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Heptakis(6-deoxy-6-guanidino)-β-cyclodextrin: an artificial model for mitochondrial ADP/ATP carrier

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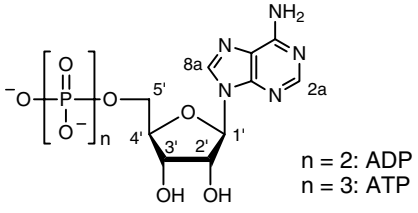
Abstract—Heptakis(6-deoxy-6-guanidino)-β-cyclodextrin, prepared by one-step reaction of heptakis(6-amino-6-deoxy)-β-cyclodextrin with 1*H*-pyrozolecarboxamidine, binds ADP/ATP very tightly.

Recently, much attention has been focused on the design and construction of artificial receptors for biologically important small molecules and anions,[1] especially for the anionic adenosine di- and triphosphates (ADP and ATP) because they are the key players in the bioenergetics of cells. Such studies on the recognition of ADP/ATP by synthetic receptors have been performed mainly in lipophilic solvents because of the strong solvation of the interacting groups in polar media. The artificial receptors include polyammoniums,[2] peptides,[3] dendrimers,[4] binuclear metallic complexes,[5] and so forth, and some systems have been developed for sensing the nucleotide phosphates in aqueous solution.[6] Several synthetic receptors with a hydrophobic cavity have also been constructed, such as polyammoniocyclodextrins and cyclophanes.[7–9] However, it still remains a challenge to build more efficient and selective artificial receptors for these very important biological anions.

ATP fuels most biochemical transformations in the cytoplasm by its hydrolysis into ADP which is reconverted to ATP in the mitochondrial matrix. The transportation of ADP and ATP between the mitochondrial matrix and the cytoplasm is accomplished by the mitochondrial ADP/ATP carrier. Recently, it has been elucidated that this protein has a funnel shaped cavity, with a maximal diameter of 20 Å and a depth of 30 Å. The channel part is 20 Å long with a diameter of 8 Å, and it is closed from the matrix side. A cationic cluster consisting of five arginine residues is located on the surface near the bottom of the cavity (Fig. 1).[10]



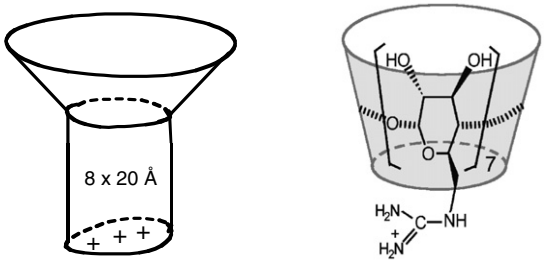


Figure 1. A simplified representation of the cavity of the mitochondrial ADP/ATP carrier (left) and its artificial model (right).

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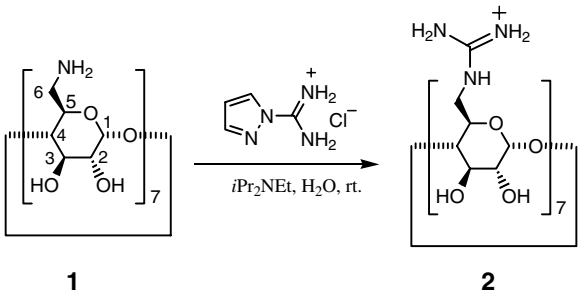
With the structural information of the natural receptor in hand, we decided to construct an artificial model of the mitochondrial ADP/ATP carrier based on β-cyclodextrin (β-CD) because it has a cavity of similar width (ca. 7 Å in diameter) to that of the natural carrier and can be easily modified. As depicted in Figure 1, the first model (**2**) for the mitochondrial ADP/ATP carrier comprises of a β-CD with seven guanidinium groups mounted to its primary side. Its synthesis includes the conversion of all the primary hydroxyl groups to amino groups (**1**) and subsequent treatment with 1*H*-pyrozolecarboxamidine (Scheme 1). The reaction of heptaamine **1** and 1*H*-pyrozolecarboxamidine proceeded smoothly in an aqueous solution in the presence of diisopropyl ethylamine and afforded the artificial receptor **2** in 92% yield.[11]

The FAB-MS spectrum of **2** showed a molecular peak at *m*/*z* 1422 which corresponds to (M+1) of the neutral species. Both the ^{1}H and ^{13}C NMR spectra are very simple, clearly demonstrating the C_7 symmetry of **2**. The C1–C6 were found at δ 102.7, 72.6, 73.3, 83.4, 71.7, and 43.1 ppm, respectively, while the guanidinium carbon resonated at δ 158.7 ppm. The seven magnetically different protons of **2** in D_2O were unambiguously assigned and the results are summarized in Table 1. Kano indicated that the energetically minimized conformation of the heptacation of **1** in water derived from the MM-MD calculations took an upturned bucket-type structure because of the electrostatic repulsion between the NH_3^+ groups. The NMR spectra of **2** did not suggest obvious changes of the shape of the saccharide skeleton.[12] This is probably because the delocalized planar guanidium moieties can separate far enough from each other without significantly altering the cavity shape.

