Short Communication

2'-Deoxyribosylzeatin: A Novel Inhibitor for DNA Polymerase I of *Escherichia coli*

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Several purine nucleoside analogues have been used as antiviral drugs, and such compounds are currently synthesized by chemical methods.¹⁾ However, the steps of chemical productions are long and complicated, and undesirable by-products are formed.²⁾ Enzymatic productions of nucleoside analogues have been developed using bacterial purine nucleoside phosphorylase (PNPase).^{3,4)} PNPase catalyzes not only the synthesis of purine nucleosides from purine bases and ribose-1-phosphate, but also the phosphorolysis of purine nucleosides.⁵⁾ The purified microbial PNPases were obtained from *Escherichia coli*,⁶⁾ *Salmonella typhimurium*,⁶⁾ *Bacillus stearothermophilus*^{7,8)} and *Klebsiella* sp. LF 1202.⁹⁾ A bacterium was screened and isolated from soil as an adenosine-assimilating bacterium by us¹⁰⁾ and the enzyme purified from the bacterium was a novel one that acted on both purine and pyrimidine nucleosides.⁹⁾ Therefore, we designated it nucleoside phosphorylase (NPase) to distinguish it from PNPase or pyrimidine nucleoside phosphorylase (PyNPase).⁹⁾ Using NPase, we demonstrated the enzymatic production of adenine arabinoside, a selective antiviral drug, from uridine arabinoside and adenine.⁹⁾

In this article, we report the synthesis of

2'-deoxyribosylzeatin (2'-dRZ), a novel purine nucleoside analogue, from 2'-deoxyribose-1-phosphate and zeatin, a plant hormone, by using NPase as shown in Fig. 1. We also show that 2'-dRZ inhibited the DNA-polymerizing reaction catalyzed by the DNA polymerase I of *E. coli*. Zeatin is one of a natural cytokinin and the molecular structure is analogous to adenine. The compound is found in a wide variety of plant cells. Ribosylzeatin is one of the intermediates in the synthetic route of zeatin from adenosine in plant cells, but the occurrence of 2'-dRZ has not been reported so far.

The synthesis of 2'-dRZ was done in a mixture (100 μ l) containing 46 mM zeatin (Sigma Chemical Co., Ltd., St. Louis, MO), 10 mM 2'-deoxyribose-1-phosphate, 100 mM potassium phosphate buffer (pH 8.0), and 8 units/ml NPase at 37°C for 12 hr. After the reaction, the mixture was chromatographed on paper [solvent system, isobutyric acid–0.5 M ammonia = 5:3, v/v] to separate 2'-dRZ and the spots were detected under UV illumination (254 nm). The spot corresponding to 2'-dRZ was cut off and extracted with 1.5 ml of sterilized water at 25°C for 16 hr. The aqueous phase was filtered on a Millipore filter (0.45 μ m) and the concentration of 2'-dRZ was calculated using the molar extinction coefficient of zeatin (16.2 $\text{mM}^{-1} \text{cm}^{-1}$) at 270 nm. Identification of

Fig. 1. Enzymatic Production of 2'-Deoxyribosylzeatin by Nucleoside Phosphorylase of *Klebsiella* sp. LF 1202.

NPase, nucleoside phosphorylase.

Abbreviations: 2'-dRZ, 2'-deoxyribosylzeatin; PNPase, purine nucleoside phosphorylase; NPase, nucleoside phosphorylase of *Klebsiella* sp. LF 1202; PyNPase, pyrimidine nucleoside phosphorylase; ssDNA, single stranded DNA; Klenow fragment, large fragment of DNA polymerase I of *Escherichia coli*; dNTPs, deoxynucleotide triphosphate mixture (dATP, dGTP, dCTP, dTTP); EDTA, ethylenediaminetetraacetic acid.

Fig. 2. Denaturing Polyacrylamide Gel Electrophoresis.

Detailed conditions for the synthesis of M13mp19 DNA by the Klenow fragment was described in the text, except for the chemicals added in the reaction mixture: lane 1, without chemical; lane 2, 0.5 mM 2'-deoxyribose-1-phosphate; lane 3, 0.5 mM zeatin and lane 4, 0.5 mM 2'-deoxyribosylzeatin. The band of synthesized M13 is indicated by an arrow.

the molecular structure of 2'-dRZ will be reported elsewhere.

