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Determination of GTP Cyclohydrolase I Activity in Stimulated Leukocyte by High Performance Liquid Chromatography

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GTP cyclohydrolase I (EC 3.45.4.16) catalyzes the first reaction in the pathway for the biosynthesis of tetrahydrobiopterin (BH_4). Defects in GTP cyclohydrolase I cause BH_4 synthesis deficient phenylketonuria (PKU) and mental retardation due to neurotransmitter deficiency in human. A method for determination of GTP cyclo-hydrolase I activity in leukocyte was established with the aid of high performance liquid chromatography (HPLC). In order to determine GTP cyclohydrolase I activity, the leukocyte was stimulated by phytohemaglutinine (20 μg/ml) during culturing for 4-5 days. The reaction mixture contained 0.5 mM GTP, 2.5 mM EDTA, 0.15 M KCl, 5% (v/v) glycrerol in 0.1 M Tris-HCl buffer (pH=7.5). The reaction was initiated by adding GTP at 37°C for 100 minutes in dim light. The reaction was stopped by addition of 1 g/dl iodine in 1M HCl. The oxidized reaction product, neopterin triphosphate, was dephosphorylated to neopterin by adding alkaline phosphatase (pH=9). Neopterin was analyzed by reverse phase C-18 HPLC with fluorescence detection (ex. 350 nm, em. 450 nm). The production of neopterin was linear within 100 minutes after the reaction started. The pH optima for the GTP cyclohydrolase I were found to be around 7.5. the Km value of GTP was determined to be 24 μM. This method might be applied for differential diagnosis of BH_4 deficient PKU.

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