The complexation of **2** with ADP and ATP was investigated by NMR spectroscopy. As shown in Figure 2, when ATP was mixed with **2** in a 1:1 molar ratio in D_2O, the chemical shift patterns of both ATP and **2** (Fig. 2b) are obviously different from those of the individual free species (Fig. 2a and d), indicating that complexation between ATP and **2** occurred. Addition of excess ATP does not cause appreciable shift of the existing signals but results in the appearance of a new set of signals which is basically the same as that of the free ATP (Fig. 2c). This observation implies that a 1:1 complex is formed and the exchange between the free and bound ATP molecules is very slow in the NMR timescale. Because the complexation equilibrium reaches very fast and no time-dependence of the complexation of **2** and ATP was confirmed by careful NMR measurement, the slow exchange between the bound and free ATP implies that the complex of ATP and **2** is very stable. Obvious dissociation of the complex was not recognized by NMR method even at a very low concentration of 0.4 mM. Conversely, complexation of polyvalent cationic species of heptaamine **1** with ATP under the same experimental conditions only shifted the signals but did not resolve the free and bound ATP molecules (Fig. 2e) although it was reported that similar heptakis(6-amino)-β-CDs bind ATP very strongly.[7,8] The very slow exchange between the complexes and the free species in the present case prevents the derivation of the binding constants by NMR titration or dilution experiments.



1 **2**

Scheme 1. Synthesis of the mitochondrial model.

The 1:1 complexation was further confirmed by MS spectroscopy. By mixing **2** and an excess of ATP in water and allowing the solution to stand for a few hours, precipitates were obtained. TOF MS spectrum of the precipitates exhibited the molecular ion peak at *m*/*z* 1931 for complex ATP·**2** together with a peak at *m*/*z* 1422 for **2**.

Similar results were also obtained with ADP. The ^{1}H NMR spectra of ATP·**2** and ADP·**2** complexes together with their individual components were completely assigned with the aid of 2D NMR experiments, and the results are listed in Table 1. Examination of the chemical shift differences of all the proton nuclei caused by complexation reveals that the H3 and H5 of the CD which are located inside the cavity are shifted to higher fields by 0.24 and 0.15 ppm in both complexes. An obvious upfield shift was also observed for one of the geminal H6 protons. All other protons of the CD moieties did not exhibit meaningful shifts. Obvious upfield shifts were observed for almost all the protons of the nucleotides except the H2′ of both guests and H4′ of ADP which are remarkably shifted to lower fields. The ROESY spectra of the complex demonstrated cross peaks for the pairs of H5–H3′, H5–H4′, H3–H1′, H3–H2a, and H3–H8a between **2** and ADP.

The above observations enable us to figure out the structure of the complexes (Fig. 3). The guest molecules bind to receptor **2** by locating their ionic phosphate part close to the guanidinium residues on the primary side of **2** while allowing their adenine moiety to penetrate the CD cavity. The much larger downfield shift of the H3 located near the wider opening of the CD than that of the H5 of **2** suggests that adenine moiety is not completely but partially accommodated in the cavity near the portal. Compared with the ATP complex of heptakis(6-deoxy-6-methylamino)-β-CD reported by Schneider,[7] ATP·**2** exhibited more significant shift for the H3 ($\Delta\delta$ −0.24 vs −0.16 ppm), indicating a deeper and tighter binding of the adenine moiety in the cavity.

