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Mechanical Tuning of Molecular Recognition To Discriminate the Single-Methyl-Group Difference between Thymine and Uracil

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Abstract: Construction of enzyme-like artificial cavities is a complex and challenging subject. Rather than synthesizing complicated host molecules, we have proposed mechanical adaptation of relatively simple hosts within dynamic media to determine the optimum conformation for molecular recognition. Here we have applied this concept to one of the most challenging biomolecular recognition problems, i.e., that of discriminating thymine from uracil. We synthesized the novel cholesterol-armed triazacyclononane as a host molecule and subjected it to structural tuning by compression of its Langmuir monolayers in the absence and in the presence of Li^+ cations in the subphase. Experimental results confirm that the monolayer of triazacyclononane host selectively recognizes uracil over adenine (ca. 7 times based on the binding constant) and thymine (ca. 64 times) under optimized conditions ([LiCl] = 10 mM at surface pressure of 35 mN m^{-1}). The concept of mechanical tuning of a host structure for optimization of molecular recognition offers a novel methodology in host−guest chemistry as an alternative to the more traditional molecular design strategies.

Molecular recognition is one of the most important chemical events not only in supramolecular chemistry[1] but also in biochemical systems.[2] For example, recognition of nucleobases in DNA and RNA is especially crucial in genetic transmission and protein expression. In those cases, nucleobase pairing is due to complementary hydrogen bonding that is often regarded as attractive in the design of artificial recognition systems in solution[3] and at interfaces.[4] One shortcoming of this system is that the adenine base of nucleic acids cannot discriminate thymine from uracil, both of which possess identical hydrogen-bonding sites and only a single-methyl-group difference in structure. This critical molecular discrimination can be achieved only by the enzyme uracil DNA glycosylase,[5] which can differentiate existing thymine from uracil created accidentally by deamination of cytosine within DNA sequences. However, despite the great importance of discriminating thymine from uracil, it is extremely difficult to design and synthesize enzyme-like artificial hosts for this purpose. To avoid this problem, mechanical adaptation of quite simple host molecules within dynamic media[6] might be used to determine the optimum point for molecular recognition and has the additional advantage of being relatively simple to practically implement, even in cases of subtle molecular discrimination. Recently, we have realized this concept in the chiral recognition of amino acids by applying mechanical forces to host monolayers at the air−water interface.[7] Here, we have applied this concept to one of the most challenging recognition events in biomolecules: discrimination between thymine and uracil. In our system under optimized condition, uracil can be recognized by a factor of ca. 64 times greater than thymine.

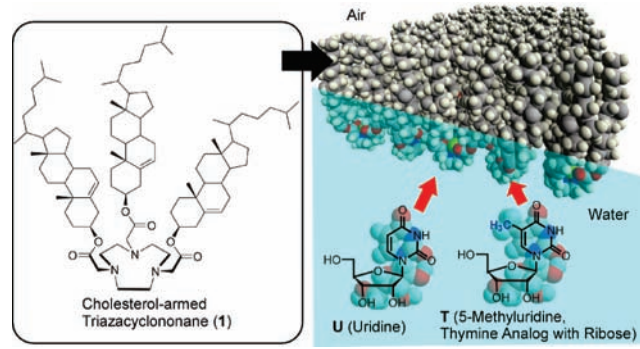

Figure 1. Structures and schematic drawing of host **1** and guests **U** and **T**.

Our strategy is summarized in Figure 1. We synthesized the novel cholesterol-armed triazacyclononane (**1**)[8] as a host molecule and prepared it as a Langmuir monolayer at the air−water interface. The triazacyclononane derivative **1** is composed of hydrophobic cholesterol moieties and a rather hydrophilic triazacyclononane. Similar cyclen-related structures have been used as recognition sites for biomolecules including nucleobases and amino acids.[9] Because cholesteryl moieties are known to form densely packed assemblies, the triazacyclononane recognition site can be continuously deformed during monolayer compression processes. During continuous changes of the host structure in a monolayer, binding of the aqueous guests, ribonucleoside uridine (**U**) and a ribonucleoside analogue of thymine (5-methyluridine, **T**), was evaluated using surface pressure measurements. It should be noted that the only structural difference between these guests is a single methyl group, since the same ribose sugar moiety is present in both cases.

