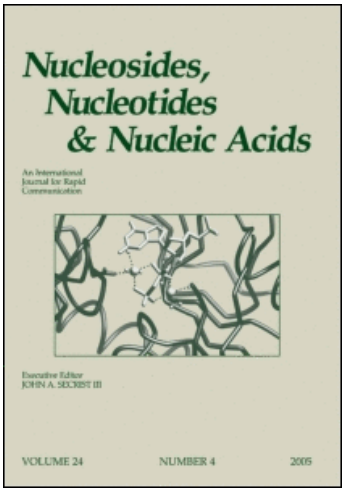

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Synthesis of 2-Chloro-2'-Deoxyadenosine by Microbiological Transglycosylation

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SYNTHESIS OF 2-CHLORO-2'-DEOXYADENOSINE BY MICROBIOLOGICAL TRANSGLYCOSYLATION

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Abstract. The title compound have been synthesized by an enzymatic trans-2'-deoxyribosylation of 2-chloroadenine using the whole cells of *E. coli* BMT-1D/1A as a biocatalyst and 2'-deoxyguanosine as a donor of glycosyl moiety.

Introduction

The potent antitumor activity of 2-chloro-2'-deoxyadenosine (Cl-dAdo) (*e.g.*, [1-5]) has led to the development of enzymatic and chemical methods for its preparation. Enzymatic synthesis of Cl-dAdo was achieved by direct transfer of 2-deoxyribofuranose moiety from 2'-deoxythymidine to 2-chloroadenine (Cl-Ade) catalyzed by partially purified *trans*-N-deoxyribosylase [1,3]. The efficient chemical method consists of stereospecific glycosylation of sodium salt of 2,6-dichloropurine followed by ammonolysis [6,7].

Considerable success has recently been achieved in the enzymatic transglycosylation of both purine and pyrimidine bases using natural or modified in carbohydrate moiety nucleosides as glycosyl donors and bacterial nucleoside phosphorylases (purified or in the whole cells) as biocatalysts (for recent reviews, see Refs. [8,9]). Earlier we selected an *E. coli* BMT-1D/1A strain, the cells of which contain highly active uridine and thymidine phosphorylases, and purine nucleoside phosphorylase (PNP) [10,11].

This paper is dedicated to the 65th birthday of Prof. Dr. Wolfgang Pfleiderer.

The present paper is aimed at investigation of the possibility of using the cells of this bacterial strain for trans-2'-deoxyribosylation of Cl-Ade employing 2'-deoxyguanosine (dGuo) as a donor of carbohydrate moiety.

Materials and Methods

Reagents. 2'-Deoxyguanosine (dGuo) was purchased from Serva (Heidelberg, Germany) and glutaraldehyde (GA) from Reanal (Budapest, Hungary). 2-Chloroadenine (Cl-Ade) was synthesized as described [12]. Standard Silufol UV_{254} (Serva, Germany) plates were used for TLC. Column chromatography was performed on silica gel L 40/100 μ (Chemapol, Czechoslovakia).

Microorganism. E. coli BMT-1D/1A was selected according to the technique offered by Munch-Petersen *et al.* [13]. The cells were grown and treated with GA essentially as described earlier [14,15].

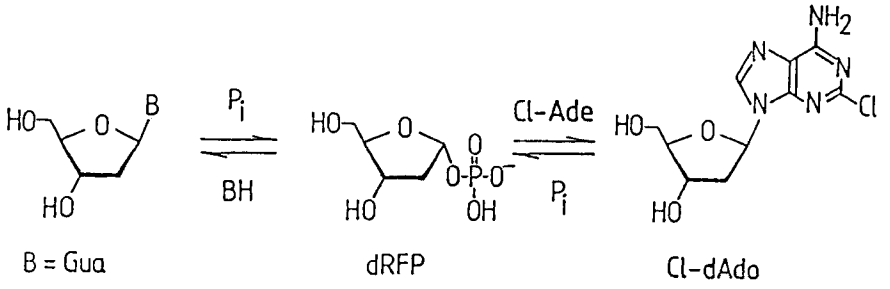
Standard reaction conditions for Cl-dAdo synthesis. The reaction mixture (1 mL) consisting of wet paste of GA-treated cells (0.2 mg, calculated as abs. dry wt.), 10 mM K-phosphate buffer (pH 7.25), 10% DMSO (v/v), 5 mM Cl-Ade and 15 mM dGuo was incubated at 60°C for 21 h with gentle mixing. Formation of Cl-dAdo was monitored by TLC. The solvent used was BuOH - 25% NH4OH (7:2; v/v). Cl-dAdo was eluted from TLC plates with water and quantified spectrophotometrically.

