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Hydrolysis of Linear DNA Duplex Catalyzed by Co(III) Complex of Cyclen Attached to Polystyrene

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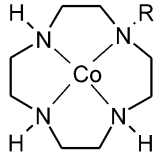
Abstract—To design artificial restriction enzymes, synthetic catalytic centers that effectively hydrolyze linear double-stranded polydeoxyribonucleotides are needed. The Co(III) complex of cyclen (CoCyc) attached to polystyrene derivatives hydrolyzes linearized pUC18 DNA with half-lives as short as 30 min at 25 °C. The catalytic activity of CoCyc is enhanced by >150 times on attachment to the resin.

Bacterial restriction enzymes are not useful for sequence-specific cleavage of chromosomal DNAs as they recognize only 4–8 nucleotides. To design artificial restriction enzymes for chromosomal DNAs, it is necessary to secure both a binding site recognizing 15–20 or more[1] nucleotides and a catalytic center hydrolyzing phosphodiester linkages of double-stranded polydeoxyribonucleotides.

Phosphodiester linkages of DNA are very stable to hydrolysis. The half-life for spontaneous hydrolysis of the phosphodiester has been estimated as about 10^{11} years at pH 7 and 25 °C.[2] Most of known synthetic catalysts for DNA hydrolysis are metal complexes.[3–15] Although many metal complexes have been reported to promote the cleavage of supercoiled and open circular DNA and single- or double-stranded oligodeoxyribonucleotides, hydrolytic cleavage of linear double-stranded polydeoxyribonucleotides by metal complexes has been seldom reported. The only synthetic catalyst reported thus far for hydrolysis of a long linear DNA duplex is the dicerium complex which hydrolyzed a 192-base pair restriction fragment.[15] The difficulties encountered in hydrolyzing long linear DNA duplexes indicate higher stability of their phosphodiester bonds compared with those of circular DNA duplexes, linear oligo-DNA duplexes, or single-stranded DNAs. This is possibly due to differences in the conformation of the DNA backbone and the concomitant differences in the steric environments of the phosphodiester bonds.

The Co(III) complex of cyclen (CoCyc: **1**) is one of the most effective synthetic catalysts discovered so far for DNA hydrolysis. It has been estimated that $>10^7$-fold rate increase was achieved with CoCyc in hydrolysis of phosphodiester linkages leading to the conversion of a supercoiled DNA to the corresponding open circular form.[10] Hydrolytic nature of the DNA cleavage by the Co(III) complexes of polyamines including cyclen was evidenced by chemical or enzymatic religation[11] of the cleavage products as well as by the detection[7] of 3′-OH and 5′-OH termini of the cleavage products. A mechanism (**2**) has been proposed[7] for the catalytic action of the Co(III) complexes: the Co(III) center binds the phosphate anion of the phosphodiester linkage and the adjacent Co(III)-bound hydroxide ion makes intramolecular attack at the phosphorus atom of the bound substrate.



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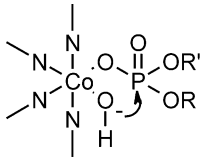

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Our previous study revealed that the catalytic activity of CoCyc in phosphodiester hydrolysis of a supercoiled

