Supramolecular modulation of action of polyamine on enzyme/DNA interactions

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Thread-in-hole complexation of cucurbit[6]uril (CB[6]) with spermidine and spermine either enhances or reduces the activity of the polyamine on the enzymatic reactions of DNA.

Though structurally simple and trivial, polyamines are important molecules in biological systems. Spermidine and spermine are distributed widely in prokaryotic and eukaryotic cells and take part in the control of cell growth and differentiation.[1] In addition, some polyamines such as spider toxins show strong physiological activities.[2] Despite such biological activities of polyamines, their mechanism of action is not fully understood. Thus, it is interesting from the chemical biology point of view to find small molecules that can recognize the polyamine structures, and either inhibit or enhance their activities. Such molecules have thus far been unknown. Focusing on the characteristic 1,4-diamine structure inherent to spermidine and spermine, we conjectured that the unique ability of cucurbit[6]uril (CB[6]) can be utilized for such a purpose; that is, the ability to selectively complex with a 1,4-diamine to form a pseudorotaxane such as **1** and **2** (Fig. 1) in preference to binding to simple amines or even to 1,2- and 1,3-diamines.[3,4] We report herein a finding that CB[6] modulates the activity of spermidine and spermine in the reaction of DNA with a topoisomerase and a nuclease.

Among numerous targets of the action of spermidine and spermine are the reactions of DNA, where these amines take part in the enzymatic processing of DNA molecules.[1] We can view such a reaction in the simplest way as a trimolecular interaction of an enzyme, DNA and the polyamine, where the latter interacts either with the enzyme and/or DNA to modulate the reaction in question.

We first describe topoisomerization of a supercoiled plasmid DNA, pBR322, catalyzed by calf thymus topoisomerase I (Topo I) in the presence of spermidine or the pseudorotaxane **1**. It is known that the relaxation of supercoiled plasmid DNA into its open circular form by the action of Topo I is accelerated slightly by a small amount of spermidine and significantly by a larger amount.[5] When Topo I was allowed to react with a plasmid DNA composed of a mixture of a supercoiled form (63%) and an open circular form (37%; Lane 1, Fig. 2a), the supercoiled DNA was converted to the open circular form to make a 48 : 52 mixture (Lane 4). In the presence of spermidine at a spermidine/base pair ratio (R) of 13, the reaction became slightly faster and the amount of the open circular form became 58% under otherwise the same conditions (Lane 3). When a large amount (R = 130) of spermidine was present in the medium (Lane 5), most of the supercoiled DNA was converted to the open form (95%). When the same reaction was performed in the presence of pseudorotaxane **1** rather than spermidine, 100% conversion into the open circular form was achieved only with a molar ratio of R = 13 (Lane 2). Thus, it is clear that the CB/spermidine complex is much more effective than spermidine itself in accelerating the Topo I-catalyzed topoisomerization of the plasmid DNA.

Spermine is known to accelerate the enzymatic hydrolysis of plasmid pBR322 by restriction endonuclease *Ban*II,[6] and, this time, the use of pseudorotaxane in place of spermine was found to act against the acceleration of the hydrolysis. Under the conditions of a control experiment (Fig. 2b, Lane 1), the *Ban*II cleavage reaction gives a mixture of 73% linear and 19% open circular forms. In the presence of spermine at a molar ratio of R = 2.6, the double strand cleavage reaction is accelerated to give 94% linear DNA (Lane 2). On the other hand, the pseudorotaxane **2** at the

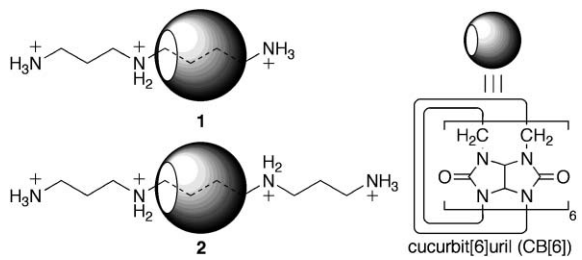

Fig. 1 Pseudorotaxanes of CB[6] with spermidine **1** and with spermine **2**.

