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Drastically Decreased Reactivity of Thiols and Disulfides Complexed by Cucurbit[6]uril

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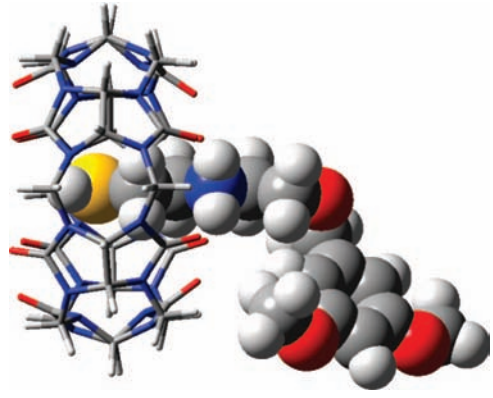
ABSTRACT



The cucurbit[6]uril (CB6) host forms stable complexes with 2-aminoethanethiol (cysteamine) and a derivative that contains a bulky terminal group attached to the amine group, as well as with the related disulfide cystamine. In these complexes, the thiol or the disulfide group is encapsulated inside the host cavity. The CB6-complexed thiols show drastically decreased reactivity with several oxidants, while the CB6-bound disulfide also exhibits hindered reactivity with reducing agents, such as dithiothreitol.

The thiol−disulfide interconversion equilibrium has considerable biological importance.[1] Disulfide bridges, formed by cysteine's thiols, constitute an important structural element for the stabilization of a number of protein folded states.[2] Thiol−disulfide interchange reactions play a key role in maintaining an appropriate redox balance in living tissues, and current thinking links these reactions to levels of oxidative stress,[3] effective platelet function,[4] and even cancer chemoprevention.[5] It is thus important to understand any factors that may affect the reactivity of biologically relevant thiols and disulfides.

The growing interest in the host family of the cucurbit[*n*]urils (CBn, see structures in Figure 1) is well documented.[6−8] While most of this rapidly expanding body of work focuses on the larger cavity hosts CB7, CB8, CB10, and related compounds, the much better known CB6 also has very interesting properties.

In this work, we focus our attention on the binding interactions between the CB6 host and the amino acid L-cysteine, the main source of thiol groups in proteins, the related thiol cysteamine (2-aminoethanethiol), and its corresponding disulfide, cystamine. We also investigate a

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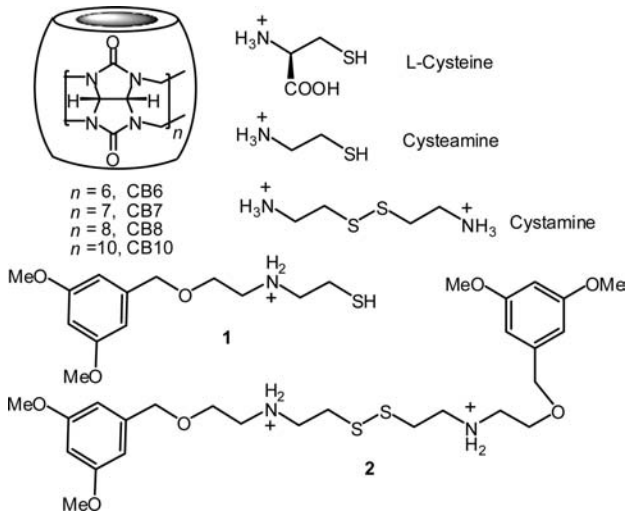

Figure 1. Structures of cucurbit[*n*]uril hosts and thiol and disulfide guests used in this work.

derivative of cysteamine (compound **1**), which has a bulky group attached to the amine group. The structures of all these compounds are shown in Figure 1.

CB6 was prepared as previously reported.[9] The synthesis of compound **1** is described in the Supporting Information. Disulfide **2** was prepared by oxidation of **1** (vide infra). All other guests are commercially available. We carried out the majority of the experiments in 0.2 M NaCl/D_2O solution adjusted to pD 2 to enhance the solubility of CB6 and to ensure protonation of the amine functional groups on the guests.

