ENZYMATIC SYNTHESIS OF PURINE 2'-AMINO-2'-DEOXYRIBOSIDE

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1. Introduction

An antibiotic, 2'-aminonucleoside, was first found in the culture broth of *Aerobacter* as 2'-amino-2'-deoxyguanosine which had an antibacterial activity against *Esherichia coli*, and antitumor activity against Sarcoma 180 in vivo [1].

Recently 2'-amino-2'-deoxyadenosine was isolated from the culture broth of *Actinomycetes* which suppressed the growth of SV-40 transformed cell [2], or mycoplasma [3].

We have reported the enzymatic synthesis of purine arabinoside as ara-A which is a potent antiviral substance [4,5]. The scheme for ara-A preparation was as in fig.1A. In this scheme cyclouridine (Cyclo-U), an intermediate for the synthesis of uracil arabinoside (Ara-U), was easily converted to 2'-amino-2'-deoxyuridine (2AU) [6]. Here, we describe a microbial or enzymatic synthesis of 2'-amino-2'-deoxyinosine (2AI) [10] from 2'-amino-2'-deoxyuridine and hypoxanthine by a transaminoribosylation as shown in fig.1B.

2. Materials and methods

2AI-forming microorganisms were selected from stock cultures of our laboratory. The cells of *Erwinia herbicola* AJ 2803 were used as a likely source of enzyme for transaminoribosylation. They were grown in a medium containing 1% polypepton, 1% meat extract, 0.5% yeast extract and 0.5% NaCl in tap water, and the medium adjusted to pH 7.0 by adding 1 N KOH. The microbial strain was cultured aerobically at 30°C for 36 h with shaking in a 500 ml Erlenmeyer flask containing 100 ml medium. 2AU was easily prepared by the reaction of uridine with ethylene carbonate followed by azide cleavage and subsequent reduction [6]. All other chemicals were commercial products. Amounts of 2AI was determined using high pressure liquid chromatography as in [4], with KH_2PO_4 (0.01 M) as solvent.

3. Result

3.1. *Distribution of 2AI production in microorganisms*

A total of 365 strains from stock culture were tested for their 2AI producing abilities from 2AU and hypoxanthine. 2AI-producing activity was widely distributed in various genera of bacteria, and the amounts of 2AI produced by bacteria active in this synthesis are shown in table 1. The genera *Arthrobacter, Enterobacter, Erwinia, Salmonella, Flavobacterium* and *Esherichia,* contained large amounts of activity; *Erwinia herbicola* AJ 2803 was selected as the best producer, giving the highest activity of transaminoribosylation.

3.2. *Optimum temperature*

The optimum temperature for 2AI formation by *Erwinia hercibola* AJ 2803 was studied. As shown in fig.1, 2AI was produced in high yields at 50–65°C with an optimum around 60°C. Thus the enzyme is stable at relatively high temperature.

3.3. *Effect of phosphate*

The effect of phosphate was investigated by using various potassium phosphate buffer (pH 7.0) concentrations. No 2AI was formed without phosphate buffer (fig.2). The optimum potassium phosphate buffer level was ~40 mM. This indicates that the reaction might be catalyzed by nucleoside phosphorylase.

Table 1
Distribution of 2AI production in various microorganisms

Strain	2AI formed (mg/l)
Micrococcus luteus AJ 1055	261.0
Micrococcus glutamicus AJ 1519	60.0
Staphyrococcus epidermidis AJ 1184	102.0
Sarcina variabilis AJ 1214	85.5
Bacillus subtilis AJ 3289	185.0
Bacillus sphaericus AJ 1309	261.0
Arthrobacter simplex AJ 3223	928.0
Pseudomonas diminuta AJ 2067	254.0
Enterobacter aerogenes AJ 11125	1290.0
Erwinia herbicola AJ 2803	1450.0
Erwinia carotovora AJ 2591	551.0
Xanthomonas campestris AJ 2797	724.0
Salmonella schottmuelleri AJ 2930	1260.0
Brevibacterium lactfermentum AJ 1511	30.0
Bacterium cadaveris AJ 3264	928.0
Flavobacterium rhenanum AJ 2468	1175.0
Esherichia coli AJ 2599	1218.0
Serratia marcescens AJ 2599	957.0
Citrobacter freundii AJ 2619	435.0

The incubation mixture containing 5% wet cells, 30 mM 2AU, 10 mM hypoxanthine and 30 mM potassium phosphate buffer (pH 7.0) was incubated at 63°C for 20 h with gentle agitation. The reaction was terminated by heating at 100°C for 5 min. 2AI formed was determined by high pressure liquid chromatography in [4].