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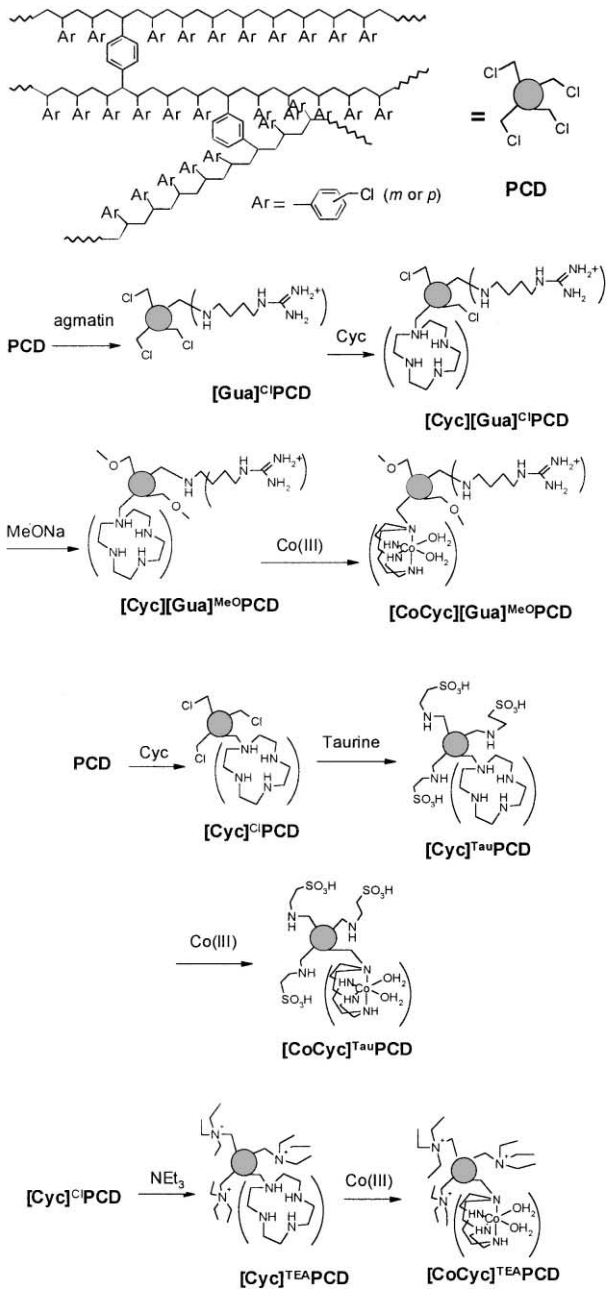
plasmid DNA is enhanced by >200 times upon attachment of CoCyc to poly(chloromethylstyrene-co-divinylbenzene) (PCD).[16] Since synthetic metallonuclease hydrolyzing double-stranded polydeoxyribonucleotides is very rare although it is essential to designing artificial restriction enzymes, we tested whether the reactivity of CoCyc is sufficiently enhanced on attachment to PCD leading to hydrolysis of a linearized plasmid DNA.

The synthetic pathways for various PCD derivatives with or without CoCyc are indicated in Scheme 1. PCD with 2% cross-linkage, [Cyc]^{Cl}PCD, [Gua]^{Cl}PCD, [Cyc][Gua]^{Cl}PCD, and [Cyc][Gua]^{MeO}PCD were synthesized according to the general procedure reported previously.[16,17] [Cyc]^{Tau}PCD or [Cyc]^{TEA}PCD was prepared by shaking [Cyc]^{Cl}PCD with taurine (Tau) and



Scheme 1. Synthetic routes to PCD-based CoCyc catalysts.

N,N-diisopropylethylamine in 1:1 (v/v) dimethyl sulfoxide-acetonitrile or with triethylamine (TEA) in acetonitrile, respectively, at 50 °C for 3 days. MeO (methoxy) and Tau pendants provide neutral and zwitterionic microenvironments, respectively, to the resin surface whereas as guanidinium (Gua) and TEA pendants create cationic microenvironments. Co(III) ion was incorporated by heating the precursor PCD (2 g) suspended in 100 mL 1:1 (v/v) methanol/water for 2 h at 70 °C with 0.52 g (1.3 mmol) tris(sodium carbonato) cobalt(III) trihydrate,[18] followed by addition of 10 mL concd HCl and evaporation of the solvent in vacuo. CoCyc (**1**) was prepared according to the literature.[10]

The contents (residue mol% relative to styryl moiety) of Cyc and Gua in the PCD-based catalysts were determined as reported previously,[16] and those of TEA and Tau were estimated by elemental analysis: 0.95 residue mol% for Cyc, 0.81 residue mol% for Gua, 35 residue mol% for TEA, and 15 residue mol% for Tau. Some chloromethyl groups were left unsubstituted on the surface of [Cyc]^{Tau}PCD, [Cyc]^{TEA}PCD, and [Cyc][Gua]^{MeO}PCD but were also unaffected under the conditions of hydrolysis of the DNA substrates as checked by Electron Probe Micro Analysis. Dry beads of the CoCyc-containing PCD derivatives were swollen in water for 1 h at 80 °C prior to incubation with DNA substrates. Upon swelling, the color of beads changed from green to yellow presumably reflecting exchange of the Co(III)-bound chloride ion[19] with water molecule.

The linearized form of plasmid pUC18 DNA (2,686 base pairs) was used as the linear DNA substrate. Plasmid pUC18 DNA was prepared and isolated according to standard protocols.[20] The *Escherichia coli* cell line used for infection was XL1-Blue. The purity of pUC18 DNA was confirmed via both agarose gel electrophoresis and UV spectroscopy by determining the ratio of absorbance at 260 nm to the absorbance at 280 nm. The concentration of DNA was determined from the absorbance at 260 nm ($A_{260}=1.0$ for 50 μg/mL). The linear DNA substrate was prepared by treatment of pUC18 DNA with EcoR I digest.[20] Treatment of the linearized pUC18 with nuclease S1 indicated the absence of nicks. Stock solutions of pUC18 were stored frozen at −20 °C.

