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# Synthesis of 9-β-D-arabinofuranosylguanine by combined use of two whole cell biocatalysts

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ABSTRACT

Unlike the preparation of other purine nucleosides, transglycosylation from a pyrimidine nucleoside and guanine is difficult because of the low solubility of this base. Thus, another strategy, based on the coupled action of two whole cell biocatalyzed reactions, transglycosylation and deamination, was used. *Enterobacter gergoviae* and *Arthrobacter oxydans* were employed to synthesize 9-β-D-arabinofuranosylguanine (AraG), an efficient anti leukemic drug.



Guanosine analogues are frequently used as antiviral and anticancer agents and as building blocks for oligonucleotide therapeutics.[1] AraG as well as their prodrugs, the 6-modified analogues, are powerful anti leukemic agents.[2]

Chemical syntheses of guanine nucleosides have been achieved stereoselectively, but using expensive and polluting reagents and several steps including protection and deprotection procedures.[3] Alternatively, the synthesis of purine nucleosides by enzymatic transglycosylation between a pyrimidine nucleoside and a purine base, catalyzed by nucleoside phosphorylases or microorganisms that contain them, has attracted considerable attention[4,5] since the reaction is regio- and stereoselective and affords reduced by-products content.

Unlike other purine bases, the poor solubility of guanine is a drawback to carry out microbial transglycosylations in the pH range in which the enzymes are active. Some strategies were reported to overcome this disadvantage including the use of more soluble guanine derivatives like glyoxal-guanine[6] and guanosine[7] or immobilized biocatalysts stable in high pH media where guanine is more soluble.[8,9]

A good alternative for the synthesis of guanine nucleosides consists in the combination of transglycosylation and deamination reactions. Adenosine deaminase (ADA) catalyzes the hydrolysis of adenosine to inosine and ammonia. It is commercially available from mammalian sources (calf spleen or calf intestine) but, even though the use of easily over-expressed bacterial enzymes is considered more attractive,[10] this approach has not been extensively studied. Several purine nucleoside analogues are substrates of ADA with different degrees of efficacy.[11] Thus, employing soluble 2-amino-6-substituted purine bases and 2′-deoxyuridine (dU) or thymidine, the corresponding 2′-deoxynucleosides were prepared by transglycosylation using bacterial whole cells or isolated enzymes. Afterwards, the sequential hydrolytic action of ADA afforded 2′-deoxyguanosine (dG).[10,12,13]

One of our first attempts to obtain guanosine derivatives, based on a previously reported strategy[7] that employed guanosine as a more soluble base source, consisted in the use of *Escherichia coli* BL21, an efficient whole cell biocatalyst widely studied in our group[14] to perform microbial transglycosylation reactions. Employing dU as sugar donor, dG was obtained in 85% yield after 3.5 h reaction. The same strategy was applied to the preparation of 9-β-D-arabinofuranosylguanine (AraG) starting from arabinofuranosyluracil (AraU) and guanosine. However, the yield was poor probably as consequence of the lower hydrolysis rate of AraU compared to that of guanosine and the consumption of the resulting guanine by other metabolic pathways. To avoid this problem, pulses of guanosine were added every 4 h. but only 17% of AraG (2.5 mM) was achieved after 12 h.

Recently, the use of whole cells of *Arthrobacter oxydans* as ADA source has been reported by our group.[15] This bacterium showed high ADA activity and was utilized as biocatalyst for the preparation of guanosine and dG from the corresponding 2,6-diaminopurine analogues. However, the reaction time must be rigorously controlled since the products are substrates of endogenous PNP. In particular, purine arabinosides are poor substrates of the *A. oxydans* PNP;