An isotherm between surface pressure and molecular area (π−A isotherm) of the cholesterol-armed triazacyclononane (**1**) on pure water indicates a well-condensed phase with collapse pressure of 45 mN m^{-1} and a limiting area of 1.12 nm^2 (Figure 2A), which is almost equal to that required for three well-packed cholesteryl moieties. Addition of 10 mM LiCl into the subphase forming the Li^+ complex at the triazacyclononane core[8] caused a reduction in collapse pressure to 40 mN m^{-1}, while the limiting area remained unchanged. The reduction of collapse pressure may be caused by

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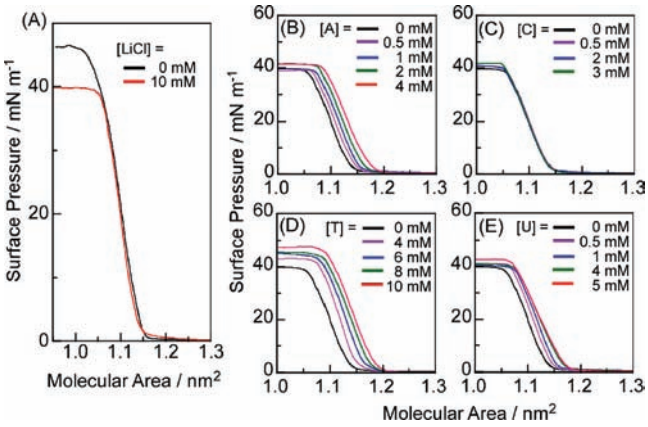

Figure 2. (A) $\pi-A$ isotherms of **1** without guests at 20 °C. (B−E) $\pi-A$ isotherms of **1** with guests at 20 °C with 10 mM of LiCl: (B) adenosine, **A**; (C) cytidine, **C**; (D) 5-methyluridine (thymine analogue), **T**; (E) uridine, **U**.

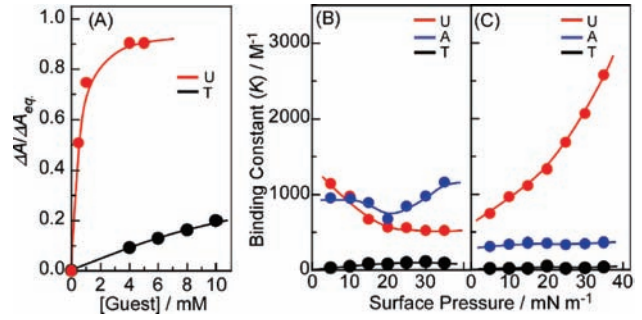

Figure 3. (A) Typical binding curve of **U** and **T** to **1** (35 mN m^{-1}, 20 °C, and [LiCl] = 10 mM). (B,C) Binding constants of **U**, **A**, and **T** to **1** at various surface pressures at 20 °C: (B) without LiCl and (C) with 10 mM LiCl.

increased structural inflexibility upon Li^+ binding. Binding constants of **1** with $LiClO_4$ and $NaClO_4$ in $CDCl_3-CD_3CN$ (2:1, v/v) at 25 °C were determined to be 6.8 (log K_{Li}) and 3.9 (log K_{Na}) by ^1H NMR measurements. Proton signals for the side-arm methylene protons appear as an AB quartet with the Li^+ complex ($\Delta\delta = 0.09$ ppm) but as a singlet with the Na^+ complex, which suggests that **1** forms a more static complex with Li^+ than with Na^+. Therefore, we selected Li^+ for modifying the mechanical properties of **1** at the air−water interface. Addition of nucleosides (adenosine **A**, **T**, and **U**) in the absence[8] and in the presence (Figure 2B, D, and E, respectively) of 10 mM LiCl caused significant shifts of molecular area of **1**,while faint shifts were detected for cytidine **C** (Figure 2C).

Plots of increments in molecular areas as a function of guest concentrations in the subphase fit well to equimolar binding curves,as exemplified in Figure 3A,where shifts of molecular area (ΔA) per that at binding maximum (ΔA_{eq}) were plotted as a function of guest concentration in the subphase. Calculated binding constants (K) clearly depend on the type of nucleosides in the absence (Figure 3B)[7] and in the presence (Figure 3C) of LiCl (10 mM). In both cases, interestingly, the binding constant for **U** is consistently much larger than that of **T**, even though they have very similar structures. In the absence of Li^+, binding constants for **U** and **A** are quite similar. Conversely, in the presence of subphase LiCl, differences in the binding constants of **U** and **A** become much more significant due to the large pressure dependence of the binding constants. These experimental results confirm that the monolayer of **1** selectively recognizes **U** over **A** (by a factor of ca. 7 times based on the binding

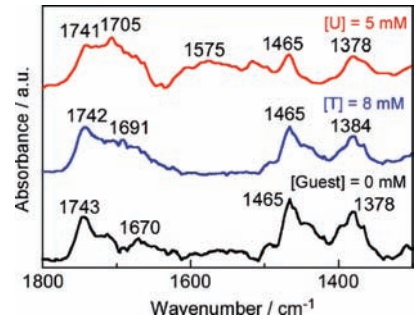

Figure 4. FTIR spectra in reflection−absorption mode of LB films of **1** (10 layers, 35 mN m^{-1}, 20 °C, and [LiCl] = 10 mM) transferred from surfaces of the subphases containing no guests, 8 mM **T**, and 5 mM **U**.

