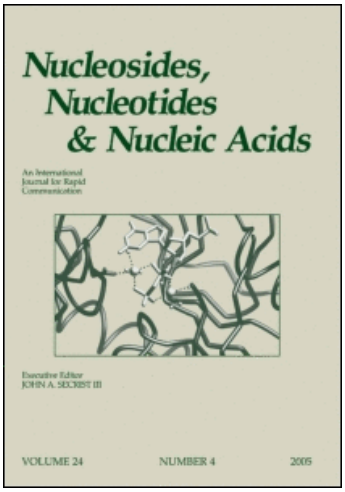

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BENZIMIDAZOLES IN THE REACTION OF ENZYMATIC TRANSGLYCOSYLATION

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Abstract. Substrate activity of a broad spectrum of derivatives of benzimidazole in the reaction of enzymatic *ribo*- and 2-*deoxyribo*sylation catalyzed by purine nucleoside phosphorylase of whole cells of *E. coli* BMT-1D/1A has been studied. Guanosine or 2'-deoxyguanosine were used as glycosyl donors.

INTRODUCTION

We have recently shown[1] that 1-deazapurines are good substrates in the reactions of trans*deoxyribo*sylation and trans*ribo*sylation using 2'-deoxyguanosine (dG) and guanosine (G), respectively, as pentofuranosyl donors. In contrast, 3-deazapurines are very effective as acceptors in the first reaction, but not in the second one. In order to ascertain the possible role of N(1) and N(3) atoms (purine numbering) in these reactions, we have investigated the substrate properties of benzimidazole. It was shown that it displays high substrate effectiveness in both reactions suggesting that both nitrogen atoms of the pyrimidine ring are not essential for the transglycosylation reactions. In view of this result, we have become interested in a systematic examination of the structure-activity relationship of substituted benzimidazoles and related substrates for transglycosylation reaction catalyzed by purine nucleoside phosphorylase (PNPase) of whole bacterial cells.

RESULTS AND DISCUSSION

High acceptor ability of benzimidazole in the reaction of enzymatic transglycosylation catalyzed by PNPase of whole bacterial cells clearly indicates that it does have good affinity for the enzyme. On the other hand, it was also shown that 1-(β-D-ribofuranosyl)benzimidazole (BI-Rib) is a very feeble substrate for phosphorolysis by PNPase from *E. coli*[2], consistent with the higher stability of the glycosidic bond of BI-Rib *vs.* that of purine nucleosides[3]. The foregoing patterns are relevant to the similar properties of 8-aminoadenine and 8-azaguanine, on the one hand, and their ribosides, on the other hand[4]. Thus, the high substrate effectiveness of benzimidazole implies that, besides a good affinity of the heterocycle for the enzyme, once formed, benzimidazole nucleoside does not readily undergo phosphorolysis.

From the foregoing, it is evident that an enzymatic methodology may be applied for the synthesis of nucleosides of benzimidazole derivatives. With this aim in view, a series of benzimidazole derivatives were tested as substrates for PNPase of whole cells *E. coli* BMT-1D/1A. Bearing in mind our previous considerations on enzymatic synthesis of 2-chloro-2'-deoxyadenosine[5], we have employed in the present work glutaraldehyde-treated cells. Guanosine as well as 2'-deoxyguanosine were chosen as glycosyl donors because only PNPase is involved in this case of transglycosylation. The results are listed in the Table.

Benzimidazole and its 5,6-disubstituted derivatives are good substrates in both reactions albeit *ribo*sylation needs more prolonged incubation to reach comparable yields as in the case of 2-*deoxyribo*sylation. The latter is consistent with the data on enzymatic glycosylation of 6-nitro- and 6-chloro-1-deazapurines[1].

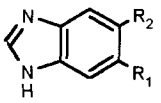
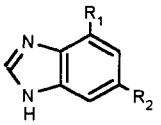
In the foregoing group of disubstituted derivatives, electron-donating methyl groups enhance acceptor ability, whereas electron-withdrawing nitro groups decrease it considering the effects of benzimidazole substitution in a qualitative manner (yield of nucleosides and time of reaction).

Highly impressive is the regioselectivity of enzymatic glycosylation of 5-nitrobenzimidazole affording N(1) glycosides of 6-nitrobenzimidazole. It should be noted that 2'-deoxyribosylation of 5-nitrobenzimidazole under phase-transfer conditions resulted in the formation of both 5- and 6-nitrobenzimidazole 2-deoxy-β-D-*erythro*-pentofuranosides (≈1:1; 85%, combined)[6], thus pointing to similar nucleophilic properties of N(1) and N(3) atoms. In this context, the role of 5-substituents in orientation of an acceptor upon binding by the PNPase is worthy of more detailed investigation.

A second group of interesting derivatives consists of 4,6-dibromo- and -dinitrobenzimidazoles and 4-aminobenzimidazole (1,3-dideazaadenine). The former displayed good acceptor ability in both reactions giving rise to 4,6-dibromobenzimidazole nucleosides, consistent with an analogous regioselectivity of enzyme-catalyzed transglycosylation of purine as well as 1- and 3-deazapurine bases (*e.g.*,[1,5]). The lack of substrate activity of 4,6-dinitrobenzimidazole *vs.* 5,6-isomer in the trans*ribo*sylation reaction and very low in the case of trans*deoxyribo*sylation may be attributed either to the changes in electron densities within imidazole ring or to the decreased ability to bind to the enzyme. Finally, similar to 3-deazaadenine[1], 1,3-dideazaadenine revealed substantially different reactivities in *ribo*sylation *vs.* 2-*deoxyribo*sylation: it exhibits no acceptor ability in the former reaction.

