A NOVEL AND SIMPLE METHOD FOR THE PREPARATION OF ADENINE ARABINOSIDE BY BACTERIAL TRANSGLYCOSYLATION REACTION

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1. Introduction

The antibiotic, ara-A (adenine arabinoside, 9-β-D-arabinofuranosyladenine), was originally synthesized as a potential anticancer agent [1] and also found to possess significant activities against DNA viruses including cytomegalovirus in vitro, vaccinia virus in vivo and Epstein-Barr virus in vitro [2–5]. Ara-A is clinically effective against various manifestations of herpes simplex and herpes zoster virus infection in humans as shown [6,7]. Thus, the development of a new method for the preparation of ara-A is of considerable interest. Fermentation [8] and chemical synthesis [9–11] methods have been used for the preparation of ara-A. These methods were low yielding, laborious and time-consuming. Here, we describe a simple method for the production of ara-A by a bacterial or enzymatic arabinose-transfer reaction (transarabinosylation, fig.1).

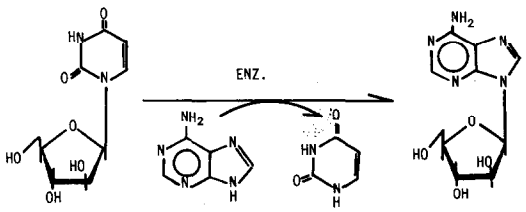

Fig.1. Transarabinosylation.

2. Materials and methods

Ara-A forming microorganisms were selected from stock cultures of our laboratory. The cells of *Enterobacter aerogenes* AJ 11125 were used as a likely source of enzyme for transarabinosylation; 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. Ara-U was easily prepared by reaction of uridine with ethylene carbonate followed by acidic treatment as in [12,13]. All other chemicals were commercial products. Ara-A was determined using high pressure liquid chromatography: resin, Zipax SCX; column size, 2.1 × 500 mm; solvent, 0.05 N HNO_3; pressure, 50 kg/cm^2; flowrate, 1.5 ml/min; detecter, ultraviolet monitor (260 nm).

3. Results

3.1. *Distribution of ara-A producer in microorganisms*

Ara-A-producing activity was widely distributed in various strains of bacteria, especially in the genera *Enterobacter, Erwinia* and *Escherichia* (table 1) where *Enterobacter aerogenes* AJ 11125 was selected as the best producer which gave the highest activity in its transarabinosylation reaction. Intact cells (50 mg as wet paste) of this organism produced 2 mg/ml of ara-A in the standard incubation mixture.

3.2. *Optimum pH*

The relative activity for ara-A production by *Enterobacter aerogenes* AJ 11125 at various pH values was determined by adjusting the incubation mixture pH with 1 N KOH or HCl. The optimum pH was ~7.0 at 60°C as shown in fig.2.

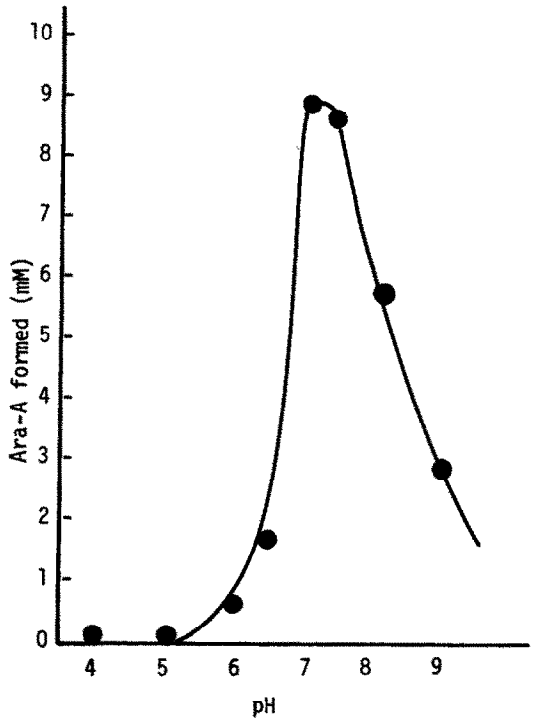

Fig.2. Effect of pH on ara-A formation. *Enterobacter aerogenes* AJ 11125 was cultured as in the text. Each incubation mixture containing 5% intact cells as wet paste, 30 mM ara-U, 10 mM adenine and 25 mM potassium phosphate and adjusted to various pH values with 1 N KOH or HCl, was incubated for 5 h at 60°C.

Table 1
Distribution of ara-A producer in microorganisms

Genera	No. strain tested	No. strain forming ara-A
Pseudomonas	40	1
Xanthomonas	6	0
Agrobacterium	6	0
Alcaligenes	6	0
Escherichia	33	31
Citrobacter	2	2
Salmonells	3	3
Kluyvera	5	5
Enterobacter	13	10
Serratia	4	3
Proteus	11	10
Erwinia	7	6
Aeromonas	4	3
Flavobacterium	31	4
Achromobacter	4	1
Bacterium	2	2
Micrococcus	8	0
Staphylococcus	2	0
Microbacterium	3	0
Planoccoccus	6	0
Sarcina	6	0
Bacillus	13	0
Sporoscarcina	1	0
Corynebacterium	7	0
Arthrobacter	3	0
Brevibacterium	17	0

The incubation mixture containing 5% wet cells, 0.5% ara-U, 0.2% adenine and 0.5% KH_2PO_4 was adjusted to pH 7 with 1 N KOH and was incubated at 60°C for 15 h with gentle agitation. The reaction was terminated by heating at 100°C for 5 min. Bacteria which formed >10 mg/l of ara-A were judged as positive strain

3.3. *Optimum temperature.*

As shown in fig.3, the optimum temperature for ara-A formation by *Entrobacter aerogenes* AJ 11125 was 60–65°C, at pH 7.0. If the reaction was at <50°C, adenine was deaminated to hypoxanthine and no ara-A was formed at ~37°C.

