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Determination of Propionyl-CoA Carboxylase Activity in Human Lymphocytes by High Performance Liquid Chromatography

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Objective: Propionyl-CoA carboxylase (EC 6.4.1.3; PCC) is a biotin-dependent enzyme which converts propionyl-CoA to D-methylmalonyl CoA mitochondrial matrix. PCC is composed of two subunits, alpha and beta subunit. Defects in either subunits result in deficient PCC activity and lead to propionic acidemia (PA, MIM 232000, 232050). Determination of PCC activity from cell culture or mutation screening on the genes responsible for the alpha and beta subunits of PCC are the two methods of choice to confirm the disease status. Conventional method for PCC activity assay requires radioisotope-labeled reagents and is time-consuming. Previously, methylmalonyl-CoA mutase was shown to be determined with the aid of high performance liquid chromatography (HPLC) for confirmatory diagnosis of methylmalonic acidemia. In this study we applied this non-radioisotope method for PCC measurement to test the application of HPLC in PCC enzyme assay for diagnosis of PA.

Methods: Phytohaemagglutinin (PHA) stimulation of lymphocytes were lysed by sonication 5mM (pH 7.0) phosphate buffer and used for PCC assay. The reaction mixture containing Tris buffer, NaHCO3, ATP, and propionyl-CoA was incubated at 37oC for 60 minutes, and the reaction was stopped by HClO4. After centrifugation, the supernatant was analyzed by reverse-phase HPLC to determine the methylmalonyl-CoA production of the enzyme reaction.

Results: The mobile phase of HPLC was composed of 100mM phosphate buffer (pH 4.0) with 7.5% (v/v) acetonitrile at 1.0 ml/min flow rate using Finepak SIL C18-5 column to separate methylmalonyl-CoA and propionyl-CoA. Normal PHA-stimulated lymphocytes had activities from 0.5 to 0.9 nmol/mg protein/min whilst PHA-stimulated lymphocytes from PA patient had undetectable PCC activity.

Conclusions: We have established a non-radioisotope assay for determining the activity of PCC in PHA-stimulated lymphocytes for diagnosis of PA. This method is clinically applied in the differential diagnosis for an affected baby detected by MS/MS with elevated propionylcarnitine.

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