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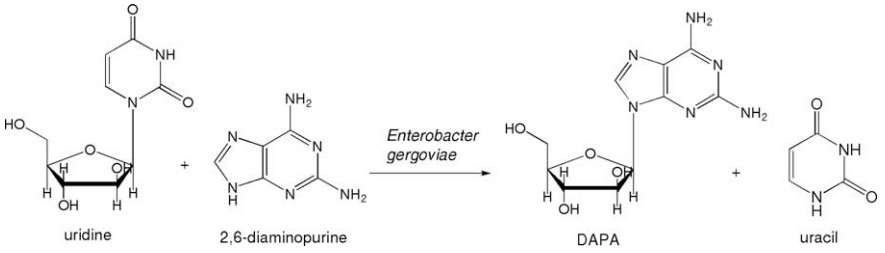



**Scheme 1.** Synthesis of 2,6-diaminopurine arabinoside by microbial transglycosylation. The reaction mixture comprising wet cell paste of *E. aerogenes* containing $1.0 \times 10^{10}$ cells/ml, 10 mM 2,6-diaminopurine, 30 mM uracil arabinoside and 30 mM potassium phosphate buffer pH7 was stirred 48 h at 200 rpm and 60 °C.

therefore, the aim of this work was to analyze the use of *A. oxydans*, instead of commercial ADA, in combination with microbial transglycosylation to prepare AraG.

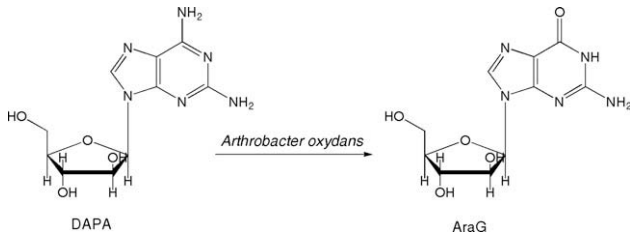
Several microorganisms were screened in order to obtain, by whole cell biocatalyzed transglycosylation, 2,6-diaminopurine arabinoside (DAPA) from 2,6-diaminopurine and AraU[16] (Scheme 1). The strain selected was *Enterobacter gergoviae* that afforded DAPA in 76% yield at 60 °C (Table 1, entry 1). As expected, the transglycosylation carried out at 45 °C was less efficient[5] and the product partially deaminated to AraG as consequence of the action of *E. gergoviae* ADA (Table 1, entry 4). The products were confirmed by HPLC–MS and the reaction mixtures quantified by HPLC.

The reaction medium containing 7.6 mM of DAPA was centrifuged and $1.0 \times 10^{10}$ cells /ml of *A. oxydans* or 10 U/ml of commercial ADA were added to the supernatant. The temperature was maintained at 37 °C to carry out the deamination reaction. After 48 h, 1.6 mM of AraG (28% yield) was obtained using *A. oxydans* as biocatalyst while 7.6 mM (100% yield) was produced with commercial ADA. To improve the deamination yield with *A. oxydans* whole cells, some experimental conditions were analyzed. In this way, the number of added cells was increased. Using $3.0 \times 10^{10}$ cells/ml, 46% deamination yield in 48 h was achieved (2.5 mM AraG) (Scheme 2). Substrate specificity or diffusional restrictions could be the reason of the lower deamination activity observed with *A. oxydans* whole cells.

**Table 1**
Synthesis of guanine arabinoside by one-pot transglycosylation and deamination

| Entry | Biocatalyst | Temperature (°C) | DAPA[a] (mM) | AraG[a] (mM) |
|---|---|---|---|---|
| 1 | *E. gergoviae* | 60 | 7.6 | — |
| 2 | *E. gergoviae*+*A. oxydans*[b] | 60 | 5.1 | 1.4 |
| 3 | *E. gergoviae*+ADA[c] | 60 | 1 | 4.6 |
| 4 | *E. gergoviae* | 45 | 2.4 | 1.3 |
| 5 | *E. gergoviae*+*A. oxydans*[b] | 45 | 0.25 | 0.04 |
| 6 | *E. gergoviae*+ADA[c] | 45 | 0 | 2.2 |