The ^{1}H NMR spectrum of cysteamine exhibits two triplets at 2.83 and 3.20 ppm, which correspond to the methylene protons adjacent to the thiol and amine functional groups, respectively. In the presence of CB6 both peaks broaden and shift to higher fields, clearly indicating the formation of an inclusion complex (see the Supporting Information). The CB6-induced shift is more pronounced for the protons adjacent to the thiol group, suggesting that they are inserted more deeply in the host cavity. The chemical exchange between free and CB6-bound cysteamine is fast on the NMR time scale and we only observed a set of proton signals, shifting gradually to higher field as the host concentration increases. The chemical shift of the methylene adjacent to the amino group was measured as a function of added CB6 concentration. Optimization of the fitting of these data to a 1:1 binding isotherm yields an equilibrium association constant (K) of 5.6($\pm$2.0) $\times$ 10^3 M^{-1} at 25 °C (see the Supporting Information). In stark contrast to cysteamine, L-cysteine is not bound by CB6, as evidenced by the complete lack of CB6-induced changes, under identical experimental conditions, on the ^{1}H NMR spectrum of the amino acid.

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Thiol **1** is a cysteamine derivative that was designed to force the binding approach of CB6 to the guest from the thiol end. As in the case of cysteamine, the two peaks corresponding to the methylene protons between the thiol and amine groups (labeled a and b in Figure 2), shift to higher

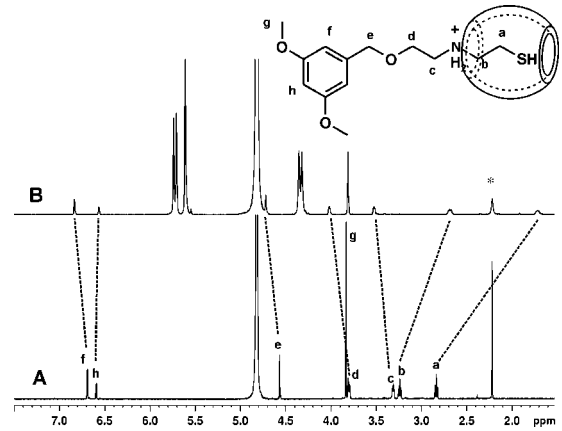

Figure 2. ^{1}H NMR spectrum (500 MHz, D_2O/0.20 M NaCl, pD 2.0) of thiol **1** (a) in the absence and (b) in the presence of 2.0 equiv of CB6.

field as the concentration of CB6 increases. The signal for protons c, on the other side of the amine group, experiences CB6-induced shifts to lower fields, as do the d, e, and f protons. Again, the CB6-induced chemical shifts of the c protons were used to determine the corresponding K value, which was found to be 7.3($\pm$1.0) $\times$ 10^4 M^{-1} (see the Supporting Information). This inclusion complex is sufficiently stable to be observed in MALDI-TOF mass spectrometric experiments (m/z 1,268). We were initially surprised by the CB6-induced shifts to lower field on protons e and f, which appear to be far removed from the cavity portal in the complex. However, semiempirical PM3 computations with the CB6·**1** complex yielded the minimized stucture shown in the graphical sbstract, in which the guest adopts a bent conformation that brings these protons relatively close to the host portal and its rim of carbonyls, a finding that is consistent with the observed NMR complexation-induced shifts.

Oxidation of thiol **1** with trichloronitromethane in acetonitrile solution yields disulfide **2**, which was easily isolated after recrystallization from ethanol. A comparison of the ^{1}H NMR spectra of both compounds reveals that protons a and b, which resonate at 2.83 and 3.24 ppm, respectively, in thiol **1**, appear at lower field (3.00 and 3.42 ppm) in **2** (see the Supporting Information). Addition of excess CB6 to a D_2O solution containing **2** does not cause any significant shifts in the proton resonances of the disulfide, suggesting the absence of binding interactions between these two compounds. This was anticipated, given the size of the 3,5-dimethoxybenzene terminal groups in **2**, which prevent access by CB6 to the central binding site in the disulfide.