3.4. *Isolation and identification of the product.*

Intact cells (30 g as wet paste) of *Enterobacter aerogenes* AJ 11125 were suspended in the incubation mixture containing ara-U (7.3 g), adenine (1.3 g) and KH_2PO_4 (3.4 g) in 1 l total vol. (pH 7.0) and incubated at 60°C for 15 h with gentle agitation. Under these conditions, 92% of the adenine on a molar basis was transformed into ara-A (2.4 g). The incubation mixture was boiled for 5 min to terminate the reaction, then centrifuged to remove the bacterial cells. The supernatant was kept for several hours at 4°C, and the precipitant was collected by filtration and recrystallized from water to give white needles (2.0 g).

This compound was proved to be identical with an authentic ara-A sample in all physical properties: melting point 256°C (dec.); $[\alpha]_D^{40}$ −5.2° (0.25% in water); $UV_{max}^{pH\ 7}$ 259 nm (ε 14 500), $_{max}^{pH\ 11}$ 259 nm (ε 14 300); NMR data (DMSO-d_6), 8.19 ppm (1 H, s, H-8), 8.25 ppm (1 H, s, H-2), 7.35 ppm (2 H, s, NH_2), 6.33 ppm (1 H, d, H-1′, $J_{1'2'}$, 4.5 Hz): Elemental analysis for $C_{10}H_{13}N_5O_4$ calc. C, 44.94; H, 4.90; N, 26.21; found. C, 44.60; H, 5.01, N, 26.15.

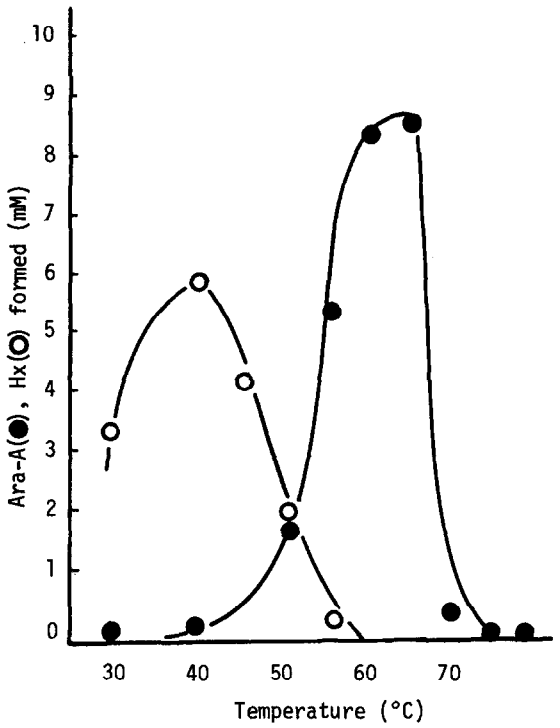

Fig.3. Effect of temperature on ara-A formation. *Enterobacter aerogenes* AJ 11125 was cultured as in the text. Each incubation mixture containing 5% intact cells as wet paste, 30 mM ara-U, 10 mM adenine and 25 mM potassium phosphate buffer (pH 7.0) was incubated at various temperatures for 5 h. Hx, hypoxanthine.

4. Discussion

Arabinose-transfer activity from ara-U to adenine is distributed in many microorganisms. In particular, *Enterobacter aerogenes* AJ 11125 was found to have the highest activity.

A chemical transribosylation reaction from pyrimidine to purine has been reported by some investigators [14,15]. The synthetic method for purine nucleosides by chemical transglycosylation, however, has inevitable defects such as the formation of α or β anomers or 7 and 9 isomers in some cases. On the contrary, the microbial or enzymatic transarabinosylation gives stereospecifically the 9-β-D-arabinofuranosyl derivative alone.

Of special interest is the optimal temperature of the catalytic reaction. At ~37°C, hypoxanthine was formed without any ara-A formation. This suggested that the adenine-deaminase activity in bacterial cells was high at 37°C. On the other hand, the deaminase activity was completely depressed at ~60–65°C, and ara-A production took place favourably.

In addition, it must be pointed out that arabinofuranose-1-phosphate isolated from the incubation mixture may be an intermediate in the formation of ara-A because no ara-A was produced in the absence of P_i (unpublished). From these results, it seems that the transarabinosyl reaction may be catalyzed by a nucleoside phosphorylase.

The present method, using a combination of chemical and enzymatic reactions, offers the most preferable route for the preparation of ara-A because of its stereospecificity and simplicity.

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