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301 DETERMINATION OF HUMAN PLACENTA METHYLMALONYL-COA MUTASE ACTIVITY BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.

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A high-performance liquid chromatography system was developed with nearly baseline seperation of succinyl-CoA from methylmalonyl-CoA to determine the activity of methylmalonyl-CoA mutase in the lysate of human placenta. This method was much simpler than the conventional ones which used radioactive substrate, [^{14}C]methylmalonyl-CoA, and required time-consuming methods, e.g. paper chromatography, high voltage electrophoresis or permangnate oxidation, to seperate [^{14}C]methylmalonate and [^{14}C]succinate. Five grams of placenta sample was prepared by homogenization and sonication for 6 x 10 sec (40 W) in 5 ml reaction buffer, and the debris was removed by centrifugation at 600 x *g* for 10 min. The placental lysate was preincubated in the reaction mixture, 100 mM phosphate buffer, pH 7.5, with 7.5 μM adenosylcobalamin at 30°C for 10 min and then the reaction was initiated by addition of 0.4 mM methylmalonyl CoA into the mixture and terminated by 4.2 N perchloric acid after 5 minutes. The chromatographic seperation was achieved in a reversed-phase (C18) system with 220 mM sodium phosphate buffer (pH 4.0), 15% methanol and 0.4% chloroform at 35°C. The eluate was monitored at 254 nm with a detecting limit around 5 *p*mol. The human placenta methylmalonyl-CoA mutase had a specific activity of 6.0 mU/mg protein and its *Km*'s were 1.8 x 10^{-5} M and 1.0 x 10^{-7} M for the substrate methylmalonyl-CoA and the cofactor adenosylcobalamin, respectively. This method offers a quick, sensitive and accurate determination of methylmalonyl-CoA mutase activity and could be adopted to assay the enzyme activity in human lymphoblasts and fibroblasts for differential diagnosis of methylmalonic acidemia.

302 DETERMINATION OF SEPIAPTERIN REDUCTASE ACTIVITY IN ERYTHROCYTE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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From our previous studies, it was found that most tetrahydrobiopterin (BH_4) deficient phenylketonuria(PKU) in Taiwan were caused by "dihydrobiopterin synthetase" deficiency, which may be defect in 6-pyruvoyl-tetrahydropterin synthase or in sepiapterin reductase. A method for determination of sepiapterin reductase activity in erythrocyte was established with the aid of high performance liquid chromatography(HPLC). Hemolysate were prepared by adding potassium phosphate buffer (5mM, pH7.5) to red blood cells (9:1, v/v). The reaction mixture contained Tris buffer (0.1M, pH7.0), NADPH (100μM), and sepiapterin (50μM). The reaction was initiated by adding sepiapterin at 37°C in dim light and stopped by addition of 40% trichloroacetic acid. Dihydrobiopterin (BH_2) produced from the enzyme reaction was oxidized to biopterin by manganese dioxide. After centrifugation, biopterin was analyzed by reverse phase C-18 HPLC with fluorescence detection(Ex.350nm, Em.450nm). The production of biopterin showed a good linearity within 30 min after the reaction started. The optimal pH was found to be around 7.0 with Tris buffer. This HPLC-fluorometric method was more sensitive than the conventional photometric method measured by decreasing amount of sepiapterin. The method might can be applied in aid to differential diagnosis of tetrahydrobiopterin deficient PKU.