Disappearance of the linear DNA during incubation with the PCD-based catalysts was monitored by electrophoresis, as exemplified by Figure 1. It is possible that the decrease in the intensity of the electrophoretic band (Fig. 1) was due to simple adsorption of the DNA substrate onto the resin. This possibility was excluded

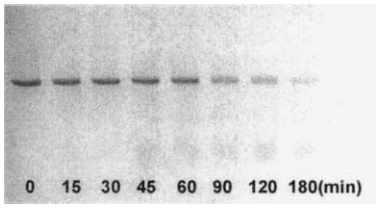


Figure 1. Negative image of electrophoretic separation of the linearized pUC18 DNA incubated with [CoCyc]^{TEA}PCD ($C_0=0.46$ mM) at 25°C and pH 5.5.

since a substantial amount of the nucleotides was recovered in the buffer solution when the PCD resins were separated by filtration after the DNA substrate disappeared completely. This is based on the fact that the absorbance at 260 nm of the product solution was greater than 93% of the absorbance of the substrate DNA solution. When the same DNA was hydrolyzed completely into mononucleotides with DNase I, the absorbance increased by 20%. Considering possible hypochromicity of the cleavage products obtained with the PCD derivatives, recovery of nucleotides in the buffer solution is more than 80% when the DNA is cleaved with the PCD derivatives.

The pseudo-first-order rate constant (k_0) for disappearance of the linear DNA substrates was calculated from the intensity of its electrophoretic bands measured at various time intervals as described previously.[16] Kinetic data were collected at 25 °C under the conditions of C_0 (concentration of CoCyc moiety obtainable when the PCD derivatives are assumed to be dissolved) $> S_0$ (the initially added concentration of DNA). The reaction rate increased as stirring speed was raised, reaching a plateau value at 800 rpm, and, thus, all of the kinetic measurements were conducted at the stirring speed of 800 rpm.

Linear dependence of k_0 on C_0 was observed for the linear DNA substrate (Fig. 2). The pH dependence of k_0 was measured at a fixed C_0 concentration for each CoCyc-containing PCD derivative and the results are summarized in Figure 3. The highest value of k_0 indicated in Figures 2 and 3 corresponds to the half-life 30 min at 25 °C. Comparison of the rate constants measured for CoCyc and for the PCD-based CoCyc at the same C_0 concentration reveals that the reactivity of CoCyc is enhanced upon attachment to PCD by at least 150 times toward the linear DNA ([CoCyc]^{TEA}PCD at pH 5.5). Considering that only a small portion of the CoCyc moieties attached to PCD can attack the DNA,[16] the degree of activation should be much greater than 150-fold. As discussed previously,[16] activation of CoCyc on attachment to PCD may be attributed to close contact between the phosphate linkage of DNA and the CoCyc center or favorable medium provided by the resin. The catalytic rate is considerably affected by the nature of the pendants (MeO/Gua, Tau, and TEA) attached to PCD (Figs 2 and 3), which may be related to different microenvironments introduced by the pendants.

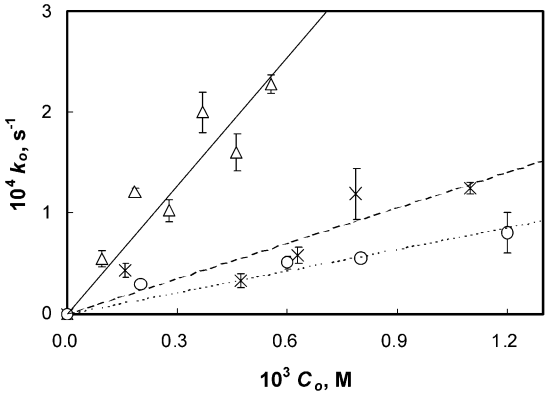


Figure 2. Dependence of k_0 on C_0 for cleavage of the linearized pUC18 DNA by [CoCyc]^{TEA}PCD (△), [CoCyc]^{Tau}PCD (×), and [CoCyc][Gua]^{MeO}PCD (○) at 25°C and pH 5.5.

