TAYLOR-MADE PRODUCTION OF PYRIMIDINE NUCLEOSIDE-5′-MONOPHOSPHATE ANALOGUES BY HIGHLY STABILIZED MUTANT URACIL PHOSPHORIBOSYLTRANSFERASE FROM TOXOPLASMA GONDII

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Abstract

Nowadays, enzymatic synthesis of nucleotides is an efficient and sustainable alternative to chemical methodologies. In this regard, after the biochemical characterization of wild-type and mutant uracil phosphoribosyltransferases from Toxoplasma gondii (TgUPRT, TgUPRT2, and TgUPRT3), TgUPRT2 was selected as the optimal candidate (69.5 IU mg−1, UMP synthesis) for structure-guided immobilization onto Ni2+ chelate (MNiUPRT2) and onto glutaraldehyde-activated microparticles (MGlUPRT2). Among resulting derivatives, MNiUPRT23 (6127 IU g−1biocat; 92% retained activity; 3–5 fold enhanced stability at 50–60 °C) and MGlUPRT2N (3711 IU g−1biocat; 27% retained activity; 8–20 fold enhanced stability at 50–60 °C) displayed the best operability. Moreover, the enzymatic synthesis of different pyrimidine NMPs was performed. Finally, the reusability of both derivatives in 5-FUMP synthesis (MNiUPRT23, 80% retained activity after 7 cycles, 5 min; MGlUPRT2N, 70% retained activity after 10 cycles, 20 min) was carried out at short times.

Keywords

Nucleoside-5′-monophosphates; Phosphoribosyltransferases; Structure-guided immobilization