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Phosphatase-Triggered Guest Release from a Cyclodextrin Complex

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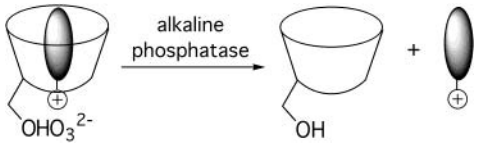
ABSTRACT

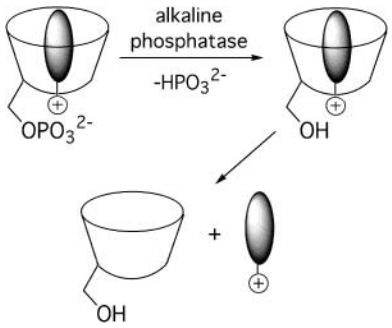


A synthetic supramolecular system is described that models the effect of phosphoryl transfer in molecular recognition. β-Cyclodextrin-6A-phosphate (pCD), which is shown to be a substrate of alkaline phosphatase, binds cationic aromatic guests, including anticancer agents, up to 100-fold better than native β-CD. The above observations demonstrate that pCD is capable of releasing the guests from its cavity upon hydrolysis with the phosphatase, as also confirmed by monitoring the hydrolysis in the presence of a guest.

Phosphoryl transfer reactions are commonly involved in the regulation of biological molecular recognition. Phosphorylation and dephosphorylation of proteins play key roles in the signal transduction[1] and cell proliferation[2] by affecting protein−protein recognition,[3] forcing conformational changes,[4] and altering the biopolymer dynamics.[5] These processes are catalyzed by various phosphatases and kinases, the unique balance of which is often characteristic for specific extra- and intracellular environments. Chemical modeling of the effect of these and other enzymes on molecular recognition is not only fundamentally interesting but may result in potential applications, e.g., in site-specific drug delivery. Examples of enzyme-controlled molecular recognition models have been previously reported for vesicular systems[6] and synthetic host−guest complexes.[7]

We have developed a system in which a phosphorylated macrocyclic host (β-cyclodextrin) releases its guest compound from the internal cavity as a result of enzymatic hydrolysis. As shown in Scheme 1, the phosphorylated macrocycle forms a complex with a positively charged aromatic guest molecule, which is stabilized by the formation of phosphate−cation salt bridges. If the phosphate group is then cleaved in the phosphatase-catalyzed hydrolysis, the binding affinity of the host to guest will drop and, under certain conditions, the guest will be released from the cavity.

Scheme 1



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(1) Pawson, T.; Scott, J. D. *Science* **1997**, *278*, 2075−2080.
(2) Radha, V.; Swarup, G. *Curr. Sci.* **1997**, *73*, 418−429.
(3) Eisenmessers, E. Z.; Post, C. B. *Biochemistry* **1998**, *37*, 867−877.
(4) Jones, B. E.; Rajagopal, P.; Klevit, R. E. *Protein Sci.* **1997**, *6*, 2107−2119.
(5) Schutkowski, M.; Bernhardt, A.; Zhou, X. Z.; Shen, M. H.; Reimer, U.; Rahfeld, J. U.; Lu, K. P.; Fischer, G. *Biochemistry* **1998**, *37*, 5566−5575.

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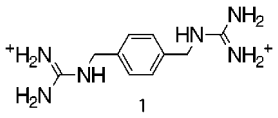
β-Cyclodextrin-6-phosphate (**pCD**) was synthesized from β-cyclodextrin (β-CD) by reaction with phosphoryl chloride under controlled conditions and purified by ion exchange chromatography.[8] To test the relative binding affinities of the native and phosphorylated cyclodextrin forms, we measured their association constants with three cationic guests. Bis-guanidinium derivative **1** was used as a model



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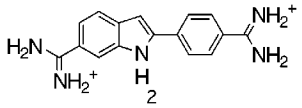
compound that would utilize two binding mechanisms common for charged cyclodextrins: electrostatic and hydrophobic.[9] To explore potential applications of **pCD** in drug delivery, we also studied antineoplastic agents DAPI **2** and



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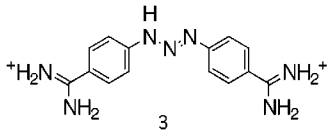
berenil **3**[10] that bear common structural features with **1**: cationic amidinium groups and aromatic moieties capable