The adenosine phosphates are known to adopt several different conformations: the adenine base can be orientated either *anti* or *syn* to the ribose moiety which puckers between the C3′-*endo* and C3′-*exo* conformations.[13]

Table 1. Chemical shifts (δ, ppm) of **2**, ADP, ATP, ADP·**2**, and ATP·**2**, and the chemical shift differences ($\Delta\delta = \delta_{complex} - \delta_{free}$, ppm) induced by complexation[a]

	2	ATP·**2**		ADP·**2**	
	δ_{free}	$\delta_{complex}$	$\Delta\delta$	$\delta_{complex}$	$\Delta\delta$
Host					
H1	5.04 (3.8)	5.01 (3.4)	−0.03	5.02 (3.2)	−0.02
H2	3.61 (9.5)	3.63 (9.8)	0.02	3.64 (9.8)	0.03
H3	3.90 (9.5)	3.66 (9.8)	−0.24	3.66 (9.8)	−0.24
H4	3.46 (9.5)	3.38 (9.8)	−0.08	3.38 (9.8)	−0.08
H5	4.01	3.85 (9.8)	−0.16	3.87 (9.8)	−0.14
H6	3.46	3.35	−0.11	3.38	−0.08
	3.58	3.56	−0.02	3.56	−0.02

	ATP			ADP		
	δ_{free}	$\delta_{complex}$	$\Delta\delta$	δ_{free}	$\delta_{complex}$	$\Delta\delta$
Guest						
Ribose						
H1′	6.03	5.99	−0.04	6.06	5.98	−0.08
	(5.2)	(4.0)		(6.0)	(4.6)	
H2′	4.61	4.95	0.34	4.70	5.05	0.35
	(4.5)	(5.5)		(5.1)		
H3′	4.47	4.41	−0.06	4.48	4.39	−0.09
H4′	4.34	4.29	−0.05	4.30	4.45	0.15
H5′	4.26	4.00	−0.26	4.10	4.03	−0.07
	4.26	4.21	−0.05	4.10	4.03	−0.07
Adenine						
H2a	8.33	8.18	−0.15	8.18	8.08	−0.10
H8a	8.51	8.41	−0.10	8.44	8.36	−0.08

[a] The data in parentheses denote the $^3J_{Hx,Hx+1}$ values in Hz.

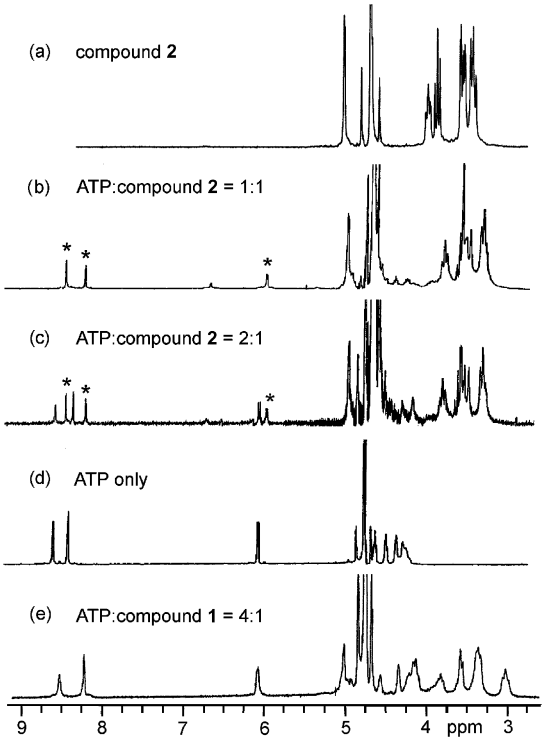


Figure 2. ^{1}H NMR spectra of the D_2O solutions of 20 mM **2** in the absence (a) and presence of 20 mM (b) and 40 mM ATP (c), the D_2O solution of 20 mM ATP (d), and the mixture of ATP and compound **1** in a molar ratio of 4:1 in D_2O. The pD was kept at 5 for all the measurements. The signals with a ∗ mark relate the ATP·**2** complex.

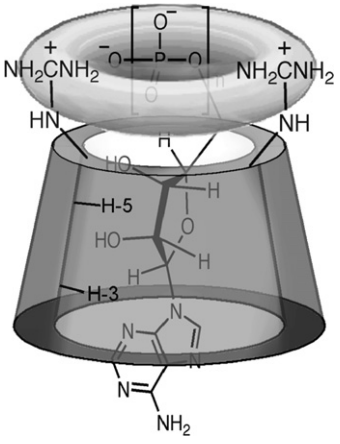


Figure 3. Schematic presentation of the ATP·**2** complex.

The *syn* and *anti* orientations are deduced to present in equal proportions around neutral pH. The remarkable downfield shift of the H2′ of both ATP and ADP should result from the orientational change of the adenine base so that the ring current effect on the H2′ becomes weakened.[14] In addition, the obvious changes in the coupling constants of $J_{H1',H2'}$ and $J_{H2',H3'}$ are indicative of conformational variations of the ribose upon binding.

