

Enzymatic Production Of Non-Natural Nucleoside-5'-Monophosphates By A Thermostable Uracil Phosphoribosyltransferase

Del Arco, Jon ; Acosta, Javier; D'Muniz Pereira, Humberto; Perona, Almudena; Lokanath, Neratur Krishnappagowda ; Kunishima, Naoki ; Fernandez Lucas, Jesus

Abstract

The use of enzymes as biocatalysts applied to synthesis of modified nucleoside-5'-monophosphates (NMPs) is an interesting alternative to traditional multistep chemical methods which offers several advantages, such as stereo-, regio-, and enantioselectivity, simple downstream processing, and mild reaction conditions. Herein we report the recombinant expression, production, and purification of uracil phosphoribosyltransferase from *Thermus thermophilus* HB8 (TtUPRT). The structure of TtUPRT has been determined by protein crystallography, and its substrate specificity and biochemical characteristics have been analyzed, providing new structural insights into the substrate-binding mode. Biochemical characterization of the recombinant protein indicates that the enzyme is a homotetramer, with activity and stability across a broad range of temperatures (50–80 °C), pH (5.5–9) and ionic strength (0–500 mM NaCl). Surprisingly, TtUPRT is able to recognize several 5 and 6-substituted pyrimidines as substrates. These experimental results suggest TtUPRT could be a valuable biocatalyst for the synthesis of modified NMPs.

Keywords

Biocatalysis; Biological Activity; Enzymes; Nucleotides; Structure Elucidation.