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The crystal structure, self-assembly, DNA-binding and cleavage studies of the [2]pseudorotaxane composed of cucurbit[6]uril

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Abstract—The [2]pseudorotaxanes of cucurbit[6]uril with guest molecule 1,6-bis(imidazol-1-yl)hexane (BIMH) were synthesized and characterized by ESI-MS spectrometry, ^{1}H NMR spectra, and X-ray diffraction crystallography. The influence of different anions on self-assembly in solid-state was discussed by X-ray diffraction crystallography. However, more interestingly, and to our amazement, we discovered the CB[6]/BIMH [2]pseudorotaxane exhibiting efficient cleavage of pBR322 DNA in physiological environment. The cleavage mechanism were studied by fluorescence spectra and the hydrolysis of bis(2,4-dinitrophenyl)-phosphate (BDNPP). From DNA-binding mode being electrostatic force and the first-order kinetics equation, we prove indirectly that the mechanism may be hydrolytic cleavage.

Cucurbit[*n*]urils ($n = 4$–12) family, abbreviated as CB[*n*] or Q[*n*], have attracted more and more attention in recent years.[1] As an incontrovertible delegate of the family, cucurbit[6]uril[2] has been well known and widely studied in various aspects including pseudorotaxane,[3] rotaxane[3a,c,4], and polyrotaxane,[5] molecular necklaces,[6] molecular switch,[7] gene transfection,[8] etc. In these works published, the guest molecules including mostly alkylammonium ions,[2d,e,3b,c] pyridylmethyl-ended alkylammonium ions[5f,g,6], and other organic ammonium ions[1c] were reported. No guest molecule containing imidazole group is reported. It is well-known that imidazole is the important biological activity molecule and functional group. And the imidazole residue of histidine as the active sites of enzymes and proteins is well recognized.[9] Considering these, we have synthesized the new guest molecule, 1,6-bis(imidazole-1-yl)hexane (BIMH), and have reported the crystal structure and self-assembly of [2]pseudorotaxane composed of CB[6] and 1,6-bis(imidazole-1-yl)hexane dihydrobromide (BIMH–Br) as a short communication.[10] In this study, to investigate the influence of the anions on the self-assembly, which is important as well as metal-coordinated to direct self-assembly,[11] we changed the different anions and got the two crystal structures with chloride (Cl^-), and trifluoroacetate (CF_3COO^-), respectively. Although phosphate diester is far more kinetically stable than other common biological functional groups such as amides or esters,[12] the backbones of nucleic acids are elegantly hydrolyzed by nucleases to facilitate their synthesis, manipulation, and repair. Due to potentials in human medicine and molecular biology, the development of effective chemical nucleases has attracted much attention.[13] Hydrolytic cleavage of DNA by small molecule is far more challenging. Most examples are metal ions or their complexes, involving transition metal ions[14] and their complexes,[15] lanthanide ions and their complexes,[16] as well as actinides. However, in the above systems as chemical nucleases sometimes exists limitation in special condition.[17] So people suggested a novel strategy to design and use non-metallic DNA artificial nuclease. Most recently, Kim's and Nakamura's groups have published the outcome that the CB[6] could affect the activity of spermine on DNA.[18] Therefore, we wonder whether the complex CB[6]/BIMH can exhibit the similar property of hydrolyzing pBR322 DNA. The experiment outcome is positive, the experiments show that the complex CB[6]/BIMH can cleave circular plasmid pBR322 DNA at pH 7.2 and 37 °C. Furthermore, the cleavage mechanism was deduced by available methods including fluorescence spectra and the hydrolysis of bis(2,4-dinitrophenyl)-phosphate (BDNPP).

Keywords: Cucurbituril; Self-assembly; DNA-cleavage; Pseudorotaxane.
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We have reported a similar crystal structure (CB[6]/BIMH–Br) and its self-assembly,[10] where the formation of superstructure was named as a pseudopolyrotaxane.[19] To explore the influence of the different anions on the self-assembly, we gained another two crystals by changing different anions (Cl^-: CCDC 601457 and CF_3COO^-: CCDC 601456).[20] The structures and self-assembly of them are shown in Figures 1 and 2, respectively. Figure 1 gives the representative crystal structure. The hexane chain moieties are located fully inside the cavity and the protonated imidazole rings resided outside the portal.