When the linear pUC18 was incubated with the Cyc-containing resins without Co(III) ion, cleavage of the DNA was negligible under the conditions of the kinetic measurements. The rate for cleavage of the linear DNA by [CoCyc][Gua]^{MeO}PCD ($C_0 = 0.6$ mM) at pH 5.5 and 25 °C was not affected appreciably by additives such as catalase (240 μg/mL), superoxide dismutase (60 μg/mL), DMSO (1.0 M), ethanol (2.0 M), and H_2O_2 (0.6 M). As mentioned above, it is well established that the Co(III) complexes of polyamines including cyclen cleave DNA by hydrolysis. This is further supported by lack of appreciable effects by the additives, which inhibit the oxidative cleavage of DNA by diffusable free radicals, hydrogen peroxide, or superoxide.[16]

The 5′-OH termini of the products obtained after incubation of the linearized pUC18 with the PCD-based CoCyc were identified by phosphorylation of the 5′-OH groups with γ-^{32}P-ATP and T4 polynucleotide kinase followed by polyacrylamide gel electrophoresis (Fig. 4). The 3′-OH termini of the products were identified by phosphorylation with α-^{32}P-ddATP and terminal transferase followed by polyacrylamide gel electrophoresis (Fig. 4). In these experiments, [CoCyc][Gua]^{MeO}PCD was chosen as the catalyst.

The cleavage products are detected by the radioisotope-labeling only when fragments of similar sizes are formed in detectable amounts. If a few cuts are made randomly in the DNA cleavage, 5′-OH and 3′-OH ends of fragments of similar sizes are formed in low concentrations and, thus, are not easily detected by the ^{32}P-labeling.

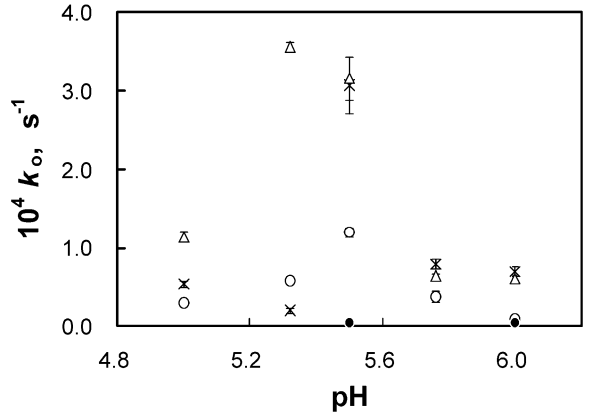


Figure 3. pH dependence of k_0 for cleavage of the linearized pUC18 DNA by [CoCyc]^{TEA}PCD (△; $C_0 = 0.48$ mM), [CoCyc]^{Tau}PCD (×; $C_0 = 0.81$ mM), and [CoCyc][Gua]^{MeO}PCD (○; $C_0 = 1.0$ mM) at 25°C. The k_0 for cleavage of the linearized pUC18 by CoCyc (●) ($C_0 = 1.0$ mM) was $(4.3 \pm 0.6) \times 10^{-6}$ s^{-1} at pH 5.5 and $(3.8 \pm 0.8) \times 10^{-6}$ s^{-1} at pH 6.0 and 25°C. At pH 5.5 or 6.0 and 25°C, intensity of the electrophoretic band of the linear DNA duplex did not decrease considerably in the absence of CoCyc over the period of time (up to 150 h) needed to measure the rate constant for CoCyc. The bell-shaped pH profiles can be related to pK_a of Co-bound water molecules as discussed previously.[16]

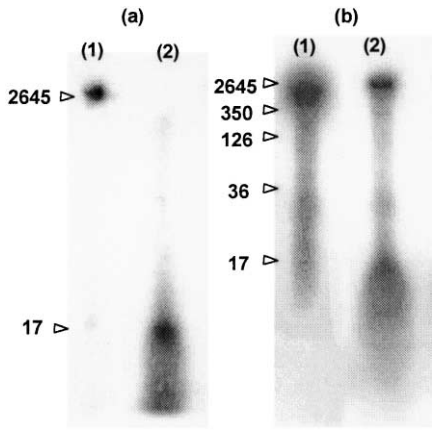


Figure 4. Autoradiogram of the linearized pUC18 ($S_0 = 24$ nM) before (lane 1) or after incubation (lane 2) with [CoCyc][Gua]^{MeO}PCD ($C_0 = 0.46$ mM) at 25 °C and pH 5.5 for 1 h: (a) after ^{32}P-labeling of the 5′-OH termini, (b) after ^{32}P-labeling of the 3′-OH termini. The numbers indicate the positions of marker DNAs with respective sizes. Control experiments showed that the unincorporated radiolabel did not interfere with the results. The small band of lane (a)-(1) is attributable to an impurity.

