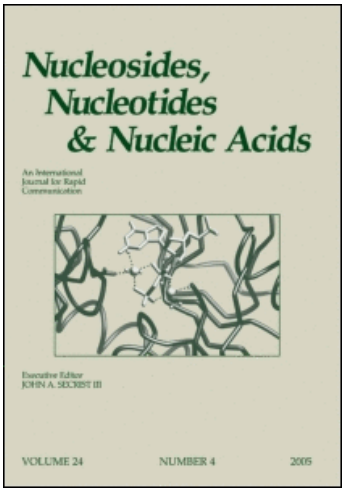

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Enzymatic Sywmesis of Dideoxyribonucleosides

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ENZYMATIC SYNTHESIS OF DIDEOXYRIBONUCLEOSIDES

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Abstract Two N-deoxyribosyltransferases from *Lactobacillus leichmanii* have been purified and the amino acids involved in the catalysis of the transfer reaction determined. A crude mixture of the transferases have been used to synthesise a purine 2',5'-dideoxynucleoside in high yield.

INTRODUCTION

Nucleoside analogues can inhibit HIV-induced reverse transcriptase as they act as chain terminators in nucleic acid synthesis and hence the synthesis of novel nucleoside analogues is of interest. The chemical synthesis of nucleoside analogues can be difficult as protecting groups on the base and the sugar residues are often required and the glycosylation of the base residue may not be stereospecific[1]. Enzymatic transglycosylations involving the transfer of a sugar residue from a donor to an acceptor base are alternative synthetic routes. Two enzymatic systems are in common use. The first, which occurs in the presence of inorganic orthophosphate, involves the use of coupled nucleoside phosphorylases[2]. This can be represented schematically as follows for the synthesis of a deoxyribonucleoside:

$$\text{Base(1)-dRib} + \text{phosphate} \rightleftarrows \text{dRib-1-phosphate} + \text{Base(1)}$$

$$\text{dRib-1-phosphate} + \text{Base(2)} \rightleftarrows \text{Base(2)-dRib} + \text{phosphate}$$

The second enzymatic method, which does not require orthophosphate, involves the transfer of 2-deoxyribose from a donor nucleoside to an acceptor base[3]. The overall reaction can be represented as follows:

$$\text{Base(1)-dRib} + \text{Base(2)} \rightleftarrows \text{Base(2)-dRib} + \text{Base(1)}$$

Both these reactions usually lead to the formation of only the natural β-anomer of the nucleoside.

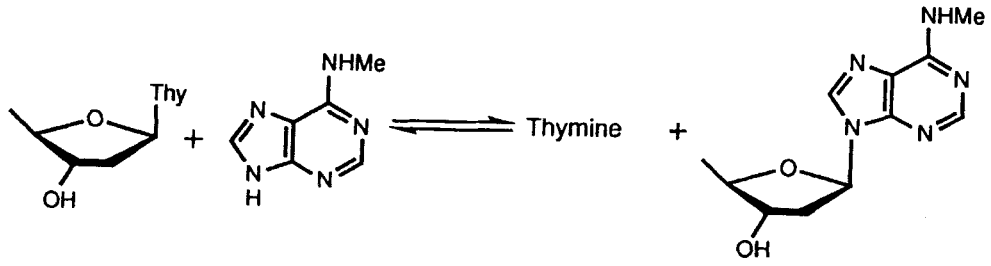
RESULTS AND DISCUSSION

We have been studying the second class of enzymes (N-deoxyribosyltransferases) from lactobacilli and related species and have developed conditions allowing us to prepare 2'-deoxynucleosides of analogues of naturally occurring bases, eg, 1-deazapurine [1][3] or imidazoles [2][4].

We find that the N-deoxyribosyltransferases occur in many species of lactobacilli[5]. Two types of enzyme are usually present. Type I, which catalyses the transfer of deoxyribose residues between purine bases, and Type II, which catalyses the transfer of deoxyribose residues either between pyrimidine bases or between purines and pyrimidines. One species *(Lactobacillus salivarius* subsp *salivarius)* contains only Type I N-deoxyribosyltransferase.

We have developed a simple procedure for preparing a crude mixture of Type I and Type II transferases from *Lactobacillus leichmanii* or *L helveticus*[3]. This crude mixture is suitable for the preparation of deoxynucleosides on a 20 mg scale and has been used, for example, to prepare 2',3'-dideoxynucleosides of N(6)-alkylated adenines which have anti-HIV properties but show little toxicity *in vitro*[6].