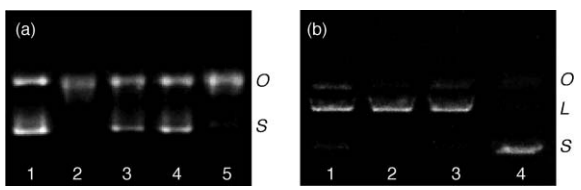

Fig. 2 Electrophoretic analysis of enzymatic reactions of plasmid DNA (pBR322) with topoisomerase (a) and endonuclease (b). S = supercoiled form, O = open circular form, L = linear form. (a) Isomerization of pBR322 with calf thymus Topo I.† Lane 1: intact pBR322; S = 63%, O = 37%. Lane 2: pBR322 + Topo I + **1** (R (reagent/bp ratio) = 13); S = 0%, O = 100%. Lane 3, pBR322 + Topo I + spermidine (R = 13); S = 42%, O = 58%. Lane 4, pBR322 + Topo I; S = 48%, O = 52%. Lane 5, pBR322 + Topo I + spermine (R = 130); S = 5%, O = 95%. (b) Hydrolysis of pBR322 with a restriction endonuclease, *Ban*II.‡ Lane 1: pBR322 + *Ban*II; S = 8%, O = 19%, L = 73%. Lane 2: pBR322 + *Ban*II + spermine (R = 2.6); S = 0%, O = 6%, L = 94%. Lane 3: pBR322 + *Ban*II + **2** (R = 2.6); S = 2%, O = 19%, L = 79%. Lane 4: intact pBR322 without hydrolysis; S = 83%, O = 16%, L = 1%.

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same molar ratio shows little effect, causing only 79% of DNA to be converted to the linear form (Lane 3).

It is established that spermidine and spermine interact with double stranded DNA through both electrostatic interaction with the phosphate groups and hydrogen bonding with the nucleobases.[1] One outcome of such interaction is the change of the morphology of the DNA molecule that can be observed by atomic force microscope (AFM) in a Mg-containing buffer solution placed on a mica surface. A typical morphology of an intact supramolecular plasmid DNA under such conditions is shown in Fig. 3a. Upon addition of spermine (R = 2.6, the same ratio used for the *Ban*II experiment), the DNA molecules aggregate to form multi-circular networks on the 2D-surface of mica (Fig. 3b; similar morphology was reported for spermidine).[7] We note in passing that this morphological change caused by water-soluble polyamine is entirely different from the globular/disc morphology observed for a DNA/fullerene tetramine complex that we reported some time ago.[8]

In contrast to the image in the presence of spermine, the AFM image of a mixture (R = 2.6) of pBR322 and pseudorotaxane **2** (Fig. 3c; or with pseudorotaxane **1**, data not shown) indicates that **2** induces little morphological change of DNA if any, and does not bind to DNA to any significant degree, either. In addition, the pseudorotaxane did not cause any appreciable change of the ratio of supercoiled and open circular forms (that is, little sign of selective stabilization of the open circular form over the supercoiled one that might account for the acceleration of topoisomerization, see below). The small dots of 1–1.5 nm height seen in Fig. 3c besides the DNA molecules are the molecules of **2** as confirmed by their own AFM image in the absence of the DNA molecules.

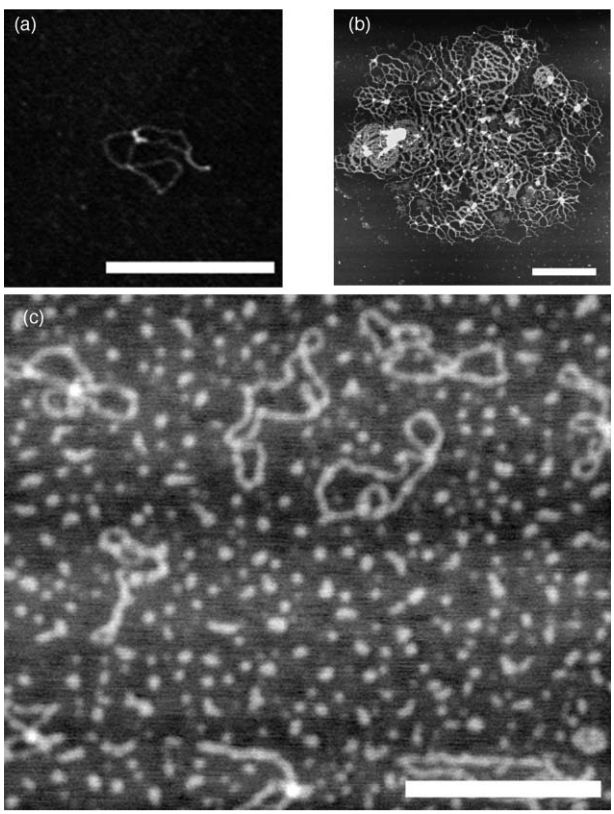

Fig. 3 AFM images of a plasmid DNA, spermine/DNA mixture and a pseudorotaxane **2**/DNA mixture. (a) An intact plasmid DNA pBR322. Scale bar shows 0.5 μm. (b) A large aggregate of plasmid DNA observed for a mixture of spermine with pBR322 at R of 2.6. Scale bar shows 2.5 μm. (c) DNA molecules and small particles observed in a mixture of pseudorotaxane **2** and pBR322 at R of 2.6. Scale bar shows 0.5 μm. The mixture was prepared under the same conditions as the hydrolysis reaction but without the endonuclease.‡

