Note

Mechanism of 2',3'-Dideoxyadenosine Synthesis by *Escherichia coli* AJ 2595

Hideyuki SHIRAE* and Kenzo YOKOZEKI

Central Research Laboratories of Ajinomoto Co., Inc.,
1-1 Suzuki-cho, Kawasaki-ku, Kawasaki 210, Japan

Received August 17, 1990

Many investigations on nucleoside *N*-transfer reactions have appeared, since MacNutt first demonstrated that a trans-*N*-glycosidase from *Lactobacillus helveticus* could catalyze the direct reaction transferring the deoxyribosyl group in purine or pyrimidine deoxyribonucleosides to other nucleic bases.¹⁾ Sakai *et al.* studied the transfer reaction of ribosyl groups in pyrimidine nucleosides to purine bases and showed that the reaction was conducted by the combination of uridine phosphorylase (UPase) and purine nucleoside phosphorylase (PNPase), and the intermediate in the reaction was ribose-1-phosphate.²⁻⁴⁾ Utagawa *et al.* reported a new method for the synthesis of adenine arabinoside from uracil arabinoside and adenine by a transarabinylation reaction⁵⁻⁸⁾ and showed that the reaction was catalyzed by the combination of UPase and PNPase in *Enterobacter*

aerogenes AJ 11125, and the intermediate in the reaction was arabinose-1-phosphate.⁹⁾

We have reported that 2',3'-dideoxyadenosine (DDA) and 2',3'-dideoxyinosine (DDI), promising anti-AIDS agents, could be synthesized from 2',3'-dideoxyuridine (DDU) and the corresponding bases with the resting cells of *Escherichia coli* AJ 2595.¹⁰⁾ In this reaction, inorganic phosphate was essential. This shows that the DDA (or DDI)-forming reaction seems to consist of two successive reactions by UPase and PNPase in *E. coli* AJ 2595.

This paper describes the mechanism of the DDA (or DDI)-forming reaction in *E. coli* AJ 2595. The organism used in this study is *E. coli* AJ 2595, which was selected from stock cultures of our laboratory as the best DDA producer. Cultivation and analytical methods were the same as described in our previous paper.¹⁰⁾ The cultivated cells were rinsed with 50 mM potassium phosphate buffer (pH 7.5), followed by centrifugation at 12,000 × *g* for 20 min at 4°C. The harvested cells were suspended in 200 ml of 50 mM potassium phosphate buffer (pH 7.5) containing 1 mM dithiothreitol (A-buffer) and then disrupted with an ultrasonic oscillator (12 Hz) at 5°C to 15°C. Cell debris was removed by centrifugation at 12,000 × *g* for 20 min. The supernatant thus obtained (225 ml) was fractionated with solid ammonium sulfate (35% saturation) by adjusting pH at 7.5 with 1 N NaOH. After the solution was stirred for 1 hr, the precipitate was removed by centrifugation at 12,000 × *g*, and the supernatant was further fractionated by adding solid ammonium sulfate to 60% saturation and stirred gently overnight. The suspension was centrifuged at 12,000 × *g*, and the precipitate was dissolved in A-buffer and then dialyzed

Table I. PARTIAL PURIFICATIONS OF DDU-PHOSPHORYLYZING ENZYME
AND DDA-PHOSPHORYLYZING ENZYME OF *E. coli* AJ 2595

Enzyme activity was assayed in a reaction mixture (1 ml) containing 20 μmol DDU or DDA, 50 μmol potassium phosphate buffer (pH 7.0), and an appropriate amount of each enzyme. The reaction was done at 40°C for 2 min and then stopped by adding 4 ml of 0.1 N HCl. The amount of uracil or adenine formed in the reaction mixture was measured by high performance liquid chromatography.¹⁰⁾ One unit of each enzyme activity was defined as the amount of enzyme that produced 1 μmol of uracil from DDU or adenine per a minute from DDA under the above conditions. The specific activity is expressed as units/mg protein. Protein was determined by the Bradford method.¹¹⁾

Purification	DDU-Phosphorylizing enzyme				DDA-Phosphorylizing enzyme			
	Protein (mg)	T.A. ^a (units)	S.A. ^b (units/mg)	Fold	Protein (mg)	T.A. ^a (units)	S.A. ^b (units/mg)	Fold
Cell-free extract	7650	641	0.084	1	7650	126	0.016	1
(NH ₄) ₂ SO ₄ (35–60% saturation)	2338	448	0.192	2.3	2338	124	0.053	3.3
DEAE-Toyopearl	455	345	0.757	9.0	328	104	0.317	19.8

^a T.A, total activity.^b S.A, specific activity.

* Corresponding author.

Fig. 1. Partial Purification of DDU-Phosphorolyzing Enzyme and DDA-Phosphorolyzing Enzyme in *E. coli* AJ 2595 by DEAE-Toyopearl PAK 650S Chromatography.

Protein (2338 mg), containing 448 units DDU-phosphorolyzing enzyme and 124 units DDA-phosphorolyzing enzyme was put on a column (ϕ 2.2 $\times$ 20 cm), equilibrated with 50 mM potassium phosphate buffer (pH 7.5) containing 1 mM dithiothreitol. Six and a half-ml fractions were collected at a flow-rate of 2 ml/min. (A) DDU-phosphorolyzing activity ($\circ$ --- $\circ$), and (B) DDA-phosphorolyzing activity ($\square$ --- $\square$). Absorbance (280 nm) and NaCl gradient were shown by solid curved line and straight solid line, respectively.