For our studies on the detailed mechanism of glycosyl transfer catalysed by the N-deoxyribosyltransferases, we have purified the enzyme mixture further using a combination of HPLC and fast protein liquid chromatography (FPLC)[7]. In this manner we have obtained two distinct proteins corresponding to the Type I and Type II enzymes. By gel filtration, Type I deoxyribosyltransferase has a molecular weight of



approximately 100,000 while Type II deoxyribosyltransferase has a molecular weight of approximately 92,000 Daltons. Comparison of the relative thermal stabilities of the two enzymes shows that the two

enzymes have widely differing temperature/activity profiles. Type II deoxyribosyltransferase is the more stable of the two enzymes and can be used at a higher temperature than Type I deoxyribosyltransferase. The two enzymes have similar pH/activity profiles suggesting that the same aminoacid residues may be involved in the glycosyl transfer reaction. Active site studies indicate that glutamic or aspartic acid residues may be involved in the glycosyl transfer reaction as Type II deoxyribosyltransferase is largely inactivated by reagents specific for carboxyl groups (carbodiimides or Woodward's reagent K). Reagents specific for the inactivation of arginine, cysteine or lysine residues have no effect on enzymatic activity, while reagents specific for histidine partially inactivate the Type II deoxyribosyltransferase.

In addition to their use in the synthesis of 2',3'-dideoxynucleosides, the crude N-deoxyribosyltransferases from *L leichmanii* provide a simple means for the preparation of 2',5'-dideoxynucleosides. Computer-assisted molecular modelling indicates that the conformation of the sugar ring in 2'-deoxythymidine and 2',5'-dideoxythymidine are very similar. We find that with N(6)-methylaminopurine as acceptor base, the corresponding 2',5'-dideoxynucleoside can be prepared from 2',5'-dideoxythymidine in almost quantitative yield. 2',5'-Dideoxynucleosides can inhibit a number of biological systems including the calmodulin-sensitive adenylate cyclase from bovine cerebral cortex[8]. The simplicity of our enzymatic route could be of interest in the preparation of a variety of therapeutic compounds.

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EXPERIMENTAL

Materials and Methods

5-Bromouracil, 2',5'-dideoxythymidine and N(6)-methylaminopurine were commercially available (Sigma). Crude nucleoside N-deoxyribosyltransferases (EC 2.4.2.6) from *Lactobacillus leichmanii* were prepared as previously described[3].

^{1}H NMR spectra were run at 400 MHz in CD_3OD solution. Chemical shifts are given in ppm relative to TMS as internal standard.

Synthesis of 9-β-D-2',5'-Dideoxyribofuranosyl N(6)-methylaminopurine.- To N(6)-methylaminopurine (9.4 mg, 0.067 mmole) and 2',5'-dideoxythymidine

(454 mg, 2.01 mmole) dissolved in citrate buffer (10 ml, pH 6) was added crude N-deoxyribosyltransferase (1 ml, 0.13 g protein) and the mixture incubated at 40°C. The progress of the reaction was followed by reverse phase HPLC (C-18 column, elution with 9-35% gradient of MeOH in H_2O). After 3 days the reaction was complete. The reaction mixture was freeze-dried and 9-β-D-2',5'-dideoxyribofuranosyl N(6)-methylaminopurine (16 mg, 98%) was isolated by flash chromatography on silica (elution with 8% EtOH in CH_2Cl_2). EI mass spectrum: found m/z 249.1211, calc for $C_{11}H_{15}N_5O_2$, $m^{+\cdot}$ = 249.1227.

^1H NMR (CD_3OD) 8.29 (1H, s, H-2), 8.23 (1H, s, H-8), 6.39 (1Ht, J1'-$2_{a'}$ = J1'-$2_{b'}$ = 5.56 Hz, H-1'), 4.33 (1Hm, H-3'), 4.08 (1Hm, H-4'), 3.20 (3H broad s, N-CH_3), 2.86 (1Hm, H-$2_{a'}$), 2.46 (1Hm, H-$2_{b'}$), 1.41 (3Hd, 5'-CH_3).

Nuclear Overhauser enhancements.- Irradiation of the signal at 6.39 ppm (H-1') caused enhancement of the signals due to H-$2_{b'}$, H-4 and H-8, irradiation of the signal at 8.23 ppm (H-8) caused enhancements of the signals due to H-1', H-$2_{a'}$ and H-3'.

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