^{31}P NMR spectra revealed interesting evolution of magnetic resonances (Fig. 4). ATP shows three broad peaks at δ −9.9 (P-γ), −10.4 (P-α), and −21.4 (P-β) ppm,

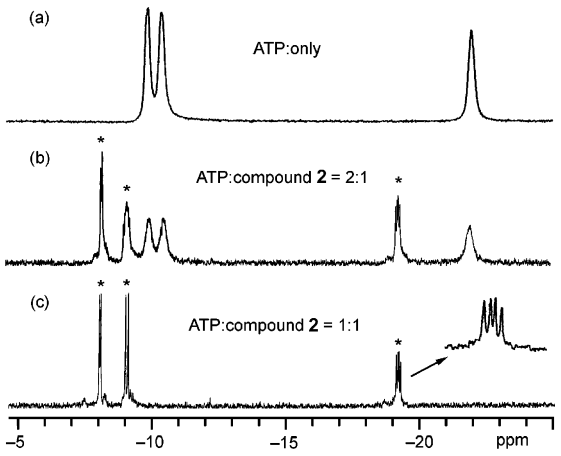


Figure 4. ^{31}P NMR spectra of 10 mM ATP in the absence (a) and presence of 5 mM (b) and 10 mM of **2** (c) in D_2O. The signals with a * mark relate the ATP·**2** complex.

respectively. The solution of **2** and ATP in a 1:1 molar ratio demonstrated another set of signals at δ −8.1 (d, $^3J_{\beta,\gamma} = 11.9$ Hz, P-γ), −9.1 (d, $^3J_{\alpha,\beta} = 20.7$ Hz, P-α), −19.2 (dd, $^3J = 20.7$, 11.9 Hz, P-β), respectively. When ATP exists in excess, the spectrum clearly displays well-resolved two sets of signals which correspond to the 1:1 complex and free ATP, respectively. The broadened peaks of free ATP are consistent with its large conformational variability. Peak broadening frequently occurs in host–guest interactions,[15] and this is also the case for the binding of ATP with divalent metal ion and polyamines. The broad peaks of ATP become even broader when binding divalent metal ions or polyamines.[16] However, all three peaks of the ATP·**2** complex are not only very sharp but also clearly present their coupling patterns. This observation together with the ^{1}H NMR results (changes in coupling constants, Table 1) suggests that the heptakis(6-guanidino)-β-CD **2** not only invokes obvious changes in the puckering structure of the ribose and the orientation of the adenine base but also tightly fixes the whole triphosphate region of ATP, leading to an extraordinarily tight binding.

Isothermal titration calorimetry revealed that the binding is endothermic or slightly exothermic. However, the profiles of the thermograms were very complicated, which might be caused by the formation of some weak complexes along with the main, most strong 1:1 complex as elucidated by two of the authors in the interactions of polyanions with polycations in aqueous solutions.[17] The key issue is that such weaker complexes would be associated with endothermic or exothermic enthalpy changes much higher than that of the 1:1 complex and thus even a small amount of these weak complexes makes large impact on the shape of thermogram upon ITC titration and eventually prevents the straightforward application of the existing models provided by the ORIGIN program to derive the binding strength and the thermodynamic parameters. Nevertheless, it is obvious that the binding of ADP/ATP by **2** is accompanied by a large entropic gain because the heat effect (enthalpic gain) alone is far insufficient to account for such a strong binding. Kano demonstrated that the inclusion of monovalent guest ion into the cavity of an oppositely charged polyvalent CD ion was promoted by the entropic gain acquired mainly by the desolvation from both host and guest upon complexation, and the cooperation of Coulombic interactions and inclusion might be important to cause extensive desolvation.[12] Similar inclusion mechanism can be reasonably applied to the complexation of **2** with ATP/ADP. Both adenosine phosphate and **2** are expected to be heavily hydrated. Extensive desolvation from both host and guest accompanies the inclusion of the adenine moiety in the CD cavity and electrostatic interaction between the host and guest, resulting in a large entropic gain that facilitates the complexation. The extended structure of guanidinium residues and more desolvation upon binding may account for the stronger affinity of **2** over **1**.

In conclusion, we have synthesized and elucidated the binding behavior of mimic of the mitocondrial ADP/ATP carrier. The host molecule binds ADP and ATP strongly enough so as to resolve the complex and the free individuals in dilute solutions using NMR spectrophotometry.