Preparative synthesis of Cl-dAdo. A suspension of Cl-Ade (200 mg, 1.18 mmol) in 10 mM K-phosphate buffer (pH 7.25), containing dGuo (942 mg, 3.52 mmol), 10% DMSO (v/v) and wet paste of GA-treated cells (47 mg, calculated as abs. dry wt.), was stirred at 60°C for 21 h. Total volume was 235 mL. The reaction was stopped by boiling for 5 min with subsequent withdrawal of cells and bulk of guanine by centrifugation (5000xg, 10 min). The supernatant obtained was kept at 4°C for 48 h, the precipitate formed was filtered off, washed with warm MeOH (15 mL) and then with MeOH/water (1:1, v/v; 10 mL) to give chromatographically (TLC) pure Cl-Ade (63 mg). The combined filtrates were evaporated, co-evaporated with EtOH, and residue was purified by silica gel (200 mL) column chromatography, eluted with a linear MeOH gradient (5-45%, v/v; 2x1000 mL) in $CHCl_3$. The fractions containing individual products were collected and evaporated to yield Cl-Ade (8 mg), Cl-dAdo (200 mg, 59% calculated for the Cl-Ade taken in the reaction; 92% based on the consumed Cl-Ade), and dGuo (370 mg). Crystallization of Cl-dAdo from EtOH gave

product (117 mg; 35 and 54%, respectively, *vide supra*) as a white powder that was identical with chemically synthesized Cl-dAdo [6] in all respects (^{1}NMR, UV at different pH values, and TLC).

Results and Discussion

The bacterial transdeoxyribosylation process studied here should include two coupled reversible reactions which occur *in situ:* (*i*) the phosphorolysis of the glycosyl donor, dGuo, in the presence of inorganic phosphate resulting in the formation of guanine and 2-deoxy-D-ribofuranose-1-α-phosphate (dRFP) as an intermediate, and (*ii*) the condensation of the latter with Cl-Ade with the release of phosphate to give the desired nucleoside, Cl-dAdo. It is clear that only PNP is involved in this transglycosylation process.



The cells of *E. coli* BMT-1D/1A have been selected according to [13], carry two regulatory mutations, and are able to synthesize constitutively the nucleoside-catabolizing enzymes. We have earlier shown that adenine exhibits very high efficiency as 2-deoxyribofuranose acceptor in the reaction catalyzed by *E. coli* BMT-1D/1A cells [10,11]. It should be stressed that the whole cells offer some obvious advantages over purified enzyme(s) from a viewpoint of the cost of biocatalyst and the simple operation.

The optimal reaction conditions for 2'-deoxyadenosine production were also studied and essentially employed in the present investigation. As previously stated, treatment of *E. coli* cells with glutaraldehyde (GA) enhances the stability of their phosphorylases and significantly improves the yields of desired nucleosides [14,15]. Taking this into account together with the aim to develop a practical method for Cl-dAdo preparation, we have employed in this work GA-treated cells.