As shown in Figure 2, the self-assembly of [2]pseudorotaxane with Cl^- anion is consistent with the one we reported.[10] The highly ordered linear pseudopolyrotaxane formation was aggregated by intermolecular $\pi \cdots \pi$ (face-to-face) stacking between neighboring imidazole rings with a centroid–centroid 3.961 Å (0.0°), interpseudorotaxane C–H $\cdots$ O and N^+–H $\cdots$ O hydrogen bond interactions (Table S1). The separation between neighboring CB[6] molecules is 12.578 Å (*c* axis)

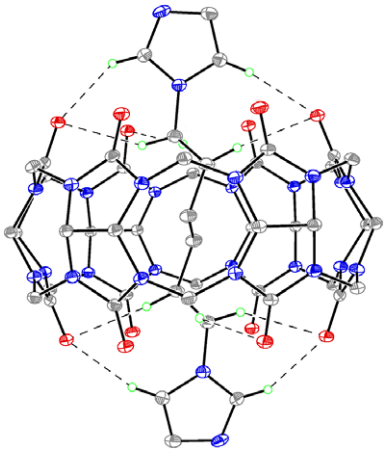

Figure 1. The represented crystal structure of CB[6]/BIMH [2]pseudorotaxanes. C atoms, gray; N atoms, light blue; O atoms, red. Side view of the structure, all H atoms, except for H atoms involved in hydrogen bonding. The minor disorder, anions, and solvent (H_2O) are omitted for clarity. Only atoms involving hydrogen bonds are labeled.

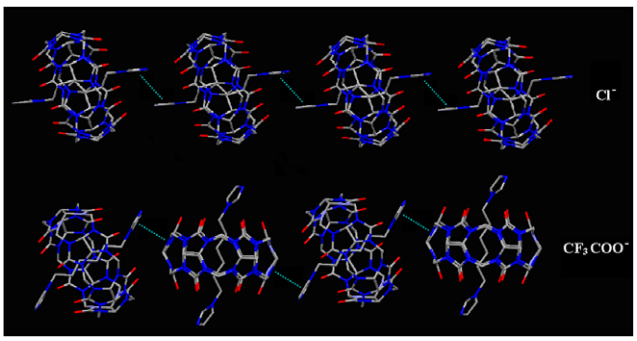

Figure 2. The self-assembly of [2]pseudorotaxane was effected by different anions. Top (Cl^-): the linear superstructure formation, may been named pseudopolyrotaxane; bottom (CF_3COO^-): zig-zag chain formation.

along $\pi \cdots \pi$ orient. However, the self-assembly of CB[6]/BIMH–CF_3COO^- complex exhibited the zig-zag chain formation due to CF_3COO^- anions' influence. The superstructure formation is mainly oriented via the other $\pi \cdots \pi$ between the guest imidazole rings from one [2]pseudorotaxane and the semi-glycoluril-ring of the host CB[6] from the other [2]pseudorotaxane with a centroid–centroid 3.454 Å (0.7°). The packings of the two crystals are shown in Figures 1S and 2S (ESI). In addition, it is made out that the packing of [2]pseudorotaxane with halide anions was more close than the one of [2]pseudorotaxane with CF_3COO^-, which was reflected in lattice density (1.747 for Br^-[10] and 1.659 for Cl^- versus 1.629 for CF_3COO^-).

The ^1H NMR spectrum (D_2O, 25 °C, TMSP as an interior criterion) characterized the structure of the 1:1 complex by only one set of signals (ESI, Fig. 3S). The signals of the methylene protons located inside CB[6] shifted upfield to (δ 3.99, 1.18, 0.57) from their original resonances (δ 4.23, 1.89, 1.33, respectively). The outside protons (for hydrogen atoms on imidazole group H1, H2, H3), the changes in chemical shifts are different. For H1 and H2, the resonances were downfield (to δ 9.16, 7.92) from their original positions (δ 8.71, 7.50) due to the deshielding effect of the portal carbonyl groups. The signal of H3, however, showed a slightly upshift field from original 7.46 to 7.37. The smaller upshift ($\Delta\delta = -0.09$ ppm) change, though the shielding region was reported by Mock being about 6 Å or 4.5 methylene groups,[2d] should be also a result of shielding region of CB[6] due to less volume imidazole group relative to the portal of CB[6].[3f]

