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One-step enzymatic synthesis of nucleosides from low water-soluble purine bases in non-conventional media

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ABSTRACT

The effect of several water-miscible cosolvents on activity and stability of soluble and immobilized 2′-deoxyribosyltransferase from *Lactobacillus reuteri* on Sepabeads® has been studied in order to establish optimal conditions for enzymatic synthesis of nucleosides using purine bases with low solubility in aqueous buffer. As a rule of thumb, there was a general reduction of soluble enzyme activity when cosolvent content was gradually increased in reaction medium. In contrast, immobilized enzyme activity was enhanced 1.2–1.4-fold at 20% of methanol, ethanol, 2-propanol, diethylene glycol, and acetone; and at 10% and 30% acetonitrile. Likewise, highest increased activity (1.8-fold) was also obtained in presence of 20% acetonitrile. Immobilized enzyme was successfully used in the synthesis of 2′-deoxyxanthosine and 2′-deoxyguanosine using 2′-deoxyuridine as sugar donor and the corresponding poor water-soluble base in the presence of 30% of methanol, ethanol, 2-propanol, ethylene glycol, acetonitrile, and DMSO, giving high nucleoside yields at 4 h.

1. Introduction

Modified nucleosides are extensively used as antiviral and anticancer agents because of their ability to act as reverse transcriptase inhibitors or chain terminators in RNA or DNA synthesis (De Clercq, 2010; Robak et al., 2006). These molecules have been traditionally synthesized by different chemical methods (Boryski, 2008), but enzyme-catalyzed synthesis of nucleoside analogues is an attractive alternative since it shows many actual advantages such as one-pot reaction under mild conditions, high stereo and regioselectivity, and a friendly environmental technology (Lewkowicz and Iribarren, 2006; Mikhailopulo, 2007). Microbial nucleoside phosphorylases and *N*-deoxyribosyltransferases have been generally used in nucleosides synthesis by mediating the transfer of glycosyl residues to acceptor bases. Among them, *N*-deoxyribosyltransferases (EC 2.4.2.6) catalyze the cleavage of the glycosidic bond of a 2′-β-deoxynucleoside substrate and the subsequent transfer of the deoxyribosyl moeity to an acceptor purine (Pur) or pyrimidine (Pyr) base. Such reaction is both regioselective (they catalyze the deoxyribosyl transfer of the donor substrate to the N-1 position of a pyrimidine or the N-9 position of a purine acceptor substrate), and stereoselective (β-anomers are exclusively formed). According to their substrate specificities, *N*-deoxyribosyltransferases have been divided in purine *N*-deoxyribosyltransferases (PDTs) and nucleoside *N*-deoxyribosyltransferases (NDTs). PDTs (categorized as class I) catalyze the 2′-deoxyribose transfer exclusively between purine bases (Pur⇆Pur), whereas NDTs (categorized as class II) has substrate specificity for both purine and/or pyrimidine bases as donor and/or acceptor (Pur⇆Pur, Pur⇆Pyr and Pyr⇆Pyr) (Kaminski, 2002). From an industrial perspective, one-step transglycosilations catalyzed by NDTs are more advantageous than those based on nucleoside phosphorylases, which needs the performance of both pyrimidine and purine nucleoside phosphorylases (Lewkowicz and Iribarren, 2006). Nucleoside 2′-deoxyribosyltransferases have been mainly studied in lactic acid bacteria such as *Lactobacillus helveticus* (Kaminski, 2002), *Lactobacillus leichmannii*, *Lactobacillus fermentum* (Kaminski et al., 2008), *Lactobacillus reuteri* (Fernández-Lucas et al., 2010), and *Lactococcus lactis* subsp. *lactis* (Miyamoto et al., 2007), although the presence of NDTs has been also described in parasitic unicellular eukaryotic organisms (Steenkamp and Halbich, 1992; Bosch et al., 2006; Lawrence et al., 2009), and extremophilic bacteria (Fernández-Lucas et al., 2007).

Biochemical characterization of purified recombinant nucleoside 2′-deoxyribosyltransferase from *L. reuteri* (hereafter abbreviated as *Lr*NDT) showed that the enzyme is a homohexameric protein of 114 kDa, with high activity in a broad pH range (4.6–7.9) and very stable at different pH values ranging from 4.0 to 7.9. Likewise, *Lr*NDT is stable up to 50 °C, whereas its activity

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