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Synthesis of a Novel Cyclic Donor–Acceptor Conjugate for Selective Recognition of ATP

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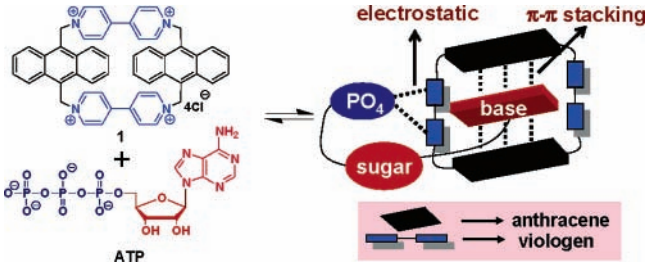
ABSTRACT



Novel cyclophane 1 was synthesized, and its interactions with phosphate, adenosine, AMP, ADP, and ATP have been investigated. With addition of ATP, significant decrease in absorbance of 1 was observed, whereas other guest molecules showed negligible effect. The complex between 1 and ATP was confirmed through cyclic voltammetry and ^{1}H NMR. The uniqueness of the system is that it complexes selectively with ATP in a cavity and involves synergistic effects of both electrostatic and π–π stacking interactions.

Detection of nucleosides and nucleotides has paramount importance as they form the fundamental units of all the life forms.[1,2] Most known molecular receptors for the nucleosides and nucleotides use complementary hydrogen bonding for their recognition.[3] Such molecular recognition in aqueous medium would be limited as a result of the competitive hydrogen bonding of the solvent.[4,5]

Moreover, the sugar moiety of the nucleosides and nucleotides can interfere in such recognition, and hence masking of the hydroxyl groups prior to the recognition event is essential.[6] Progress in this area would require new strategies for the complexation under physiological pH conditions and subsequent signaling of the host−guest complex formation.

Of all nucleotides, the recognition of adenosine 5′-triphosphate (ATP)[7] is vital since the binding of ATP by proteins is one of the most prominent molecular recognition events in the nature. Moreover, ATP plays an important role in energy transduction in organisms and controls several metabolic processes[8] including synthesis of cyclic adenosine monophosphate.[9] Sensors reported so far for the detection

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of ATP often contain functionality similar to those provided by nucleic acid binding sites on proteins and involve multiple hydrogen bonding, which prevent their use in the aqueous medium.[7] Herein, we report a novel molecular recognition system (cyclic derivative **1**, Figure 1)[10] for the selective

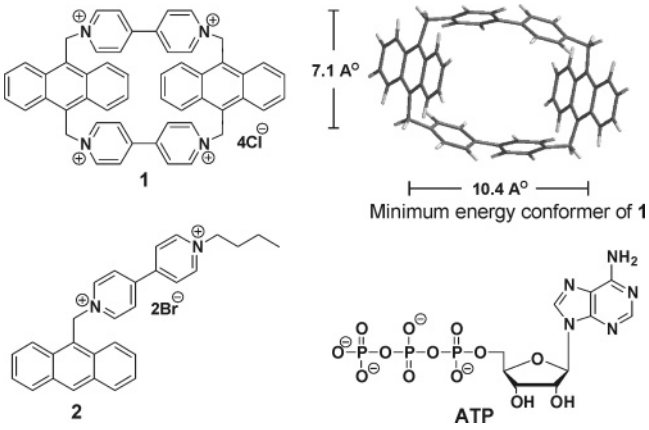

Figure 1. Structures of the cyclic system **1**, its minimum energy conformer, the model compound **2**, and ATP.

detection of ATP in buffer. This system is devoid of hydrogen bonding but recognizes the guest molecule through synergistic effects of cavity size, $\pi-\pi$ stacking, and electrostatic interactions.

The synthesis of the cyclic system **1** and the model compound **2** was achieved in moderate yields (synthesis details, Supporting Information).[11,12] An appreciable amount of rigidity was imparted to the molecule **1** by adjusting the number of the methylene spacer groups to one, which closely resembles the cyclobis(paraquat-*p*-phenylene) system reported by Stoddart and co-workers for molecular based machines.[13] The viologen moiety in **1** can be considered as a dual action molecular tool, where it can be a recognition motif for anionic guests and a signaling unit through electrochemical changes. In addition, the cyclic system **1** can stabilize the inclusion complexes through $\pi-\pi$ stacking interactions by making use of the cavity effect arising from the distinct arrangement of the anthracene and viologen moieties. In the event of binding to a suitable guest, the anthracene moiety has the capability of assuming the role of a signaling unit through changes in its photophysical properties.