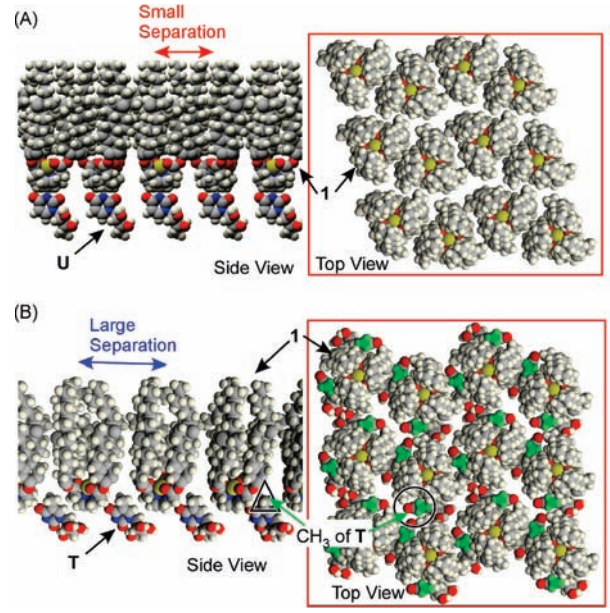

Figure 5. Plausible binding models of (A) **U** and (B) **T** to the monolayer of **1**.

constant) and **T** (by a factor of ca. 64 times) under the optimized conditions ([LiCl] = 10 mM and at surface pressure of 35 mN m^{-1}). FTIR in reflection−absorption mode also confirms the stronger interaction between **1** and **U** (Figure 4). The appearance of shoulder bands at 1670−1705 cm^{-1} in spectra of the LB films from the **U**-containing subphase corresponds to C═O stretching and N−H deformation bands of the bound **U** molecules. Moreover, distinct peaks appearing between 1500 and 1600 cm^{-1} are assignable to C═O and C−N stretching bands of the anionic form of uridine.[10] The latter suggests that electrostatic attraction between complexed Li^+ and the deprotonated imide nitrogen of **U** promotes binding, in addition to possible hydrogen bonding between nucleobases and triazacyclononane C═O groups, and this may be the major driving force for their binding under non-Li^+ conditions. In contrast, these drastic changes cannot be observed in the IR spectrum of the film transferred from the **T**-containing subphase.

In addition to these serendipitous findings, the mechanism of nucleobase discrimination can be considered from a hypothetical viewpoint (Figure 5). The air−water interfacial medium is known to be an excellent medium for discriminating hydrophilic and hydrophobic faces of molecules, as has been demonstrated in saccharide recognition by resorcinol derivatives.[11] Hydrophobic methyl groups may be oriented in the air phase and inserted between molecules of **1** (Figure 5B), causing two energetically disadvanta-

geous situations: (i) absence of strong host−guest interaction and (ii) disruption of molecular packing of **1**, leading to small binding constants. The large shift in $\pi-A$ isotherms with **T** in the subphase (Figure 2D), where a real expansion of 0.25 nm^2 at binding equilibrium can be calculated, corresponds reasonably with the cross section of the inserted methyl (CH_3) group and is also supported by molecular models. In contrast, interaction of **U** with complexed Li^+ (Figure 5A) does not significantly disrupt packing of **1** in the model. Applying pressure to the monolayer may destabilize the Li^+ complex due to mechanical deformation. The destabilized Li^+ complex then tends to promote binding of guest **U** to increase further enthalpic gain, resulting in significant increases in the binding constant to **U**.

This work strikingly demonstrates a means of molecular recognition and differentiation between structurally almost identical molecules by mechanical tuning of a simple host at an interfacial medium. Substantial discrimination between thymine and uracil bases, which have only a single-methyl-group difference, was accomplished under optimized conditions. The concept of mechanical tuning of host structure for optimization of molecular recognition offers a novel methodology in host−guest chemistry as an alternative to traditional strategies based on increasingly complex and inconvenient molecular design.

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Supporting Information Available: Experimental details, synthesis of **1**, complex formation between **1** and Li^+, $\pi-A$ isotherms without LiCl in subphase and binding constants. This material is available free of charge via the Internet at http://pubs.acs.org.

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