An attempt of studying the superstructure assembly formation (pseudopolyrotaxane) in solution of the [2]pseudorotaxane was not successful by electrospray ionization mass (ESI-MS), which is a quite soft ionization progress that can reflect the state of molecules in solution.[21] Though Osaka reported that the technique only detected the doubly charged CB[6] or CB[7] complex,[22] our result (Fig. 4S) shows that not only doubly charged ions (*m*/*z* 608.7, CB[6] + BIMH + $2H^+$) but also singly charged ions (*m*/*z* 1215.4, CB[6] + BIMH + H^+; about a half intensity) were detected. These indicate that the new CB[6]/BIMH [2]pseudorotaxane can exist stably in gas-phase as a doubly charged complex and singly charged complex.

The cleavage reaction on plasmid DNA was monitored by agarose gel electrophoresis. When circular plasmid DNA is subject to electrophoresis, relatively fast migration will be observed for the intact supercoil form (*S*, CCC form). If scission occurs on one strand (nicking), the supercoil will relax to generate a slower-moving open circular form (*C*, OC form). If both strands are cleaved, a linear form (*L*) that migrates between Form *C* and Form *S* will be generated.[23] Figure 3 (left) shows the results of cleaving superhelical pBR322 DNA in the presence of varying concentrations of the CB[6]/BIMH complex. The results indicate that the *S* form of pBR322 DNA diminishes gradually, whereas the *C* form increases with the increase of concentration of the

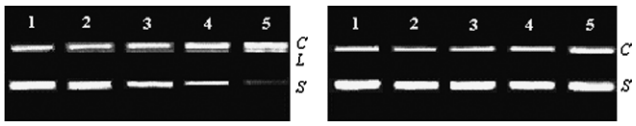

Figure 3. Results of electrophoresis of pBR322 DNA (Reagents and conditions: 10 mM Tris–HCl buffer, 6.2 mM NaCl, pH 7.18, at 37 °C for 4 h); Left: the presence of varying concentrations of CB[6]/BIMH: lanes l–5, [CB[6]/BIMH] = 0, 10, 25, 50, and 150 μM, respectively. *S* is supercoiled DNA (CCC form), *C* is nicked circular form DNA (OC form) and *L* is linear DNA, The amount distribute for each lane: lane 1 (impact pBR322: *S* = 64%, *C* = 36%, *L* = 0%); lane 2 (*S* = 58%, *C* = 40%, *L* = 2%); lane 3 (*S* = 51%, *C* = 43%, *L* = 6%); lane 4 (*S* = 32%, *C* = 55%, *L* = 13%); lane 5 (*S* = 7%, *C* = 69%, *L* = 24%); Right: only BIMH–Cl, lanes l–5, [BIMH] = 0, 10, 25, 50, and 150 μM, respectively, scarce hydrolysis.

CB[6]/BIMH complex and the linear form (*L*) is also produced. When the concentration of CB[6] goes up to 150 μM (lane 5), the pBR322 DNA gave a mixture of 24% linear form (*L*), 69% open circular form (*C*), and 7% supercoil form (*S*). However, single BIMH cleaves scarcely the DNA (Fig. 3right) in the same physiological conditions. Here, it is needed to be pointed that we only studied the CB[6]/BIMH complex having the function of incising pBR322 DNA in this text. About pH effect, ion intensity and so on, are our future work.

We know EB (ethidium bromide) was the most frequent probe of often used as probes for DNA structure detection.[24] EB emits intense fluorescent light in the presence of DNA due to its strong intercalation between the adjacent DNA base pairs. In fact, if fluorescence quenching or not for EB bound to DNA is used to determine the binding mode between another molecule and DNA. In order to investigate the mode of the CB[6]/BIMH complex binding to DNA, the fluorescence spectra experiment (ESI, Fig. 5S) of EB–DNA (1:1, 8×10^{-5} M) system was carried out in the absence and the presence of complex. The results suggest that the emission band at 600 nm of the EB–DNA system was increased in intensity with increasing the CB[6]/BIMH complex concentration until up to 1.25×10^{-4} mol/L ($Rt = C_{CB[6]/BIMH}/C_{DNA} = 1.55$). The CB[6]/BIMH complex itself does not show appreciable fluorescence in the spectral region studied. The observed fluorescence increase indicates that EB intercalated, the only fluorescent species, was bound to DNA more tightly, which indicates there exist electrostatic interactions between CB[6]/BIMH complex and DNA. This may be due to the fact that CB[6]/BIMH with positive charge, like metal cation ions,[25] can bind to the phosphate group of DNA by electrostatic forces to make a contraction in the helix axis of DNA.