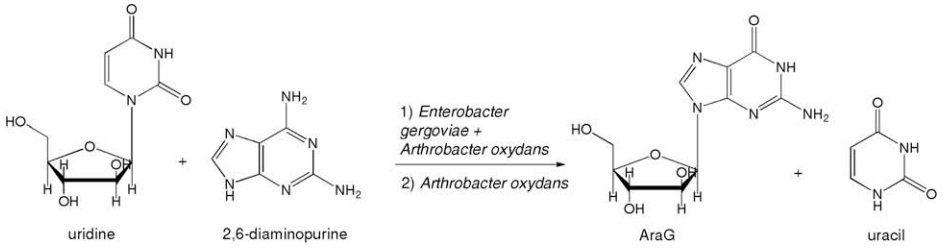
[a] Quantified after 48 h reaction by HPLC using calibration curves of the corresponding products.
[b] $1 \times 10^{10}$ cells/ml of *E. gergoviae*, $1 \times 10^{10}$ cells/ml of *A. oxydans*, 10 mM 2,6-diaminopurine, 30 mM uracil arabinoside and 30 mM potassium phosphate buffer pH7 were stirred during 48 h at 200 rpm and the corresponding temperature.
[C] Idem b using 10 U/ml of ADA instead $1 \times 10^{10}$ cells/ml of *A. oxydans*.




**Scheme 2.** Deamination of 2,6-diaminopurine arabinoside. The supernatant from the transglycosylation reaction was further incubated with $3.0 \times 10^{10}$ cells/ml of *A. oxydans* at 37 °C during 48 h.

An alternative procedure was assessed in which transglycosylation and deamination biocatalysts were added simultaneously. When one-pot biotransformation was assessed with *E. gergoviae* and *A. oxydans* at 45 °C (Table 1, entry 5), a compromise temperature for both enzymatic activities, DAPA and AraG were obtained in small quantities. The presence of xanthine as subproduct evidenced that *A. oxydans* adenine and guanine deaminases were active. Performing the simultaneous reaction at 60 °C (Table 1, entry 2), only 1.4 mM of AraG was obtained in 48 h. These unexpected low productivities may be attributed to the competitive hydrolysis of AraG by *E. gergoviae* PNP. This assumption was supported by the presence of guanine in these reaction media and further confirmed using commercially available ADA instead of *A. oxydans*, since a decrease in AraG yield (4.6 mM, and 2.2 mM, Table 1 entries 3 and 6, respectively) compared to the sequential procedure (7.6 mM) was observed.

Finally, the most effective strategy to synthesize AraG using combined two whole cells biocatalysts was achieved when one-pot transglycosylation and deamination reactions catalysed by *E. gergoviae* and *A. oxydans*, respectively, was followed by another deamination employing *A. oxydans* (Scheme 3). The first step was carried out at 60 °C during 48 h. After centrifugation, the supernatant was treated with $3 \times 10^{10}$ cells/ml of *A. oxydans* at 37 °C. After 3 days, 3.6 mM of AraG was obtained.




**Scheme 3.** Synthesis of 9-β-D-arabinofuranosylguanine by *E. gergoviae* and *A. oxydans*. The reaction mixture containing $1 \times 10^{10}$ cells/ml of *E. gergoviae*, $1 \times 10^{10}$ cells/ml of *A. oxydans*, 10 mM 2,6-diaminopurine, 30 mM uracil arabinoside and 30 mM potassium phosphate buffer pH7 was stirred at 200 rpm and 60 °C. After 48 h, the supernatant was treated with $3 \times 10^{10}$ cells/ml of *A. oxydans* during 72 h at 37 °C.

In summary, in this Letter we report the first application of two combined bacterial whole cell biocatalysts to produce AraG, a bioactive compound. Using *E. gergoviae* to carry out the transglycosylation and *A. oxydans* to perform the deamination reaction, AraG was obtained in 1 g/l yield. Moreover, the methodology here reported provides more accessible biocatalysts in view to further technological applications that could still be improved by the cloning and over-expression of the corresponding enzymes in one recombinant microorganism.

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