Table II. DDA-PRODUCTION BY THE COMBINATION OF DDU-PHOSPHOROLYZING FRACTION AND DDA-PHOSPHOROLYZING FRACTION

The two fractions A and B (see text) obtained from DEAE-Toyopearl PAK 650S column chromatography were used as the enzyme source. The reaction mixture (1 ml), containing 100 μ mol of DDU, 100 μ mol of adenine, 10 μ mol of potassium phosphate buffer (pH 7.5), and each enzyme solution (0.040 units of DDU-phosphorolyzing enzyme or 0.014 units of DDA-phosphorolyzing enzyme), was incubated at 50°C for 3 hr. Substrates and products were measured as described in our previous paper.

Fraction	Amount of base or nucleoside (mM)			
	Uracil	Adenine	DDU	DDA
A	13	100	87	0
B	0	100	100	0
A + B	14	94	86	6

against the same buffer. The dialyzate was put on a column of DEAE-Toyopearl PAK 650S (ϕ 2.2 $\times$ 20 cm; Tosoh Co., Tokyo), equilibrated with A-buffer. After it was washed with the buffer, the enzyme was eluted with a linear gradient of NaCl from 0 to 0.3 M in A-buffer (Fig. 1). The DDU-phosphorolyzing fractions and the DDA-phosphorolyzing fractions were mixed, respectively, and then

ammonium sulfate was added to each mixed fraction to bring each solution to 60% with stirring. After 2 hr, each suspension was centrifuged at 12,000 $\times g$ for 20 min at 4°C, each precipitate obtained was dissolved in a small volume of A-buffer, and then it was dialyzed against the same buffer. The degree of purifications for DDU-phosphorolyzing enzyme or DDA-phosphorolyzing enzyme was approximately 9-fold and 20-fold, respectively (Table I).

Table II shows the result of DDA formation from DDU and adenine by the partially purified enzyme fractions described above. Fraction A with DDU-phosphorolyzing activity could, of course, phosphorolyze DDU to uracil, but could not form DDA from DDU and adenine. Fraction B with DDA-phosphorolyzing activity could not phosphorolyze DDU to uracil. In contrast, DDU was phosphorolyzed to uracil and DDA was formed from DDU and adenine by the enzyme solution mixed with fractions A and B. This result indicates that DDA formation by *E. coli* AJ 2595 was catalyzed by the combination of two enzymes with DDU-phosphorolyzing activity and DDA-phosphorolyzing activity. This mechanism of DDA production was the same as those described by Sakai *et al.*,⁴⁾ Utagawa *et al.*,⁹⁾ and by us.¹²⁾ The optimal conditions were identified for DDA production by the enzyme solution mixed with fractions A and B as follows: optimal pH, 7.0; optimal temperature, 45°C; optimal phosphate concentration, 10 mM; and optimal proportion of fraction A and B, A:B=4:1. It is noteworthy that the optimal temperature of 45°C is lower than that for other nucleoside-base transfer reaction (above 60°C) previously reported.^{5,6,13-17)}

Acknowledgment. We are indebted to Drs. K. Kubota and I. Maeda, of our laboratories, for their encouragement during the course of this work.

References

- 1) W. S. MacNutt, *Biochem. J.*, **50**, 384 (1952).
- 2) T. Sakai, T. Tochikura and K. Ogata, *Agric. Biol. Chem.*, **29**, 742 (1965).
- 3) T. Sakai, T. Tochikura and K. Ogata, *Agric. Biol. Chem.*, **29**, 737 (1965).
- 4) T. Sakai, T. Tochikura and K. Ogata, *Agric. Biol. Chem.*, **30**, 245 (1966).
- 5) T. Utagawa, H. Morisawa, T. Miyoshi, F. Yoshinaga, A. Yamazaki, K. Mitsugi and Y. Hirose, *FEBS Lett.*, **109**, 261 (1980).
- 6) T. Utagawa, H. Morisawa, F. Yoshinaga, A. Yamazaki, K. Mitsugi and Y. Hirose, *Agric. Biol. Chem.*, **49**, 1053 (1985).
- 7) H. Morisawa, T. Utagawa, T. Miyoshi, F. Yoshinaga, A. Yamazaki and K. Mitsugi, *Tetrahedron Lett.*, **21**, 479 (1980).
- 8) T. Utagawa, H. Morisawa, A. Yamazaki, S. Yamanaka, F. Yoshinaga and Y. Hirose, *Agric. Biol. Chem.*, **49**, 2167 (1985).

- 9) T. Utagawa, H. Morisawa, S. Yamanaka, A. Yamazaki, F. Yoshinaga and Y. Hirose, *Agric. Biol. Chem.*, **49**, 2425 (1985).
 - 10) H. Shirae, K. Kobayashi, H. Shiragami, Y. Irie, N. Yasuda and K. Yokozeki, *Appl. Environment. Microbiol.*, **55**, 419 (1989).
 - 11) M. Bradford, *Anal. Biochem.*, **72**, 248 (1976).
 - 12) H. Shirae, K. Yokozeki and K. Kubota, *Agric. Biol. Chem.*, **52**, 1233 (1988).
 - 13) T. Utagawa, H. Morisawa, T. Nakamatsu, A. Yamanaka and S. Yamanaka, *FEBS Lett.*, **119**, 101 (1980).
 - 14) T. Utagawa, H. Morisawa, S. Yamanaka, A. Yamazaki, and Y. Hirose, *Agric. Biol. Chem.*, **50**, 121 (1986).
 - 15) H. Shirae, K. Yokozeki and K. Kubota, *Agric. Biol. Chem.*, **52**, 1499 (1988).
 - 16) H. Shirae, M. Uchiyama, K. Yokozaki and K. Kubota, *Agric. Biol. Chem.*, **52**, 1777 (1988).
 - 17) M. Ishii, H. Shirae and K. Yokozeki, *Agric. Biol. Chem.*, **53**, 3209 (1989).
-