From above DNA-binding mode being electrostatic force, we deduce that complex incising pBR322 DNA may be hydrolysis mechanism. To give an evidence, the model substrate BDNPP [bis(2,4-dinitrophenyl)-phosphate] was employed because its hydrolysis can reflect the incising mechanism of pBR322 DNA.[26] CB[6]/BIMH complex-promoted hydrolysis of BDNPP was monitored by following the visible absorbance change at 400 nm (assigned to those of 2,4-dinitrophenolate and -nitrophenolate, respectively; Fig. 6S) at 37(±0.5) °C on a diode-array spectrophotometer with a thermostated cell holder. All the reactions were carried out under second-order conditions. The rate constants were obtained by fitting the initial (<3%) concentration changes according to the first-order kinetics equation (correlation coefficient >0.99). Figure 6S is the kinetics linear spectra of reaction of complex and BDNPP. The pH of the solution was maintained by 10 mM Tris–HCl buffer (pH: 7.18). In experiment, $[BDNPP]_{int} = [complex]_{int} = 7.5 \times 10^{-5}$ mol L^{-1}, measured 11994 $(mol/L)^{-1}$ cm^{-1} of 2,4-dinitrophenolate at 37 °C. The observed second-order rate constant is $k_{obs} = 11.6$ $(mol/L)^{-1}$ min^{-1}, $t_{1/2} = 1139$ min.

According to these experiment results above, we suppose simply that the DNA-cleavage mechanism for the [2]pseudorotaxane CB[6]/BIMH should be a cooperation process of the host CB[6] and the guest BIMH molecules. Based on the previous reports,[27] the fact that the glycoluril carbonyl O atoms acted as the hydrogen acceptor[28] and the crystal structure information (Fig. 7S), the proposed hydrolysis mechanism is depicted in Figure 4. The protonated imidazole affords the proton to the phosphate anion of DNA, and synchronously the CB[6] glycoluril carbonyl O atom, acting as Lewis base on, pulls a proton from water molecule to promote its attack the phosphorus atom. The mechanism can be applicable for the CB[6]/spermidine hydrolyzing DNA molecule.[18] However, the real hydrolysis mechanism needs to be confirmed through the further detailed research.

In summary, the new [2]pseudorotaxanes of cucurbit[6]uril with new guest molecule 1,6-bis(imidazol-1-yl)hexane were synthesized and fully characterized by ESI-MS spectrometry, ^1H NMR spectra, and X-ray diffraction crystallography. The influence of different anions on supramolecular self-assembly in solid-state was discussed by X-ray diffraction crystallography.

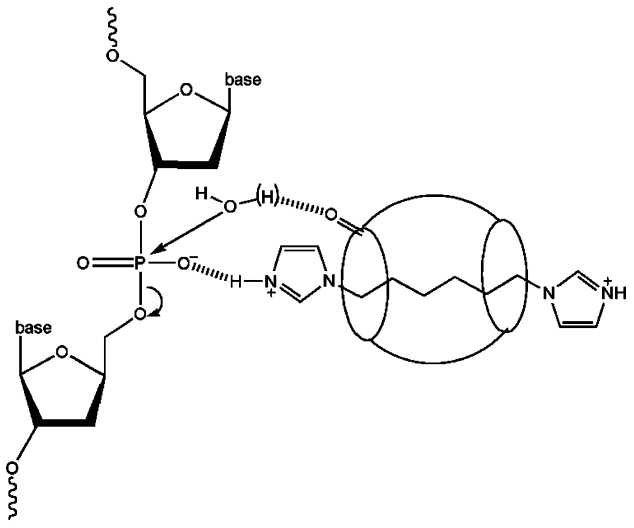

Figure 4. The proposed DNA-cleavage mechanism for CB[6]/BIMH [2]pseudorotaxane.