In aqueous and buffer media, both **1** and **2** showed characteristic absorption maximum at 378 and 372 nm, respectively, corresponding to the anthracene moiety (Figure S1, Supporting Information) and exhibited very low fluorescence quantum yields ($\Phi_F = 0.0007$ and 0.001) when compared to 9-(hydroxymethyl)anthracene. The efficient quenching of fluorescence in **1** and **2** could be attributed to the photoinduced electron transfer process from the excited anthracene chromophore to the viologen moiety, which is thermodynamically favorable with a change in free energy of $\Delta G_{ET} = -0.77$ eV (calculation details, Supporting Information).

Figure 2 shows the change in absorption spectrum of the cyclic derivative **1** in the presence of different concentrations

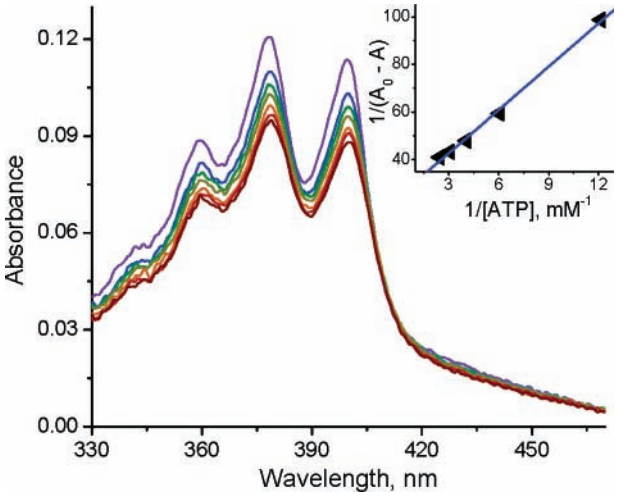

Figure 2. Effect of ATP concentration on absorption spectra of **1** (11 μM) in 10 mM phosphate buffer. Inset shows the Benesi–Hildebrand analysis.

of ATP. With increasing concentrations of ATP, **1** showed a decrease in the absorption corresponding to the anthracene moiety. In contrast, the absorption spectrum of **1** exhibited negligible changes in the presence of guest molecules such as phosphate anion, adenosine, adenosine 5′-monophosphate (AMP), and adenosine 5′- diphosphate (ADP) (Figures S2–S5, Supporting Information). The fluorescence spectrum of the cyclic system **1**, on the other hand, exhibited negligible changes with the increase in addition of ATP.

Figure 3 shows the relative changes in the absorbance of **1** with various ligands. It is evident from Figure 3 that the cyclic system **1** shows maximum selectivity for ATP, whereas all other ligands have negligible influence on the absorption properties. In contrast, the addition of ATP and

(10) The minimum energy conformer of **1** was obtained by AM1 calculations using PC TITAN software from Wave function Inc.; 18401 von Karman, Suite 370, Irvine, CA 92612.

(11) Compound **1** (26%): mp >300 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.09 (8H, s), 7.81–9.01 (32H, m); ^{13}C NMR (75 MHz, D_2O) δ 72.9, 122.2, 124.6, 125.3, 127.7, 129.3, 131.3, 142.7; HRMS (FAB) *m*/*z* calcd for $C_{52}H_{40}N_4Cl_4$ (M − 2Cl) 791.8064, found 791.8072 $[M - 2Cl]^+$.

(12) Compound **2** (79%): mp 289–290 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.88 (3H, t, $J = 7.4$ Hz), 1.28–1.29 (2H, m), 1.89–1.91 (2H, m), 4.67 (2H, t, $J = 7.4$ Hz), 7.08 (2H, s), 7.61–9.33 (17H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.3, 18.7, 32.7, 56.1, 60.5, 121.6, 123.3, 125.8, 126.7, 126.9, 128.4, 129.6, 131.1, 131.4, 131.5, 144.8, 145.7, 148.6, 149.1; HRMS (FAB) *m*/*z* calcd for $C_{29}H_{28}N_2Br_2$ (M − Br) 484.4501, found 484.4495 $[M - Br]^+$.

