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A facile whole-cell biocatalytic approach to regioselective synthesis of monoacylated 1-β-D-arabinofuranosylcytosine: Influence of organic solvents

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ABSTRACT

The lyophilized *Pseudomonas fluorescens* cell was an efficient alternative catalyst to enzymes for highly regioselective acylation of a polar nucleoside, 1-β-D-arabinofuranosylcytosine (ara-C). The cells showed an evident solvent dependence in the reaction. Among the tested solvents except for acetonitrile–pyridine, catalytic activity of the cells clearly increased with increasing the polarity of the organic solvents used. Among all the tested solvents both pure and binary, the best results were observed in isopropyl ether–pyridine system, in which the catalyst also showed good thermal and operational stabilities. For the biocataylsis in isopropyl ether–pyridine, the optimal isopropyl ether concentration, water content, acyl donor/ara-C ratio, biocatalyst dosage and reaction temperature were 30% (v/v), 4%, 45, 50 mg/mL and 30 °C, respectively, under which the initial rate, yield and 5′-regioselectivity were 2.93 mM/h, 77.1% and 97.3%, respectively. The bacterial cells exhibited comparable 5′-regioselectivity to the expensive immobilized enzyme, which could also have environmental and cost advantages.

1. Introduction

Nucleoside analogs are compounds of high significance in medicinal chemistry with antitumor, antiviral and immunosuppressive effects (Bergman et al., 2004; Li et al., 2010a; Wagner et al., 2000). Due to a fairly close resemblance in structure, they can be used as competitors of natural nucleosides, be inserted into a growing DNA/RNA strand by the polymerases after phosphorylation and thus disturb the normal DNA/RNA replication (Wagner et al., 2000). However, some nucleoside analogs with high hydrophilicity, such as 1-β-D-arabinofuranosylcytosine (ara-C), have major clinical shortfalls in the treatment of solid tumors, since they cannot easily transfer across the cell membrane by passive diffusion and might undergo a rapid enzymatic deactivation in plasma. To improve the clinical efficiency of those analogs, lipophilic modification has gained much attention as a promising strategy, which is also valuable for new nucleoside drug/prodrug discovery and development (Bergman et al., 2004; Li et al., 2010a).

Selectively modification of only one hydroxyl group of nucleosides is considered as an arduous task to chemists, due to the multiple hydroxyl groups of similar reactivity (Li et al., 2010a,b). During the past decade enormous efforts have been made in the development of synthetic methodologies for lipophilic derivatives of nucleosides (Li et al., 2006a,b, 2010a,b; Wechter et al., 1975). Among these synthetic tools available to chemists, application of enzymes in organic chemistry has become one of the most attractive alternatives to the conventional chemical methods for its high regioselectivity, and environmental friendliness (Klibanov, 2001). In contrast, regioselectively introducing acyl group to sugar moiety of nucleosides by chemical method was an arduous task needing time-consuming protection/deprotection steps and bulky acylating regents (Li et al., 2010a; Morís and Gotor, 1993).

The main hurdle to the industrial application of an enzymatic approach in the synthesis of nucleoside esters are the high cost of the procedure, low thermo- and operational stability of free enzymes in organic solvents. To cope with the limitations, solvent engineering strategies were developed to improve the stability of the enzymes. Our previous research reported the successful application of ionic liquid-containing systems and binary organic solvent mixtures to synthesis of monoesters of nucleosides (Li et al., 2006a, 2010b). However, the use of ionic liquids made handing (filtrating, etc.) more difficult and constrained mass transfer. Other methods focused on the screening of non-expensive lipases (Yang et al., 2010) and searching for effective immobilization carrier for free enzymes (Mitchell and Perez-Ramirez, 2011). The latter, however, added to the complexity and cost of the enzymatic procedures.

Compared to the typically used enzymes, whole-cell biocatalysts offer several benefits to organic synthesis (Schmid et al., 2001; Klibanov, 2001; de Carvalho, 2011). One is that whole-cell biocatalysis eliminate the need for enzyme purification and

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