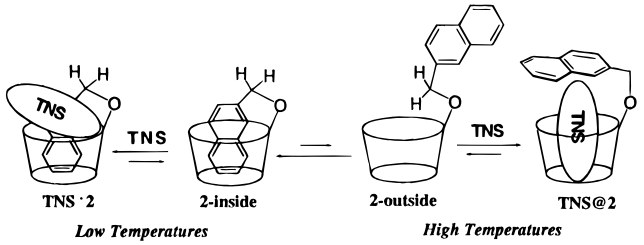
Figure 2. (a) Changes in fluorescence intensity of 10^{-5} M TNS to which varying amounts of β-CD (I), complex β-CD/**3** (II), and **2** (III) are added. (b) Intensity changes of 10^{-5} M TNS and 0.5×10^{-3} M **2** as a function of temperature. Measurements were carried out in the sequence indicated. Excitation was at 366 nm and detection at 485 nm in all cases.

parallels that of **2**. Both samples suggest an increase in water exposure and thus enhanced dissociation in the case of β-CD/**3** and conformational isomerization to the outside isomer in the case of **2** (Figure 1).

The equilibrium model of Figure 1 suggests that inclusion of external hydrophobic guests should be hindered at ambient temperatures but feasible at higher temperatures. The emission properties of **2** were measured to determine whether its fluorescence may be used to ascertain the binding state of external hosts. Unfortunately, the fluorescence spectrum of **2** is virtually identical to those of free **3** and β-CD/**3**,[13] and their lifetimes in Ar-saturated solutions were relatively short and similar (27.5, 27.4 and 22.8 ns, respectively). Since the effects of water exposure on the naphthyl methyl ether chromophore are so small, we decided to test the isomerism of **2** with an ideal extrinsic hydrophobic fluorescence probe, and 2-(*p*-toluidino)-6-naphthalenesulfonate (TNS) was selected.[14]

It is known that TNS displays negligible fluorescence in H_2O but emits strongly in hydrophobic media.[14] TNS absorbs and emits at longer wavelengths than the naphthyl methyl ether chromophore. Thus, excitation and detection present no spectral interference. Addition of aqueous solutions of β-cyclodextrin, β-CD/**3**, or covalently bound **2** to 10^{-5} M solutions of TNS at 25 °C results in increased TNS fluorescence when excitation is carried out at 360 nm and detection at 485 nm. As shown in Figure 2a, addition of up to $\sim 6 \times 10^{-3}$ M β-cyclodextrin (line I) or inclusion complex (β-CD/**3** , line II) to 10^{-5} M TNS results in a ~35- or 30-fold increase in fluorescence intensity, respectively. The lower intensity values with β-CD/**3** as compared to those for pure β-cyclodextrin are indicative of competitive binding between **3** and TNS. Surprisingly, addition of 6×10^{-3} M **2** results in a ~165-fold fluorescence increase (line III) that shows an efficient hydrophobic interaction between TNS and **2** under conditions where its hydrophobic cavity is postulated not to be fully available (i.e., **2**-inside). Nonetheless, when the temperature was increased from 25 to 85 °C to promote a faster inside−outside equilibrium and higher populations of **2**-outside, a further 2-fold increase in intensity was observed (point 1 to point 2). In contrast, control experiments with TNS and β-cyclodextrin or β-CD/**3** at up to 85 °C showed a ~5-fold *decrease* in intensity. Further differences between **2** and the two model samples were observed when temperatures were lowered to 25−30 °C. While changes in samples of TNS with β-cyclodextrin or β-CD/**3** were fully reversible, intensity changes in **2** were not. As indicated in Figure 2b, an additional ~5-fold fluorescence increase was observed when the temperature was lowered to 30 °C. After this, variations within those temperature limits reproduced the intensities between points 2 and 3.

Scheme 2



It is known that TNS forms 1:1 and 1:2 complexes with β-cyclodextrin.[14c,15] In analogy with known 1:2 complexes, one may suggest that **2** probably associates with one TNS molecule. However, the TNS intensity data are too complex, and we did not attempted to analyze their stoichiometry. We interpret the data in Figure 2 in terms of a model requiring the postulated temperature-dependent inside−outside isomerism (Scheme 2). It is clear that points 1 and 3 in Figure 2b reflect different types of complexes between **2** and TNS, and, as suggested by the model, the most hydrophobic environment becomes available only after thermal activation. Comparison of lines I and III suggests that naphthyl-containing **2**-inside offers a more hydrophobic environment to TNS than does pure β-CD (Figure 2a). This enhancement may result from a less polar environment, a higher binding constant, or a combination of the two. Increasing the temperature to 85 °C allows for larger populations of the outside−isomer, and the 2-fold intensity increase is assigned to the inclusion of TNS in the hydrophobic cavity of **2** (i.e., TNS@**2**, Scheme 2). It is important to note that similar experiments with inclusion compound β-CD/**3** result in decreased intensities, since both complex dissociation and excited state deactivation are accelerated. The final 5-fold intensity change between points 2 and 3 reflects an equilibrium with binding states that were not kinetically available when the two components are mixed at ambient temperatures and may include several species like those in Scheme 2.

While the precise nature of the structures in Scheme 2 remains speculative at this point, such structures must involve different hydrophobic environments for TNS. Thermal activation may allow for inclusion of TNS with displacement of the naphthyl group, or it may allow for co-inclusion of the two hydrophobic groups. Despite overlap between the naphthyl groups of **2** and TNS, preliminary ^{1}H NMR measurements give evidence of two distinct binding interactions before and after heating. Experiments in progress with other hydrophobic probes also support the model of Scheme 2, which we believe is an ideal model for the study of activated binding phenomena and for the development of temperature sensors.

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Supporting Information Available: ^{1}H NMR spectra (400 MHz) of **3** and β-CD/**3** as a function of temperature and summary of fluorescence properties of **2**, **3**, and β-CD/3 (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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