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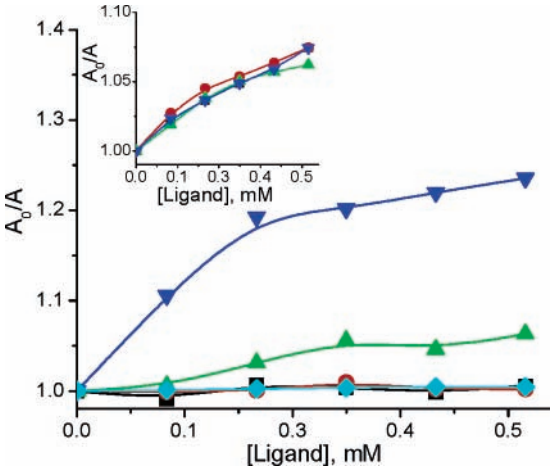

Figure 3. Change in the absorbance of **1** and **2** (inset) with addition of Na_3PO_4 (◆); adenosine (■); AMP (●); ADP (▲); and ATP (▼).

other ligands to the model system **2** exhibited negligible changes in the absorption properties (inset of Figure 3 and Figure S6, Supporting Information). Benesi−Hildebrand analysis[14] of the absorption changes (inset of Figure 2) showed a 1:1 stoichiometry for the complex formed between **1** and ATP with a binding constant of $K_{assoc} = 4040 \pm 140$ M^{-1} in buffer, whereas relatively a higher value of $K_{assoc} = 4900 \pm 200$ M^{-1} was observed in water.

Figure 4 shows the differential pulse voltammograms (DPV) of **1** (ca. 0.2 mM) in the aqueous medium, which

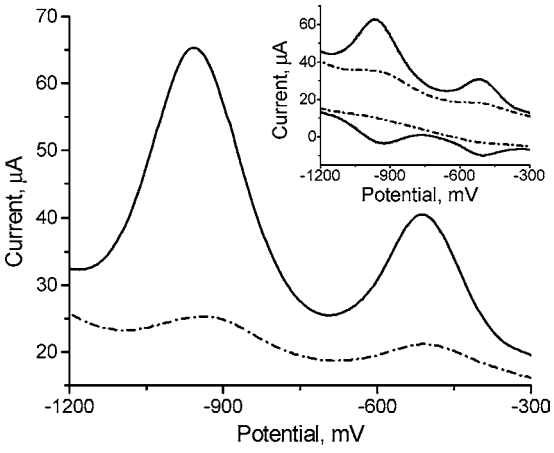

Figure 4. Differential pulse and square wave voltammograms (inset) of **1** (0.2 mM) in absence (—) and presence (- · -) of ATP (1.3 μM) in aqueous medium.

exhibited two reversible one-electron reduction processes centered at −0.50 and −0.96 V, characteristic of the viologen moiety.[15] When ATP (1.3 μM) was added, we observed a shift of reduction potentials by 16 and 8 mV, along with a significant decrease in current intensity of 40.04 μA (61%) and 19.33 μA (48%), confirming thereby the formation a stable complex between **1** and ATP.[16] Similarly, the successive addition of ATP to a solution of the cyclic system **1** in D_2O resulted in broadening of protons of the methylene group in the ^1H NMR spectrum, whereas the protons corresponding to the viologen moiety experienced an upfield shift of δ 0.03 ppm at 0.35 mM of ATP (Figure 5). The

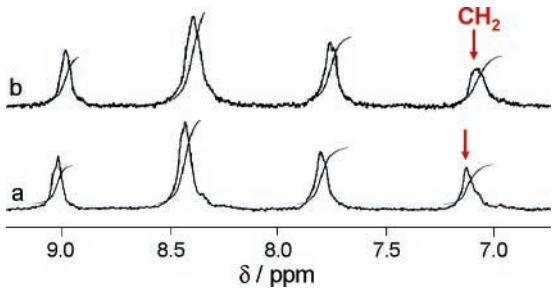

Figure 5. ^1H NMR spectra of **1** in D_2O in the absence (a) and presence (b) of ATP (0.35 mM).

binding constant ($K_{assoc} = 4700 \pm 200$ M^{-1}) determined by NMR titrations (Figure S7, Supporting Information) is in good agreement with the value obtained in water from the absorption technique.

To understand the nature and strength of the complex formed between **1** and ATP, we investigated the effects of ionic strength (Figure S8, Supporting Information) and temperature. As the salt concentration increases, the decrease in absorbance of **1** with the gradual addition of ATP becomes less and less prominent (Figure 6). The values of K_{assoc} at different ionic strengths were determined and are found to be 3558, 222, and 137 M^{-1} at 2, 50, and 500 mM of NaCl, respectively. The decrease in K_{assoc} values with increase in ionic strength indicates that the viologen unit of **1** is shielded from the phosphate ions by Na^+ ions at higher ionic strength,[17] which in turn blocks the interaction between **1** and ATP. When the temperature of the complex **1**·ATP was raised from 293 to 343 K, we observed an increase in the intensity of absorbance of **1** at 378 nm (inset of Figure 6), indicating the dissociation of the complex. However, the complex showed nearly 19% hypochromicity even at 343 K, indicating thereby the stability of the complex even at this temperature.