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References and notes

1. Pu, L. *Chem. Rev.* **2004**, *104*, 1687–1716; Martínez-Máñez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419–4476; Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486–516.
2. García-España, E.; Díaz, P.; Llinares, J. M.; Bianchi, A. *Coord. Chem. Rev.* **2006**, *250*, 2952–2986; Hosseini, M. W.; Blacker, A. J.; Lehn, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 3896–3904.
3. McCleskey, S. C.; Griffin, M. J.; Schneider, S. E.; McDevitt, J. T.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 1114–1115.
4. Aranzaes, J. R.; Belin, C.; Astruc, D. *Angew. Chem., Int. Ed.* **2006**, *45*, 132–136; Astruc, D.; Daniel, M.-C.; Ruiz, J. *Chem. Commun.* **2004**, 2637–2649.
5. Ojida, A.; Nonaka, H.; Miyahara, Y.; Tamaru, S.; Sada, K.; Hamachi, I. *Angew. Chem., Int. Ed.* **2006**, *45*, 5518–5521.
6. Li, C.; Numata, M.; Takeuchi, M.; Shinkai, S. *Angew. Chem., Int. Ed.* **2005**, *44*, 6371–6374; Kwon, J. Y.; Singh, N. J.; Kim, H. N.; Kim, S. K.; Kim, K. S.; Yoon, J. *J. Am. Chem. Soc.* **2004**, *126*, 8892–8893; Mizukami, S.; Nagano, T.; Urano, Y.; Odani, A.; Kikuchi, K. *J. Am. Chem. Soc.* **2002**, *124*, 3920–3925; Sancenón, F.; Descalzo, A. B.; Martínez-Máñez, R.; Miranda, M. A.; Soto, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 2640–2643.
7. Elissev, A. V.; Schneider, H.-J. *J. Am. Chem. Soc.* **1994**, *116*, 6081–6088; Elissev, A. V.; Schneider, H.-J. *Angew. Chem., Int. Ed.* **1993**, *32*, 1331–1333.
8. Ghosh, M.; Zhang, R.; Lawler, R. G.; Seto, C. T. *J. Org. Chem.* **2000**, *65*, 735–741; Schwinte, P.; Darcy, R.;

O'Keeffe, F. *J. Chem. Soc., Perkin Trans. 2* **1998**, 805–808.
9. Neelakandan, P. P.; Hariharan, M.; Ramaiah, D. *J. Am. Chem. Soc.* **2006**, *128*, 11334–11335; Antoniss, M. M.; Reinhoudt, D. N. *Chem. Commun.* **1998**, 443–448.
10. Pebay-Peyroula, E.; Dahout-Gonzalez, C.; Kahn, R.; Trézéguet, V.; Lauquin, G. J. M.; Brandolin, G. *Nature* **2003**, *426*, 39–44.
11. *Synthesis of* **2**: A mixture of **1** (300 mg), 1*H*-pyazole carboxamidine hydrochloride (1.95 g) and *N*,*N*-diisopropyl ethylamine (2.3 mL) in water (2 mL) was stirred at rt. The reaction was followed by ^{1}H NMR by detecting the anomeric protons at δ ~5.1 ppm for **1** and ~5.0 ppm for **2**. After the complete disappearance of the signal at δ ~5.1 ppm being confirmed (it took 2d), the reaction mixture was added to acetone and filtered. The precipitates thus collected were dissolved in water (2 mL) and precipitated with acetone. This dissolution-precipitation process was repeated twice to afford compound **2** (297 mg, 92%) that is pure enough for spectroscopic measurements. FAB-MS: *m*/*z* 1422 (M+1); ^{13}C NMR (D_2O, CH_3CN int.): δ 158.7 (guanidine C), 102.7 (C1), 72.6 (C2), 73.3 (C3), 83.4 (C4), 71.7 (C5), and 43.1 (C6) ppm. ^{1}H NMR: listed in Table 1.
12. Kano, K.; Kitae, T.; Shimofuri, Y.; Tanaka, N.; Mineta, Y. *Chem. Eur. J.* **2000**, *6*, 2705–2713.
13. Sterk, H.; Schrunner, H. *J. Chem. Soc., Perkin Trans. 2* **1986**, 1715–1720.
14. Jiang, L.; Mao, X.-A. *Acta Phys. Chim. Sinica* **2002**, *18*, 796–801.
15. Schneider, H.-J.; Hacket, F.; Rüdiger, V.; Ikeda, H. *Chem. Rev.* **1998**, *98*, 1755–1785.
16. Meksuryen, D.; Fukuchi-Shimogori, T.; Tomitori, H.; Kashiwagi, K.; Toida, T.; Imanari, T.; Kawai, G.; Igarashi, K. *J. Biol. Chem.* **1998**, *273*, 30939–30944.
17. Rekharsky, M.; Inoue, Y.; Tobey, S.; Metzger, A.; Anslin, E. *J. Am. Chem. Soc.* **2002**, *124*, 14959–14967.