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Expression of Fused Plasmodium falciparum Orotate Phosphoribosyltransferase and Orotidine 5'-Monophosphate Decarboxylase in Escherichia coli

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Abstract : Fusion of the last two enzymes in the pyrimidine biosynthetic pathway in the inversed order by having COOH-terminal orotate phosphoribosyltransferase (OPRT) and NH2-terminal orotidine 5'-monophosphate decarboxylase (OMPDC), as OMPDC-OPRT, are described in many organisms. In this study, we constructed gene fusions of Plasmodium falciparum OMPDC-OPRT (1,836 bp) in pTrcHisA vector and expressed as an 6xHis-tag bifunctional protein in three Escherichia coli strains (BL21, Rosetta, TOP10) at 18 °C, 25 °C and 37 °C. The recombinant bifunctional protein was partially purified by Ni-Nitrilotriacetic acid-affinity chromatography. Specific activities of OPRT and OMPDC domains in the bifunctional enzyme expressed in E. coli TOP10 cells were approximately 3-4-fold higher than those in BL21 cells. There were no enzymatic activities when the construct vector expressed in Rosetta cells. Maximal expression of the fused gene was observed at 18 °C and the bifunctional enzyme had specific activities of OPRT and OMPDC domains in a ratio of 1:2. These results provide greater yields and better catalytic activities of the bifunctional OMPDC-OPRT enzyme for further purification and kinetic study.

Keywords : bifunctional enzyme, orotate phosphoribosyltransferase, orotidine 5'-monophosphate decarboxylase, plasmodium falciparum
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