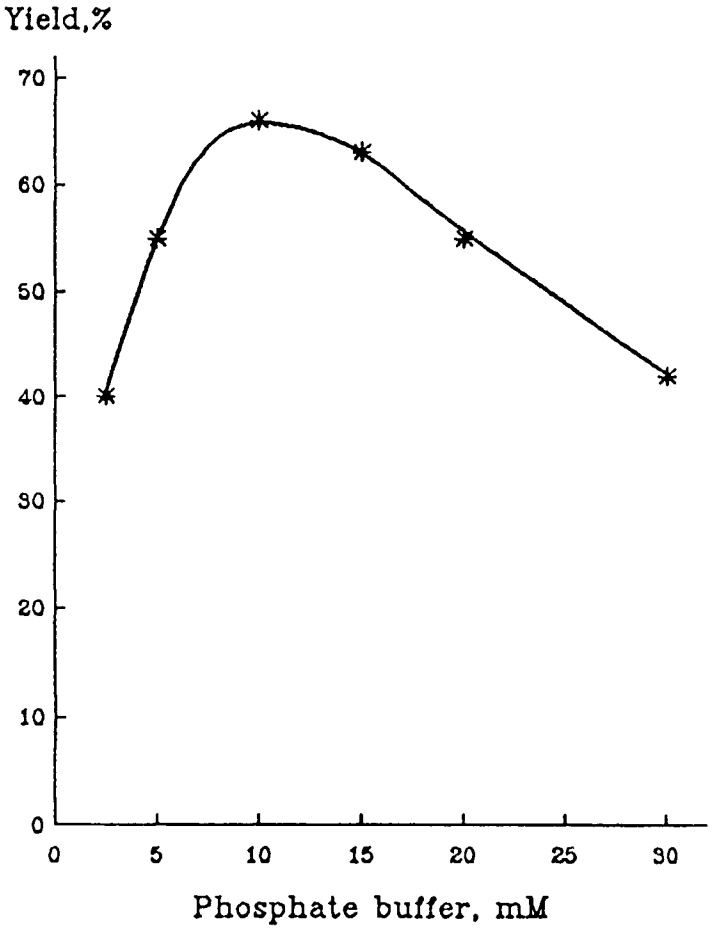


Fig. 1. Effect of the phosphate buffer concentration on the efficiency of the Cl-dAdo synthesis.

There was very little tendency towards formation of Cl-dAdo under the same reaction conditions as in the case of 2'-deoxyadenosine synthesis [11]. This result might be explained by very low solubility of Cl-Ade in the reaction mixture. To improve the yield by increasing the solubility of Cl-Ade, we have used DMSO as a component of the reaction mixture (*cf.* [14]). Indeed, an efficient conversion of Cl-Ade into Cl-dAdo was observed in the presence of DMSO in concentration of 10% (v/v). Further, we have optimized the phosphate buffer concentration in the reaction mixture.

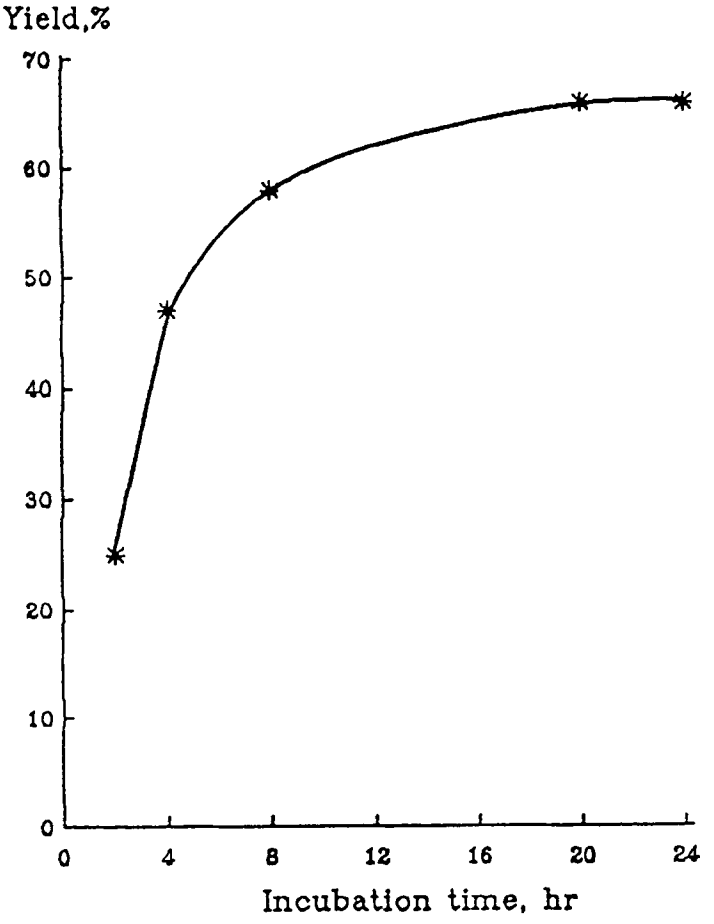


Fig. 2. Time course of the Cl-dAdo synthesis using glutaraldehyde-treated E. coli BMT-1D/1A cells.

The experimental data in Fig. 1 show that maximum Cl-dAdo yield (65%) was observed in 10 mM phosphate buffer (pH 7.25).

The formation of Cl-dAdo during the course of the microbiological transglycosylation is illustrated in Fig. 2. As can be seen, Cl-Ade is a good substrate in the transglycosylation reaction. Under conditions used (molar ratio of dGuo to Cl-Ade was 3:1), Cl-dAdo was synthesized in 54% isolated yield referred to the consumed Cl-Ade.

In conclusion, the transglycosylation method described in this communication is shown to be highly efficient for Cl-dAdo preparation. Further optimization of this reaction by the use of other donors of 2-deoxyribofuranose fragment,

on the one hand, and related bases that may be transformed into Cl-dAdo on nucleoside level, on the other hand, is now in progress.

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