Symposium date: 8th September 2008

Nucleic Acids Symposium Series No. 52 541–542
doi:10.1093/nass/nrn274

Coupled biocatalysts applied to the synthesis of nucleosides

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ABSTRACT

Biocatalytic procedures offer a good alternative to the chemical synthesis of nucleosides since biocatalyzed reactions are regio- and stereoselective and afford reduced by-products contents. Among them, enzymatic transglycosylation between a pyrimidine nucleoside and a purine base catalyzed by nucleoside phosphorylases or microorganisms that contain them, has attracted considerable attention. In addition, the combination to other enzymatic steps has been explored.

In this work we investigate the coupled action of nucleoside phosphorylases with other enzymatic activities: deaminase and phosphopentomutase.

Unlike the preparation of other purine nucleosides, transglycosylation from a pyrimidine nucleoside and guanine is difficult because of the low solubility of this base. Therefore, another strategy, based on microbial transglycosylation followed by deamination, is here explored.

The direct use of furanose 1-phosphate, the intermediate in the transglycosylation reaction, is an attractive alternative when pyrimidine nucleosides are not available. Its preparation from the more stable furanose 5-phosphate and phosphopentomutase is here applied to different sugars and bases.

INTRODUCTION

Biotechnological preparation of nucleosides is an alternative to chemical synthesis. This approach presents some advantages; usually the reaction conditions are smoother, environmentally friendly and principally regio and stereoselective. For the last three decades, nucleoside phosphorylases (NPs) have been extensively used in the preparation of purine nucleoside from pyrimidine ones[1] (called transglycosylation). The reaction proceeds via the formation of the corresponding α-furanose 1-phosphate that is subsequently used by the purine NP (PNP) as substrate. This strategy has been applied to the preparation of sugar and base modified nucleosides, using isolated enzymes or whole cells. A limitation of this approach, for sugar modified nucleosides, is that the corresponding pyrimidine analogue must be available. A way to overcome this problem is to employ furanose 1-phosphates as starting materials, which unfortunately are limited by their synthetic difficulty and intrinsic instability. Alternatively, furanose 1-phosphates can be enzymatically obtained from furanose 5-phosphates using phosphopentomutase (PPM), an enzyme of the pentose pathway. PPM catalyses the reversible transfer of a phosphate group between the hydroxyls of positions 5 and 1 of ribose and deoxyribose, in bacteria and in mammal tissues.

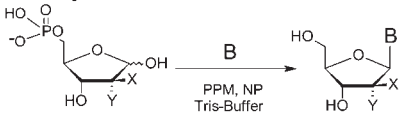
On the other hand, nowadays, there is a great interest to prepare guanine nucleoside analogues since they are efficient antiviral and anticancer agents. The industry also requires new sources of guanosine since both natural and synthetic supplies are insufficient. The preparation of guanosine analogues by means of enzymatic transglycosylation cannot be achieved due to the low solubility of guanine in the pH range in which the enzymes are active. The most effective strategy was achieved by the combination of transglycosylation and deamination reactions. Thus, using soluble 2-amino-6-substituted purine bases, several guanosine derivatives have been synthesized by nucleoside phosphorylases present in bacterial whole cells followed by hydrolysis of the substituents at position 6 by enzymatic reaction with isolated adenosine deaminase (ADA). ADA catalyses the irreversible hydrolysis of adenosine to inosine and ammonia and its broad activity on base or sugar modified nucleosides is since long known. Mammalian deaminases are commercially available, mainly from calf spleen or calf intestine, but the use of bacterial whole cells, especially as biocatalysts, is in continuous growing because of their economical benefits.

The aim of this work was to explore the usefulness of both strategies to the synthesis of modified nucleosides with pharmacological or chemical applications.

RESULTS AND DISCUSSION

In order to evaluate the production of natural and

modified nucleosides by the use of the combined action of PPM and NPs, a general chemoenzymatic approach for the preparation of furanose 5-phosphates starting from ribose, arabinose and 2-deoxyribose was developed[2]. A recombinant PPM was obtained and to determine the better conditions for the catalyzed reaction, the dependence of enzyme activity and reaction yield on pH, temperature, glucose 1,6-diphosphate, β-mercaptoethanol and phosphate concentrations was studied. For this purpose, the conversion of ribose 5-phosphate to adenosine, catalyzed by PPM and commercial PNP, was selected as the model system. Over expressed PPM was further tested coupled to two different commercial NPs, PNP and TP, and a set of natural and modified nucleosides were obtained (Table I). An interesting example was the synthesis of Ribavirine (Virazole) from ribose 5-phosphate and 1,2,4-triazole-3-carboxamide. The reaction proceeded quantitatively and with high activity in 1.5 h.