On the basis of our experimental evidence, the selective binding of ATP to the host **1** in the cavity is a result of $\pi-\pi$ stacking in combination with ionic interactions. The attraction

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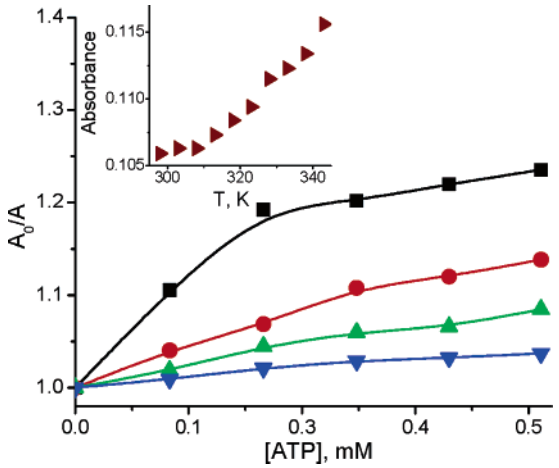

Figure 6. Relative changes in the absorbance of **1** with successive additions of ATP at different salt concentrations: 0 (■); 2 (●); 50 (▲); 500 mM (▼) of NaCl in 10 mM phosphate buffer (pH 7.4). Inset shows the effect of temperature on the absorbance of complex **1**·ATP from 298 to 343 K.

between the phosphate groups of ATP and the viologen moiety of **1** through multiple electrostatic interactions followed by $\pi-\pi$ stacking of the aromatic part of ATP inside the cavity result in the formation of a tight complex. The presence of cavity in the molecular system **1** and three phosphate groups in ATP are very essential for the selective recognition and for the formation of a stable 1:1 complex, as evidenced from the negative results obtained with the model system **2** and with various guest molecules (Figure 3). The binding interaction between **1** and ATP can be clearly understood if it is considered that the negatively charged phosphate groups of ATP interact with the N^+ centers of the viologen moiety. Once the electrostatic interaction between the phosphate groups and viologen moiety is accomplished, the aromatic part of ATP undergoes $\pi-\pi$ stacking interactions[18] inside the cavity, thereby resulting in the formation of stable complex between **1** and ATP. Evidence for this comes from the fact that **1** exhibits non-negligible interactions with ADP, containing two phosphate groups, whereas no binding interactions were obseved with AMP, adenosine, and phosphate anion.

This mode of complexation was further confirmed by making use of the effect of Debye−Huckel ionic strength function[19] of the medium on the K_{assoc} values and the thermodynamic parameters $\Delta H°$ and $\Delta S°$ (−11.15 kJ mol^{-1} and −37.41 J K^{-1} mol^{-1}), obtained using Van't Hoff's plot[20] (Figures S9 and S10, Supporting Information). Thermodynamical parameters obtained are consistent with the expected nonclassical hydrophobic interactions usually observed in the case of the cyclophane systems.[21] As a consequence of the complex formation between the cyclic system **1** and ATP, (i) current intensity decreases as observed in the differential pulse and square wave voltammograms, (ii) shielding of protons of the viologen moiety occurs due to the interactions with the phosphate groups, (iii) broadening of signals corresponding to the methylene protons is observed because of π-stacking of the aromatic part of ATP, and (iv) decrease in entropy ($\Delta S°$) is observed due to the formation of an ordered complex through nonclassical hydrophobic interactions.

In conclusion, we have developed a novel molecular recognition system that can discriminate ATP from other nucleosides, nucleotides, and phosphate anion under physiological pH conditions. The uniqueness of this system is that it complexes with ATP through synergistic effects of electrostatic and $\pi-\pi$ stacking interactions in a cavity and signals the event through the changes in UV−vis, NMR, and cyclic voltammetric techniques. Further studies are in progress to understand the effects of spacer and the nature of donor and acceptor moieties in the recognition of various nucleosides and nucleotides.

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Supporting Information Available: Details of synthesis and calculations and Figures S1−S10 showing NMR data, changes in absorbance of **1** and **2** in the presence of nucleotides, nucleosides, and phosphate anion under various experimental conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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