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Bifunctional Activity and Stability of Fused Plasmodium falciparum Orotate Phosphoribosyltransferase and Orotidine 5′-Monophosphate Decarboxylase

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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. Here, we produced gene fusions of Plasmodium falciparum OMPDC-OPRT and expressed the bifunctional protein in Escherichia coli. The enzyme was purified to homogeneity using affinity and anion-exchange chromatography, exhibited enzymatic activities and functioned as a dimer. The activities, although unstable, can be stabilized by its substrate and product during purification and long-term storage. Furthermore, the enzyme expressed a perfect catalytic efficiency (kcat/Km). The kcat was selectively enhanced up to 3 orders of magnitude, while the Km was not much affected and remained at low μM levels when compared to the monofunctional enzymes. The fusion of the two enzymes, creating a “super-enzyme” with perfect catalytic power and more flexibility, reflects cryptic relationship of enzymatic reactivaties and metabolic functions on molecular evolution.

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