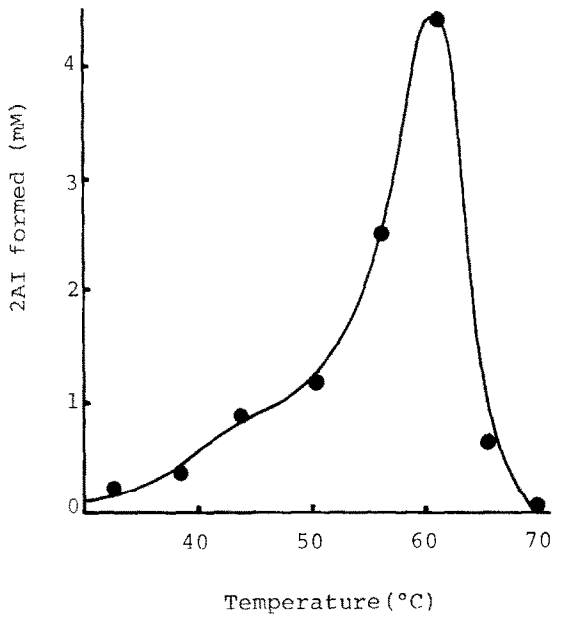

Fig.2. Effect of temperature on 2AI formation. *Erwinia herbicola* AJ 2803 was cultivated as in the text. Each incubation mixture containing 5% intact cells as wet paste, 30 mM 2AU, 10 mM hypoxanthine and 30 mM potassium phosphate buffer (pH 7.0) was incubated at various temperatures for 24 h. The reaction was terminated by heating at 100°C for 5 min.

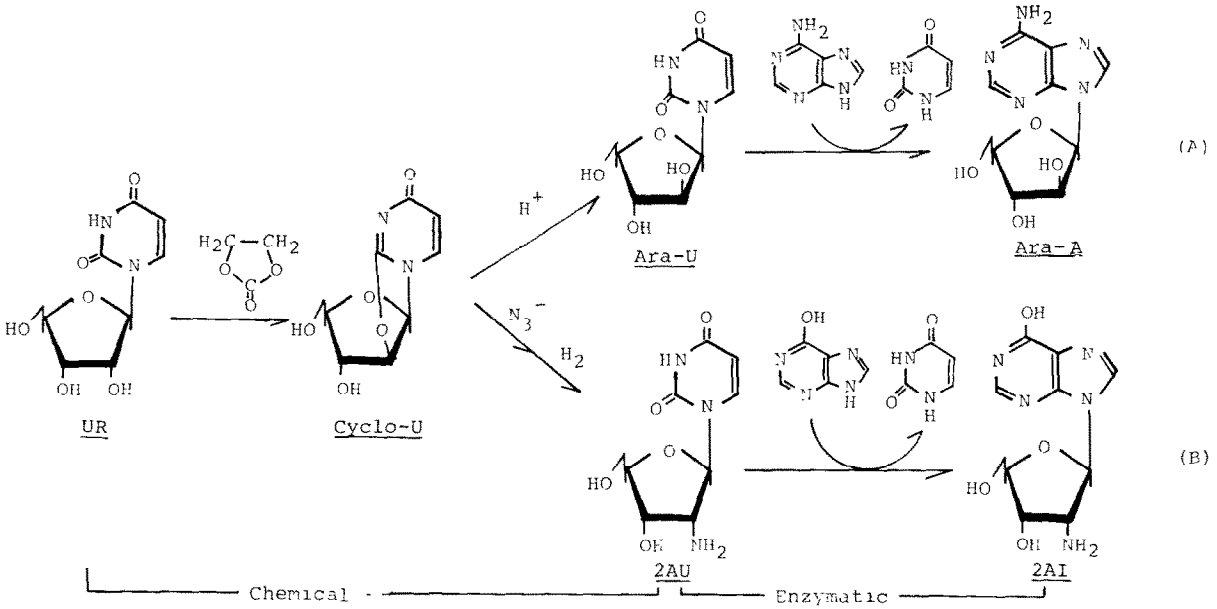

Fig.1. Combination of chemical reaction with biological reaction for the preparation of new kinds of purine nucleosides.