2'-dRZ as obtained above was then used as the assay for *in vitro* DNA synthesis with the Klenow fragment.^{11,12} The reaction of *in vitro* DNA synthesis was done in a mixture (15 μ l) containing 1 μ g M13mp19 ssDNA, 0.5 pmol primer DNA, 2 units Klenow fragment (Takara Shuzo, Co., Ltd., Kyoto, Japan), 0.2 mM dNTPs, 10 μ Ci [α -³²P]dCTP, 7 mM Tris-HCl buffer (pH 7.5), 0.1 mM EDTA, 20 mM NaCl, 7 mM MgCl₂, and 0.5 mM 2'-dRZ at 37 °C for 1 hr. The reaction mixture was analyzed by a denaturing polyacrylamide gel (4%) containing 42% urea.¹³ After the electrophoresis, DNA bands were seen by autoradiography.

As shown in Fig. 2, polymerization of DNA was not inhibited in the reaction mixture with 2'-deoxyribose-1-phosphate (lane 2), zeatin (lane 3), or without chemicals (lane 1), and

the complete size of M13 was synthesized. However, the reaction of DNA polymerization was inhibited by the addition of 2'-dRZ in the reaction mixture, and then truncated nucleotide chains were synthesized (lane 4). Therefore, we thought that 2'-dRZ would be a novel inhibitor for DNA polymerase I of *Escherichia coli*. The putative modes of actions of anti-DNA-viral nucleoside analogues are classified in 3 groups, *i.e.*, (i) competitive inhibition of DNA polymerase with deoxynucleotide triphosphates, (ii) inhibition of DNA polymerization as chain-terminator, and (iii) introduction of these drugs into DNA to reduce the activity of newly synthesized DNA as a template. In these cases, nucleoside analogues inhibit the DNA synthesis only after phosphorylation by some nucleoside kinases of host or virus, however, 2'-dRZ was not phosphorylated. Thus, 2'-dRZ may directly interact with DNA polymerase I to reduce its activity or inactivate the template DNA through some non-enzymatic modifications. We are now trying to identify the mode of action of 2'-dRZ.

References

- 1) G. Butke, K. Ouiggi and S. Chladek, *J. Carbohydr. Nucleosides Nucleotides*, **7**, 63 (1980).
- 2) W. H. Prusoff and D. C. Ward, *Biochem. Pharmacol.*, **25**, 1233 (1976).
- 3) T. Utagawa, H. Morisawa, F. Yoshinaga, A. Yamazaki, K. Mitsugi and Y. Hirose, *Agric. Biol. Chem.*, **49**, 1053 (1985).
- 4) H. Shirae, K. Kobayashi, H. Shiragami, Y. Irie, N. Yasuda and K. Yokozeki, *Appl. Environ. Microbiol.*, **55**, 419 (1989).
- 5) J. Neuhaard and P. Nygaard, in "Escherichia coli and Salmonella typhimurium. Cellular and Molecular Biology," ed. by F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter and H. E. Umbarger, American Society for Microbiology, Washington D.C., 1987, pp. 445-473.
- 6) K. F. Jansen and P. Nygaard, *Eur. J. Biochem.*, **51**, 253 (1975).
- 7) N. Hori, M. Watanabe, Y. Yamazaki and Y. Mikami, *Agric. Biol. Chem.*, **53**, 2205 (1989).
- 8) N. Hori, M. Watanabe, Y. Yamazaki and Y. Mikami, *Agric. Biol. Chem.*, **53**, 3219 (1989).
- 9) F. Ling, Y. Inoue and A. Kimura, *Appl. Environ. Microbiol.*, **56**, 3830 (1990).
- 10) F. Ling, Y. Inoue and A. Kimura, *Agric. Biol. Chem.*,

- 55**, 573 (1991).
- 11) H. Jacobsen, H. Klenow and K. Overgaard-Hansen, *J. Biol. Chem.*, **257**, 1958 (1982).
Eur. J. Biochem., **45**, 623 (1974).
- 12) C. M. Joyce, W. S. Kelley and N. D. F. Grindley, 13) B. Perbal, "A Practical Guide to Molecular Cloning,"
John Wiley and Sons, New York, 1988, pp. 612–633.
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