X	Y	NP	B	Rel. Act.	Yield/ %	Time/ h
H	OH	PNP	Hyp	100[a]	98	2
H	OH	PNP	A	14	90	48
H	OH	PNP	6-SH-Pu	66	89	2
H	OH	PNP	6-Cl-2-NH_2-Pu	1	25	32
H	OH	PNP	2-F-Pu	4.1	40	96
H	OH	PNP	TCA	127	100	1.5
OH	H	PNP	Hyp	1.15	46	46
OH	H	PNP	A	0.54	8	96
OH	H	PNP	6-SH-Pu	0.34	7	24
OH	H	PNP	TCA	0.27	19	72
H	H	TP	T	204	75	2
H	H	TP	U	148	74	2
H	H	TP	5-F-U	145	60	2.25
H	H	TP	5-Br-U	104	100	0.75

[a] The relative activity value of 100 % correspond to 0.49 μmol.min^{-1}.mg prot^{-1}

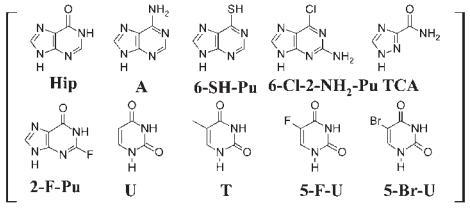
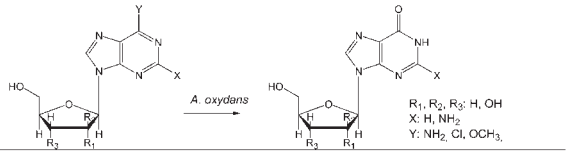


Table I Synthesis of nucleosides from furanose 5-phosphates

As mentioned above, the poor solubility of guanine is a limitation to carry out microbial transglycosylation. To solve this, guanosine was used as the base source to obtain 2'-deoxyguanosine (dG), since it has higher solubility than guanine. In spite of the increase of dG productivity, the purification was difficult due to the presence of uridine, obtained as by-product. Moreover, non-natural guanosine analogues like arabinoguanosine (AraG), cannot be prepared by this methodology because long reaction times were required and the half-life time of guanosine in the media is very short. Then, microbial transglycosylation was coupled with ADA in order to obtain this kind of compounds.

The last reaction was carried out by whole cells of *Arthrobacter oxydans* instead of isolated ADA from mammalian sources as usually. This bacteria, selected from our cell collection, showed high ADA activity and was explored as biocatalyst for the preparation of some inosine and guanosine derivatives (Table II) from the corresponding adenine and 2-amino-6-substituted purine analogues. The reaction times must be rigorously controlled since the products are probably substrates of their endogenous PNP. In particular, purine arabinosides as well as purine dideoxynucleosides are poor substrates of the *Arthrobacter oxydans* PNP and consequently, using this tool, 9-β-D-arabinofuranosyl guanine (AraG) was obtained in high yield.



Substrate	Product	Yield (%)	Time (h)
Adenosine	Inosine	37	2
2'-Deoxyadenosine	2'-Deoxyinosine	56	2
2',3'-Dideoxyadenosine	2',3' Dideoxyinosine	73	2
Arabinoadenosine	Arabinoinosine	82	24
2,6-Diaminopurine riboside	Guanosine	63	3
2-Amino-6-methoxypurine riboside	Guanosine	16	5
2-Amino-6-chloropurine riboside	Guanosine	64	5
2-Amino-6-mercaptopurine riboside	Guanosine	-	24
2,6-Diaminopurine-2'-deoxyriboside	2'-Deoxyguanosine	65	2
2,6-Diaminopurine-2',3'-dideoxyriboside	2',3'-Dideoxyguanosine	37	48
2,6-Diaminopurine arabinoside	Arabinoguanosine	70	24

Table II Biotransformation of adenine- and 2-amino-6-substituted nucleosides by *A. oxydans* ADA

CONCLUSION

The use of microbial whole cells as a novel biocatalyst in purine deamination as well as the preparative application of over expressed PPM, both coupled to the activity of NPs, is here reported.

REFERENCES

1. Lewkowicz E.S., Iribarren A.M. (2006) *Current Organic Chemistry,* **10**, 1197-1215
2. Taverna-Porro M., Bouvier L.A., Pereira C.L, Montserrat J.M, Iribarren A.M., (2008) *Tetrahedron Letters,* **49**, 2642-2645

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