The data obtained call for some comments regarding the binding of an acceptor to the enzyme and the mechanism of nucleoside synthesis by PNPase. It was previously suggested that N(7) of purine nucleosides is not a binding site, but rather serves as a site for protonation by PNPase in the phosphorolytic reaction, leading to labilization of the glycosidic bond[2,7]. By contrast, the present results as well as previously reported[1] unequivocally point to N(7) of purines or N(3) of benzimidazoles as the only binding site for PNPase *via* hydrogen bonding in the reverse synthetic reaction. It appears likely that a tautomeric proton located on a nitrogen atom is involved in the binding to the enzyme and, hence, both N(7) of purines and N(3) of benzimidazoles are the proton donors and enzyme is the acceptor. A plausible

TABLE. Effectivity of Benzimidazole (BI) Nucleoside Synthesis by Whole Cells of *E. coli* BMT-1D/1A.

Acceptor	R_1	R_2	Donor	Time [h]	Product	Yield [%]
	H	H	G	23	BI-Rib	70
			dG	3.5	BI-dRib	90
	F	F	G	24	5,6-F_2BI-Rib	75
			dG	2	5,6-F_2BI-dRib	80
	Cl	Cl	G	3	5,6-Cl_2BI-Rib	80
			dG	0.2	5,6-Cl_2BI-dRib	72
	Me	Me	G	1.5	5,6-Me_2BI-Rib	98
			dG	1.5	5,6-Me_2BI-dRib	98
	NO_2	NO_2	G	48	5,6-$(NO_2)_2$BI-Rib	32
			dG	4	5,6-$(NO_2)_2$BI-dRib	65
	H	NO_2	G	24	6-NO_2BI-Rib	75
			dG	2.5	6-NO_2BI-dRib	77
	Br	Br	G	3	4,6-Br_2BI-Rib	50
			dG	1.5	4,6-Br_2BI-dRib	66
	NO_2	NO_2	G	48	4,6-$(NO_2)_2$BI-Rib	<1
			dG	24	4,6-$(NO_2)_2$BI-dRib	7
	NH_2	H	G	48	4-NH_2BI-Rib	<1
			dG	24	4-NH_2BI-dRib	57

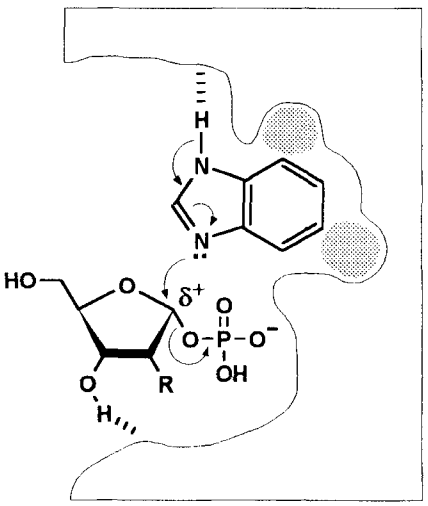


FIGURE. Hypothetical Mechanism of Nucleoside Synthesis by Purine Nucleoside Phosphorylase.

rationalization for the formation of a nucleoside in the transglycosylation reaction is a nucleophilic attack of non-bonded nitrogen atom of an acceptor at the electrophilic C(1) of RFP or dRFP with simultaneous transfer of the proton to the enzyme (Fig.).

Evidently the nucleophilicity of non-bonded nitrogen atom of an acceptor in the active center of PNPase is one of essential factors in this reaction. On the other hand, it seems probable that there are differences in the electrophilic properties of the anomeric center of RFP and dRFP. The observed differences in the substrate activity of benzimidazole derivatives should be largely attributed to both these factors.

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REFERENCES

1. Mikhailopulo, I.A.; Zinchenko, A.I.; Bokut, S.B.; Dudchik, N.V.; Barai, V.N.; Kalinichenko, E.N.; Rosemeyer, H.; Seela, F. *Biotechnol. Lett.* **1992**, *14*, 885-890.
2. Bzowska, A.; Kulikowska, E.; Shugar, D. *Z. Naturforsch.* **1990**, *45 c*, 59-70.
3. Oivanen, M.O.; Lönnberg, H.; Kazimierczuk, Z.; Shugar, D. *Nucleosides& Nucleotides* **1989**, *8*, 133-144.
4. Doskocil, J.; Holy, A. *Coll. Czech. Chem. Comm.* **1977**, *42*, 370-383.
5. Mikhailopulo, I.A.; Zinchenko, A.I.; Kazimierczuk, Z.; Barai, V.N.; Bokut, S.B.; Kalinichenko, E.N. *Nucleosides & Nucleotides* **1993**, *12*, 417-422.
6. Seela, F.; Bourgeois, W. *Synthesis* **1989**, 912-916.
7. Bzowska, A.; Kulikowska, E.; Darzynkiewicz, E.; Shugar, D. *J. Biol. Chem.* **1988**, *263*, 9212-9217.