A Facile Fluorometric Assay of Orotate Phosphoribosyltransferase Activity Using a Selective Fluorogenic Reaction for Orotic Acid

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Abstract

Orotate phosphoribosyltransferase (OPRT) exists as a bifunctional enzyme, uridine 5′ -monophosphate synthase, in mammalian cells and plays an important role in pyrimidine biosynthesis. Measuring OPRT activity has been considered important for understanding biological events and development of molecular-targeting drugs. In this study, we demonstrate a novel fluorescence method for measuring OPRT activity in living cells. The technique utilizes 4-trifluoromethylbenzamidoxime (4-TFMBAO) as a fluorogenic reagent, which produces selective fluorescence for orotic acid. To perform the OPRT reaction, orotic acid was added to HeLa cell lysate, and a portion of the enzyme reaction mixture was heated at 80 ° C for 4 min in the presence of 4-TFMBAO under basic conditions. The resulting fluorescence was measured using a spectrofluorometer, which reflects the consumption of orotic acid by the OPRT. After optimization of the reaction conditions, the OPRT activity was successfully determined in 15 min of enzyme reaction time without further procedures such as purification of OPRT or deproteination for the analysis. The activity obtained was compatible with the value measured by the radiometric method with [^{3}H]-5-FU as the substrate. The present method provides a reliable and facile measurement of OPRT activity and could be useful for a variety of research fields targeting pyrimidine metabolism.