The intense bands near the 17-mer marker obtained for the products in Figure 4 demonstrate that both 5′-OH and 3′-OH ends of the small DNA fragments are formed in high concentrations. That both 5′-OH and 3′-OH ends are formed in high concentrations provides another piece of evidence for the hydrolytic nature of the DNA cleavage.

In phosphodiester hydrolysis, either 3′-OH or 5′-OH can be expelled from phosphorus atom of the penta-coordinate addition intermediate. Many endonucleases hydrolyze DNA randomly without recognizing special nucleotide sequences. Most endonucleases produce 3′-OH and 5′-monophosphate ends after cleavage of phosphodiester bonds, but some endonucleases such as micrococcal nuclease and spleen phosphodiesterase form 3′-monophosphate and 5′-OH ends.[21] The PCD-based CoCyc produces both 3′-OH and 5′-OH ends whereas enzymes specifically form either 3′-OH or 5′-OH ends. The PCD-based CoCyc complexes lack specificity in the cleavage mode just as Co-tetramine complexes[7] unattached to resins. Nevertheless, they can be considered as artificial endonucleases.

With the dicerium complex, the only synthetic catalyst for hydrolysis a long linear DNA duplex reported prior to this study, it took 24 h at 37 °C or 5 h at 55 °C to obtain hydrolysis products of a 192-base pair DNA.[15] On the other hand, the PCD-based CoCyc derivatives degraded a DNA duplex into small fragments in few hours at 25 °C.

Acknowledgement

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References and Notes

1. Dervan, P. B. *Science* **1986**, *232*, 464.
2. Williams, N. H.; Takasaki, B.; Wall, M.; Chin, J. *Acc. Chem. Res.* **1999**, *32*, 485.
3. Takasaki, B. K.; Chin, J. *J. Am. Chem. Soc.* **1994**, *116*, 1121.
4. Schnaith, L. M. T.; Hanson, R. S.; Que, L., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 569.
5. Komiyama, M.; Takeda, N.; Takahashi, Y.; Uchida, H.; Shiiba, T.; Kodama, T.; Yashiro, M. *J. Chem. Soc., Perkin Trans. 2* **1995**, 269.
6. Rammo, J.; Hettich, R.; Roigk, A.; Schneider, H. J. *Chem. Commun.* **1996**, 105.
7. Dixon, N. E.; Geue, R. J.; Lambert, J. N.; Moghaddas, S.; Pearce, D. A.; Sargeson, A. M. *Chem. Commun.* **1996**, 1287.
8. Ragunathan, K. G.; Schneider, H. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1219.
9. Fitzsimons, M. P.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 3379.
10. Hettich, R.; Schneider, J.-J. *J. Am. Chem. Soc.* **1997**, *119*, 5638.
11. Hettich, R.; Schneider, J.-J. *J. Chem. Soc., Perkin Trans. 2* **1997**, 2069.
12. Hegg, E. L.; Burstyn, J. N. *Inorg. Chem.* **1998**, *37*, 751.
13. Berg, T.; Simeonov, A.; Janda, K. D. *J. Comb. Chem.* **1999**, *1*, 96.
14. Sreedhara, A.; Freed, J. D.; Cowan, J. A. *J. Am. Chem. Soc.* **2000**, *122*, 8814.
15. Branum, M. E.; Tipton, A. K.; Zhu, S.; Que, L., Jr. *J. Am. Chem. Soc.* **2001**, *123*, 1898.
16. Jeung, C.-S.; Kim, C. H.; Min, K.; Suh, S. W.; Suh, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2401.
17. Jang, B.-B.; Lee, K. P.; Min, D. H.; Suh, J. *J. Am. Chem. Soc.* **1998**, *120*, 12008.
18. Bauer, H. F.; Drinkard, W. C. *J. Am. Chem. Soc.* **1960**, *82*, 5031.
19. Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 5th ed.; Wiley: New York, 1988; p 732.
20. Sambrook, J.; Fritsch, E. F.; Moniatis, T. In *Molecular Cloning*: *A Laboratory Manual*, 2nd ed.; Ford, N., Ed.; Cold Spring Harbor Laboratory: Cold Spring Harbor, 1989.
21. Stryer, L., *Biochemistry*, 1st ed., Freeman: San Francisco, 1975; p 576.