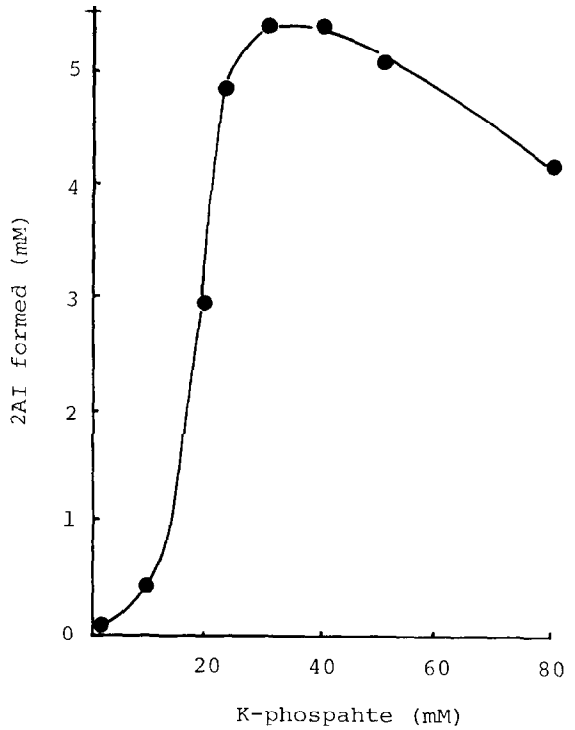

Fig.3. Effect of phosphate on 2AI formation. Each incubation mixture containing 5% intact cells of *Erwinia hervicola* AJ 2803 as wet paste, 30 mM 2AU, 10 mM hypoxanthine, 30 mM Tris–malate buffer (pH 7.0) and various concentration of potassium phosphate buffer (pH 7.0) was incubated at 63°C for 24 h. The reaction was terminated by heating at 100°C for 5 min.

3.4. *Isolation and identification of the product*

The incubation mixture containing intact cells of *Erwinia hebicola* AJ 2803 (50 g wet paste), 2AU (7.5 g), hypoxanthine (1.3 g) and KH_2PO_4 (4.0 g) in 1 liter total vol. (pH 7.0 adjusted by KOH) was incubated at 63°C for 20 h with gentle agitation. Under these conditions, ~50% of hypoxanthine on a molar basis was transformed into 2AI. The incubation mixture was boiled for 5 min to terminate the reaction, then centrifuged to remove the bacterial cells. Chromatographic separation of the products was carried out on a column of Dowex 1 × 4 (OH form, 4 × 20 cm), which was eluted by 0.05 N NH_4-formate buffer (pH 9.0). The fraction (~500 ml) containing the product was concentrated to 50 ml in vacuo and left overnight at 4°C. The precipitate was collected by filtration and recrystallized from water to give white needles (880 mg). This compound was identified as 2AI by physical determinations: melting point 205°C, $UV_{max}^{pH\ 7.0}$ 248 nm (ϵ = 13 200); NMR data (in D_2O, pD 7.9), 8.37(s, 1H,H-8), 8.18(s, 1H-H-2), 5.99(d, 1H, $J_{H1',H2'}$ = 8.4 Hz), 4.07(q, 1H,H-2', $J_{H2',H3'}$ = 5.4 Hz); elementary analysis for $C_{10}H_{13}N_5O_4$ calc., C, 44.94; H, 4.90; N, 26.21; found, C, 44.64; H, 5.01; N, 26.15.

4. Discussion

A large number of nucleosidic antibiotics have been found in Nature, some of which contain an amino group in the sugar moiety. 3'-Amino-3'deoxyadenosine has been reported to inhibit the synthesis of RNA and shows antitumor activity [7,8]. Puromycin, an inhibitor of protein synthesis, also has an amino group at the 3'-position of the sugar [9], and has been used as a biochemical reagent.

2'-Aminonucleosides were found in the culture broth of *Aerobacter* and *Actinomycetes*. These compounds were identified as 2'-amino-2'-deoxyguanosine [1] and 2'-amino-2'-deoxyadenosine [2,3]; both showed antitumor activity, but narrow antibacterial activity. For the synthesis of these compounds, chemical methods have been reported [10–12], but these methods require many steps and produce some by-products such as α-anomer or 7-isomer. As shown in table 1, many bacteria can synthesize 2 AI from 2AU and hypoxanthine by transaminoribosylation. In this case 9-(2-amino-2-deoxy-β-D-ribofuranosyl)hypoxanthine was formed in high yield. This biological transaminoribosylation reaction was catalyzed at high temperature (60°C), when most other enzymes such as nucleosidases which decompose the substrate or product are inactivated. Thus intact cells or crude enzymes can be employed as catalyst for the transaminoribosylation reaction. As shown in fig.2, this enzymatic reaction was not catalyzed in the absence of phosphate. This suggests that nucleoside phosphorylase catalyzes the reaction. As for transarabinosylation reaction, *Enterobacter aerogenes* AJ 11125 showed the highest activity in ara-A production [4], but in the case of transaminoribosylation, *Erwinia herbicola* AJ 2803 was more active than *Enterobacter aerogenes*. This also suggests that there are different kinds of nucleoside phosphorylases with different substrate specificities. We are now purifying the enzyme from *Enterobacter aerogenes* to study the substrate specificity.

This enzymatic method was extended successfully to the synthesis of other 2′-amino-2′deoxynucleosides [13]. One can expect to obtain a new type of 2′-aminonucleoside with biological activity by this chemical modification.

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