On the other hand cystamine, lacking any bulky terminal groups, forms a stable inclusion complex with CB6. The two

methylene triplets of cystamine resonate at 3.02 and 3.40 ppm (Figure 3). Upon addition of 0.4 equiv of CB6 a new

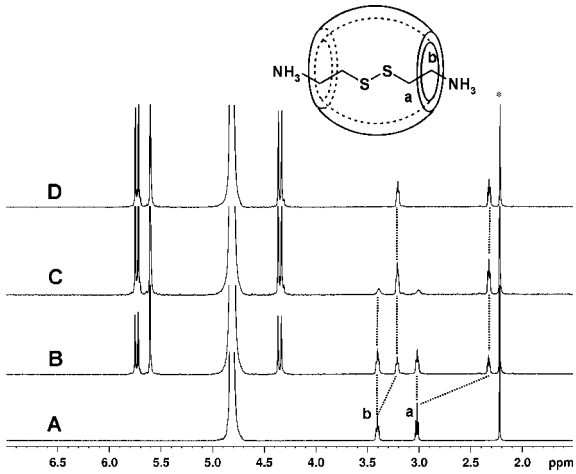

Figure 3. ^{1}H NMR spectra (500 MHz, D_2O/0.20 M NaCl, pD 2.0) of 1.0 mM cystamine (a) in the absence and in the presence of (b) 0.4, (c) 0.8, and (d) 1.0 equiv of CB6. The asterisk labels the residual acetone peak.

set of peaks at 2.32 and 3.21 ppm were observed. The chemical exchange between free and bound cystamine is slow in the NMR time scale, which allows the simultaneous observation of both sets of peaks. As more CB6 is added, the methylene peaks corresponding to the bound guest grow at the expense of free guest peaks. The complexation is quantitative at the millimolar concentration levels used in these experiments. When 1.0 equiv of CB6 or more is present, only the peaks corresponding to bound cystamine were observed. We performed dilution experiments with a solution containing 1.0 mM CB6 and 1.0 mM cystamine, in which only methylene resonances corresponding to the inclusion complex were observed. However, even after 10-fold dilution, no resonances corresponding to the free guest were observed. If we assume that a maximum of 10% of the complex may undergo dissociation and remain undetected by ^{1}H NMR spectroscopy under these conditions, we can estimate that the minimum K value must be 10^6 M^{-1}.

A computational investigation of the cystamine−CB6 complex, using DFT methods (B3LYP/3-21G), leads to the energy-minimized structure shown in Figure 4, in which the disulfide bond, as expected, is located in the center of the host cavity, while the ammonium groups at the ends of the guest interact, via ion-dipole forces, with the rims of carbonyl oxygens on the cavity portals.

The formation of such a stable inclusion complex with the disulfide bond included and seemingly protected close to the middle of the host cavity led us to question the reactivity of the S−S linkage toward reductive cleavage. The reagent of choice for R−S−S−R cleavage is dithiothreitol (DTT).[10,11] Indeed treatment of cystamine with a slight excess of DTT (1.25 equiv) leads to the production of cysteamine and the cyclic disulfide that results from DTT

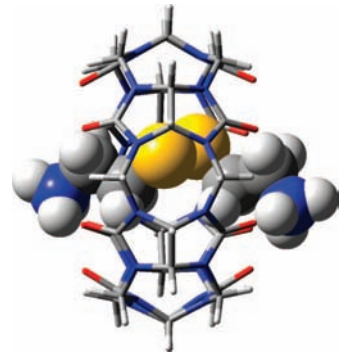

Figure 4. Energy-minimized (B3LYP/3-21G method) structure of the cystamine−CB6 inclusion complex.

oxidation. In aqueous solution at neutral pH this reaction was completed in ca. 1 h at 25 °C. Figure 5A shows the

