Purification of Sepiapterin Reductase from Rat Erythrocytes

S.-S. Wang[1]*, T.-T. Liu[2] and K.-J. Hsiao[1,3]

Insts. of Genetics[1] and Biochem[2], National Yang-Ming University; Dept. of Medical Research[3], Veterans General Hospital-Taipei, Taipei, Taiwan 112, R.O.C.

Phenylketonuria (PKU) caused by tetrahydrobiopterin (BH_4) deficiency was found to account for approximate 20% PKU detected in Taiwan. Defect in 6-pyruvoyl tetrahydropterin synthase (PTPS; EC 4.6.1.10), the second enzyme involved in BH_4 synthesis, was found to be the most frequent form of BH_4 deficiency. The product of this enzyme is unstable. In order to establish a direct assay of PTPS activity for differential diagnosis of variant forms of PKU, sepiapterin reductase (EC 1.1.1.153), the down stream enzyme in BH_4 synthesis, was purified for converting the unstable product of PTPS reaction to BH_4 for measurement. The sepiapterin reductase activity in different tissues, including human placenta and RBC, and liver and RBC of chicken, mice, rat, and pig, were measured. The rat erythrocytes were chosen for our purpose because of the highest specific activity (6.0 mU/mg protein) The enzyme from rat hemolysate was purified 240 fold with 6% yield by ammonium sulfate fractionation (20~60%), hydroxyapatite chromatography and nucleotide-analog affinity chromatography (Matrex Red A gel). The specific activity of the punfied enzyme was determined to be 4050 mU/mg protein. The SDS-PAGE analysis of the purified enzyme showed a major band (80%) at the position of 27.5 KDa, which consists with the 55 KDa homodimer as previously reported.

Journal of Biomedical & Laboratory Sciences. 1995;7:A24.

158