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of inclusion into the cyclodextrin cavities. The binding was studied by NMR titrations in buffer solutions optimal for alkaline phosphatase catalysis. The *K* values determined by curve fitting (Table 1) confirm the expected significant difference in binding affinities between the two hosts. Apparently, the relative contributions of the electrostatics and the intracavity binding forces[11] are sensitive to the structure of the guest compounds, which is consistent with earlier studies of inclusion complexes with charged cyclodextrins.[12]

Table 1. Binding Constants of Cationic Guests to Native and Phosphorylated β-Cyclodextrins

	K (M^{-1})[a]	
guest	pCD	β-CD
1	250	29
2	170	100
3	820	<10[b]

[a] Determined by ^{1}H and, for **pCD**, by ^{31}P NMR titrations at pD 9.4 in the buffer used for the phosphatase cleavage experiment (see Figure 1). The data were fitted according to a 1:1 complexation model. Values obtained from different nuclei were consistent within the standard deviation (usually <10%). [b] Only the upper limit of the constant could be estimated because of limited solubility.

The host **pCD** was then tested as a substrate of alkaline phosphatase. The phosphate cleavage reaction monitored by ^{31}P NMR yielded the trace presented in Figure 1, showing that the reaction was almost complete within several hours.[13] A similar run performed in the presence of **1** demonstrated

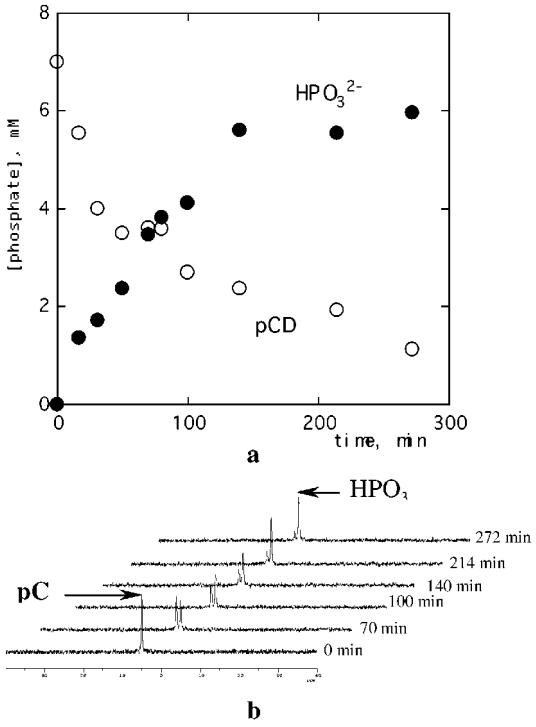

Figure 1. Kinetics (a) and ^{31}P NMR time course (b) of hydrolysis of **pCD** (7 mM) catalyzed by alkaline bovine phosphatase (1000 DEA units) at 37 °C. Buffer: 0.1 M diethanolamine, 0.5 mM $MgCl_2$ in D_2O, pD 9.4.