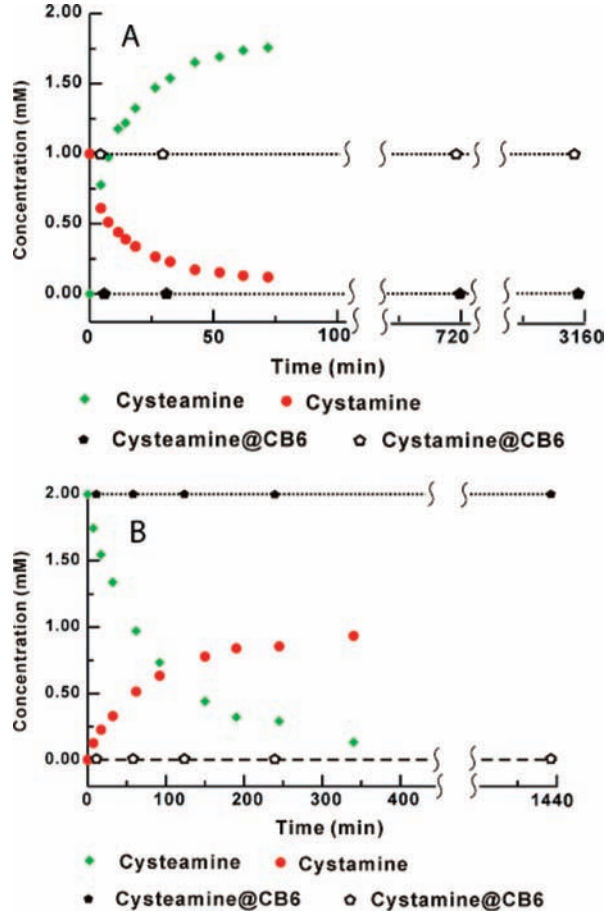

Figure 5. Time evolution of the cystamine and cysteamine concentrations. (A) Reaction of 1.0 mM cystamine with 1.25 mM DTT in D_2O in the absence and in the presence of 1.05 equiv of CB6. (B) Reaction of 2.0 mM cysteamine with 4.0 mM $FeCl_3$ in D_2O in the absence and in the presence of 1.25 equiv of CB6.

time evolution of the cystamine and cysteamine concentrations recorded in this reaction. However, if the same reaction

is carried out in the presence of 1.0 equiv of CB6, no cysteamine (free or CB6-bound) formation was observed after 50 h (Figure 5A and the Supporting Information). Clearly the inclusion complexation of cystamine inside the cavity of CB6 results in a pronounced stabilization effect and decreased reactivity against reductive cleavage by DTT. The kinetic stability of the CB6•cystamine complex suggests that its reactivity versus thiol scrambling reactions should also be strongly diminished. Cystamine readily reacts with the methyl ester of L-cysteine to produce a mixture of thiols and disulfides (see the Supporting Information for possible product structures). However, the presence of 1 equiv of CB6, under otherwise identical experimental conditions, fully prevents this reaction from taking place in a period of 24 h. Therefore, the reactivity of the CB6 complex in thiol scrambling reactions is also greatly decreased.

After collecting these data on the reactivity of the CB6•cystamine complex, we decided to investigate the effect of CB6 complexation on the reactivity toward oxidation of included thiol guests. For instance, cysteamine can be readily oxidized to cystamine with $FeCl_3$ in aqueous solution. As an example, the time evolution of the cysteamine and cystamine concentrations in a solution initially containing 2.0 mM cysteamine and 4 mM $FeCl_3$ is shown in Figure 5B. The reaction is essentially complete in ca. 7 h. In contrast to this, the presence of CB6 leads again to effective thiol protection. No oxidation was detected for a period of ca. 24 h.

We also ran additional experiments to investigate cysteamine oxidation using other oxidizing agents, such as dissolved oxygen and Cl_3CNO_2. In an aqueous solution (pH 7) equilibrated with the laboratory atmosphere we monitored cysteamine oxidation using ^{1}H NMR spectroscopy and found that it takes ca. 3 h for the cysteamine peaks to disappear completely (as the cystamine peaks reach full development). However, in the presence of a slight stoichiometric excess of CB6 (1.25 equiv), only a trace of the disulfide was observed after a period of 24 h. Similar results were obtained for the oxidation of thiol **1**. For instance, oxidation of **1** by Cl_3CNO_2 is essentially complete in ca. 40 min, while, under the same experimental conditions, no product (disulfide **2**) was detected after a period of 40 min in the presence of CB6.

In the field of supramolecular kinetics the primary goal is the development of supramolecular catalysts.[12−14] However, the stabilization of reactive compounds is also of interest,[15] and considerable work with cyclodextrin hosts has targeted the stabilization and/or protection of included guests.[16] We must also note that unique molecular behavior is emerging from recent work on molecular encapsulation.[17,18] The CB6-induced, kinetic stabilization effects reported here are remarkable not only because of their magnitude, but also because the same host affects very strongly the kinetics of the forward and reverse reactions (the reductive cleavage of cystamine and the oxidation of cysteamine, see Figure 5). These kinetic effects are primarily attributed to reactant stabilization by formation of an inclusion complex with the CB6 host. We are currently investigating in more detail the mechanistic effect of CB6 on the kinetics and thermodynamics of these reactions.

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Supporting Information Available: Synthetic details for compounds **1** and **2** and additional NMR spectroscopic data as mentioned in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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