Enzymatic Synthesis Of Therapeutic Nucleosides Using A Highly Versatile Purine Nucleoside 2'-Deoxyribosyltransferase From Trypanosoma Brucei

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Abstract

The use of enzymes for the synthesis of nucleoside analogues offers several advantages over multistep chemical methods, including chemo-, regio- and stereoselectivity as well as milder reaction conditions. Herein, the production, characterization and utilization of a purine nucleoside 2'-deoxyribosyltransferase (PDT) from Trypanosoma brucei are reported. TbPDT is a dimer which displays not only excellent activity and stability over a broad range of temperatures (50–70 °C), pH (4–7) and ionic strength (0–500 mM NaCl) but also an unusual high stability under alkaline conditions (pH 8–10). TbPDT is shown to be proficient in the biosynthesis of numerous therapeutic nucleosides, including didanosine, vidarabine, cladribine, fludarabine and nelarabine. The structure-guided replacement of Val11 with either Ala or Ser resulted in variants with 2.8-fold greater activity. TbPDT was also covalently immobilized on glutaraldehyde-activated magnetic microspheres. MTbPDT3 was selected as the best derivative (4200 IU/g, activity recovery of 22 %), and could be easily recaptured and recycled for >25 reactions with negligible loss of activity. Finally, MTbPDT3 was successfully employed in the expedient synthesis of several nucleoside analogues. Taken together, our results support the notion that TbPDT has good potential as an industrial biocatalyst for the synthesis of a wide range of therapeutic nucleosides through an efficient and environmentally friendly methodology.

Keywords

2'-Deoxy-Ribosyltransferase; Biocatalysis; Enzyme Immobilization; Molecular Dynamics; Nucleoside Analogues