Significantly, the CB[6]/BIMH [2]pseudorotaxane exhibit efficient cleavage of pBR322 DNA in physiological environment. The cleavage mechanism was studied by fluorescence spectra and the hydrolysis experiment of bis(2,4-dinitrophenyl)-phosphate (BDNPP). These results can introduce the new developments for new supermolecular material and supramolecular biochemistry on cucurbituril analogues including CB[7],[1a] CB[8],[1a] CB[10],[29]and other derivatives[30] even inverted CBs.[31] The further wide investigation is in press.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.11.054.

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20. The guest molecule 1,6-bis(imidazol-1-yl)hexane (BIMH with Cl^-): Yield 85%. ^1H NMR (300 MHz, 25 °C, D_2O, TMSP): δ 8.71 (s, 2H), 7.50 (s, 1H), 7.46 (s, 1H), 4.23 (t, 4H, α-CH_2), 1.89 (m, 4H, β-CH_2), 1.33 (m, 4H, γ-CH_2); ^{13}C NMR (300 MHz, D_2O): δ 134.13, 121.56, 119.48, 49.05, 28.94, 24.75; Elemental analysis (calcd %): C, 49.48; N, 19.24; H, 6.87. Found: C, 49.86; N, 19.44; H, 6.53. The procedure for preparation of the [2]pseudorotaxane CB[6]/BIMH is same as the one we reported.[10] For CB[6]/BIMH–Cl: ^1H NMR (300 MHz, 25 °C, D_2O, TMSP): δ 9.16 (s, 2H), 7.92(s, 2H), 7.37 (s, 2H), 5.78 (d, 12H), 5.58 (s, 12H), 4.32 (d, 12H), 3.99 (t, 4H),

1.18(m, 4H), 0.57 (m, 4H); ^{13}C NMR (300 MHz, D_2O): δ 155.8, 135.2, 123.3, 116.8, 71.1, 51.2, 48.6, 28.6, 27.3; ESI-MS: 608.7 for doubly charged cations, 1215.4 for single-charged cations. Elemental analysis (calcd %) for $C_{36}H_{36}N_{24}O_{12}\cdot C_{12}H_{18}N_4\cdot 2HCl\cdot 8H_2O$: C, 40.22; N, 27.37; H, 5.03. Found: C, 39.99; N, 27.12; H, 4.97. Crystallographic data for CB[6]/BIMH–Cl: $C_{48}H_{72}Cl_2N_{28}O_{20}$, Mr = 1432.24, monoclinic, space group $P2_1$/c(No.14), a = 12.369(4) Å, b = 20.115(6) Å, c = 12.578(4) Å, β = 113.664(3)°, V = 2866.3(15) Å^3, Z = 2, ρ_{calcd} = 1.659 g/cm^3, $\mu(Mo_{k\alpha})$ = 0.220 mm^{-1}, $F(000)$ = 1500, T = 203 K, θ_{max} = 25.01°, 13538 reflections measured, 4969 unique (R_{int} = 0.0223). Final residual for 452 parameters and 4969 reflections with $I > 2\sigma(I)$: R_1 = 0.0671, wR_2 = 0.1922, and GOF = 1.057. CCDC 601457 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223/336 033; E-mail: data_request@ccdc.cam.ac.uk. Crystallagrophic data for CB[6]/BIMH–CF_3COO: $C_{52}H_{80}F_6N_{28}O_{28}$, Mr = 1659.44, monoclinic, space group $P2_1$/c(No.14), a = 14.456(2) Å, b = 19.016(3) Å, c = 13.285(2) Å, β = 112.138(2)°, V = 3382.7(10) Å^3, Z = 2, ρ_{calcd} = 1.629 g/cm^3, $\mu(Mo_{k\alpha})$ = 0.143 mm^{-1}, $F(000)$ = 1732, T = 203 K, θ_{max} = 25.01°, 16109 reflections measured, 5913 unique (R_{int} = 0.0325). Final residual for 523 parameters and 5913 reflections with $I > 2\sigma(I)$: R_1 = 0.0990, wR_2 = 0.2524, and GOF = 1.084. CCDC 601456 contains the supplementary crystallographic data for this paper.

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