(6) Menger, F. M.; Johnston, D. E. *J. Am. Chem. Soc.* **1991**, *113*, 5467−5468.
(7) Rudra, S.; Eliseev, A. V. *J. Am. Chem. Soc.* **1998**, *120*, 11543−11547.
(8) See Supporting Information for synthetic procedure and analytical data.
(9) Yatsimirsky, A. K.; Eliseev, A. V. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1769−1772.
(10) Wakelin, L. P. G.; Waring, M. J. DNA Intercalating Agents. In *Comprehensive Medicinal Chemistry*; Kennewell, P. D., Hansch, C., Sammes, P. G., Taylor, J. B., Eds.; Pergamon Press: Oxford; New York, 1990; Vol. 2, pp 703−724.
(11) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: Berlin; New York, 1978.
(12) (a) Eliseev, A. V.; Schneider, H. J. *J. Am. Chem. Soc.* **1994**, *116*, 6081−6088. (b) Kano, K.; Arimoto, S.; Ishimura, T. *J. Chem. Soc., Perkin Trans. 2* **1995**, *8*, 1661−1666. (c)Kano, K.; Kitae, T.; Takashima, H. *J. Inclusion Phenom. Mol. Recogn.* **1996**, *25*, 243−248. (c) Ito, N.; Yoshida, N.; Ichikawa, K. *J. Chem. Soc., Perkin Trans. 2* **1996**, *5*, 965−972. (d) Kitae, T.; Nakayama, T.; Kano, K. *J. Chem. Soc., Perkin Trans. 2* **1998**, *2*, 207−212. (e) Vizitiu, D.; Thatcher, G. R. J. *J. Org. Chem.* **1999**, *64*, 6235−6238. (f) Baudoin, O.; Gonnet, F.; Teulade-Fichou, M. P.; Vigneron, J. P.; Tabet, J. C.; Lehn, J.-M. *Chem. Eur. J.* **1999**, *5*, 2762−2771.
(13) Similar reaction performed at lower Mg^{2+} concentration (0.25 mM) was noticeably slower ($t_{1/2}$ 6.5 h).
(14) The phosphatase-catalyzed cleavage of 16.5 mM **pCD** in the presence of equimolar **1** proceeded somewhat slower than that of pure host. A likely reason for that was the inhibitory effect of methyl phosphonic acid that was used in the former case as an internal standard. PF_6^- that was used as a standard in all other cases formed a precipitate with the cation of **1**.

that the cleavage reaction could also occur in the presence of the guest.[14] The kinetics of all reactions was close to first order.

The observed rates of **pCD** dephosphorylation are generally lower than those of specific phosphatase substrates,[15] apparently because bulkiness of the cyclodextrin molecule renders it a weak binder for the enzyme active site.[16] However, with a ca. 1.5 h half-life time (conditions of Figure 1), the **pCD** can still be considered a "successful" synthetic host for our approach. To compare it with other plausible macrocyclic scaffolds, we tested two phosphorylated calix-4-arenes[17,18] which showed no detectable hydrolysis within 3 days under similar conditions.

The combination of the binding and cleavage results demonstrates that **pCD** is indeed capable of releasing the cationic guests upon phosphatase-catalyzed hydrolysis. Although it is not possible to determine whether in the **pCD**−guest mixture the enzyme reacts only with free but not with the guest-bound form of **pCD**, both possible pathways lead to the same final result: decreasing complexation degree of the guest. For example, it is estimated that a mixture of 30 mM **pCD** and 30 mM **3** contains ca. 85% of the guest in the complexed form, while after complete hydrolysis of the host to β-CD, less than 20% of **3** remains complexed.

In summary, the enzyme-controlled recognition system described above can serve as a simple model of complex biochemical recognition processes modulated by phosphoryl transfer. Because cyclodextrins are widespread as drug-complexing agents,[19] the **pCD**−guest complexes also have straightforward implications for creation of drug delivery systems sensitive to specific enzymatic environment. Alkaline phosphatase is present in elevated concentrations in certain tumors[20] and can act as a trigger, increasing the concentration of the free form of the guest−drug. In this respect, we are currently studying similar systems based on polyphosphorylated cyclodextrins that are expected to maximize the selectivity in binding of cationic guests.

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Supporting Information Available: Detailed experimental procedures for synthesis and purification, NMR, and electrospray mass spectra of **pCD**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL005915X

(15) McComb, R. B.; Bowers, G. N.; Posen, S. *Alkaline Phosphatase*; Plenum Press: New York, 1979.

(16) Hydrolysis runs performed at five different **pCD** concentrations in the range 2−25 mM yielded the values of K_m of 2.9×10^{-2} M and V_{max} of 2.8×10^{-4} M/min.

(17) (a) Lipkowski, J.; Simonov, Y.; Kalchenko, V. I.; Vysotsky, M. A.; Markovsky, L. N. *Anal. Quim.* **1998**, *94*, 328−331. (b)Markovsky, L. N.; Vysotsky, M. A.; Tairov, M. A.; Kalchenko, V. I. *Phosph. Sulf. Silicon Relat. Elem.* **1999**, *146*, 89−92.

(18) Generously provided by Dr. Vitaly Kalchenko.

(19) Fromming, K.-H.; Szejtli, J. *Cyclodextrins in Pharmacy*; Kluwer Academic Publishers: Dordrecht, Boston, 1994.

(20) Schwartz, M. *Clin. Chem.* **1973**, *19*, 10−22.