On the basis of this AFM analysis of the **2**/DNA mixture, we can consider at least two possibilities to account for the acceleration of the Topo I reaction: the pseudorotaxane increases the reactivity of the enzyme or it forms a low-concentration pseudorotaxane/DNA complex that is activated toward enzyme action possibly through deformation of the DNA structure.[9] In either scenario, the enzyme, DNA and pseudorotaxane are likely engaged in the transition state of the relaxation process. The subtlety of the effect in the *Ban*II reaction is difficult to interpret, but may have been caused simply by the change of the chemical reactivities of spermine caused by the loss of the two internal amine sites upon conversion of spermine to the pseudorotaxane.

In summary, we have found that CB[6] changes the activity of spermidine and spermine upon supramolecular complexation. An application of the present supramolecular protocol to the real biological systems would be interesting. However, this is impossible by the use of the water-insoluble parent CB[6], but would be feasible with the aid of water-soluble derivatives,[10] which are to be injected to the target site in the cell to control the action of polyamines.

This work is dedicated to Prof. Iwao Ojima on the occasion of his 60th birthday.

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Notes and references

† Reaction conditions: [bp] = 38 μM; [Topo I] = 0.5 U/μL; 20 mM tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 100 mM KCl, 0.01% BSA, pH 8.5; 37 °C for 30 min. The reaction was analyzed by agarose gel electrophoresis after purification of DNA through phenol treatment and ethanol precipitation. The ratio of each form was measured by integrated optical density using ImageJ (ref. 4).
‡ Reaction conditions: [bp] = 38 μM; [*Ban*II] = 0.02 U/μL; 50 mM tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 100 mM NaCl, pH 7.5; 35 °C for 1 h.

1 S. S. Cohen, *A Guide to The Polyamines*, Oxford University Press, 1998.
2 I. R. Mellor and P. N. R. Usherwood, *Toxicon*, 2004, **43**, 493.
3 J. W. Lee, S. Samal, N. Selvapalam, H.-J. Kim and K. Kim, *Acc. Chem. Res.*, 2003, **36**, 621; W. L. Mock, in *Comprehensive Supramolecular Chemistry*, Vol. 2, ed. F. Vogtle, Pergamon, 1996.
4 Y.-M. Jeon, D. Whang, J. Kim and K. Kim, *Chem. Lett.*, 1996, 503; H. Isobe, N. Tomita, J. W. Lee, H.-J. Kim, K. Kim and E. Nakamura, *Angew. Chem., Int. Ed.*, 2000, **39**, 4257.

5 K. S. Srivenugopal, D. E. Wemmer and D. R. Morris, *Nucleic Acids Res.*, 1987, **15**, 2563.
6 H. Kirino, R. Kuwahara, N. Hamasaki and T. Oshima, *J. Biochem.*, 1990, **107**, 661.
7 Y. Fang and J. H. Hoh, *J. Am. Chem. Soc.*, 1998, **120**, 8903.
8 H. Isobe, S. Sugiyama, K.-i. Fukui, Y. Iwasawa and E. Nakamura, *Angew. Chem., Int. Ed.*, 2001, **40**, 3364.
9 X. Wang, G. F. Short III, W. D. Kingsbury, R. K. Johnson and S. M. Hecht, *Chem. Res. Toxicol.*, 1998, **11**, 1352.
10 J. Kim, I.-S. Jung, S.-Y. Kim, E. Lee, J.-K. Kang, S. Sakamoto, K. Yamaguchi and K. Kim, *J. Am. Chem. Soc.*, 2000, **122**, 540; J. Zhao, H.-J. Kim, J. Oh, S.-Y. Kim, J. W. Lee, S. Sakamoto, K. Yamaguchi and K. Kim, *Angew. Chem., Int. Ed.*, 2001, **40**, 4233; A. I. Day, R. J. Blanch, A. P. Arnold, S. Lorenzo, G. R. Lewis and I. Dance, *Angew. Chem., Int. Ed.*, 2002, **41**, 275; H. Isobe, S. Sato and E. Nakamura, *Org. Lett.*, 2002, **4**, 1287; S. Y. Jon, N. Selvapalam, D. H. Oh, J.-K. Kang, S.-Y. Kim, Y. J. Jeon, J. W. Lee and K. Kim, *J. Am. Chem. Soc.*, 2003, **125**, 10186.