Thesis Title Purification of Delta-aminolevulinate Dehydrase from Human Erythrocytes.

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ABSTRACT

Generally, in the industrial field lead has been recognized for years as an environmental pollutant. Lead poisoning is a major health problem of national importance. The adverse effects of lead on behavioral and intellectual developments have been recognized for many years. Recent data suggests that prenatal exposure to lead may be related to minor congenital abnormalities, tumors of kidney, and growth abnormalities. The most serious toxic effects result from the effects of lead on the brain and peripheral nervous system. Recently, most of the studies were suggested that red cell δ-aminolevulinate dehydrase (EC 4.2.1.24) is shown to be a sensitive index of subclinical lead poisoning. Assays for this enzyme will serve both

as a practical screening test for unrecognized plumbism and as an rapid diagnosis of acute lead intoxication.

This study describes a method of purification of δ-aminolevulinate dehydrase from human erythrocytes. Human δ-aminolevulinate dehydrase (5-aminolaevulinate hydro-lyase, EC 4.2.1.24) was isolated and purified from erythrocyte lysate of human outdated blood giving more than 10,000-fold with 48% yield. The purification procedure included DEAE-cellulose chromatography, ammonium sulphate fractionation and gel filtration by sephadex G-200. The purified enzyme had specific activity of 10.5 U/mg of protein, the molecular weight of the native enzyme was estimated to be 290 KDa by gel filtration. Under denaturing conditions ,the dissociated enzyme had a subunit of approximately 36 KDa, indicating that the enzyme was composed of eight apparently identical subunits. The enzyme had pH optimum of 6.8, temperature optimum of 55°C. The study of thermal stability was found that at 55°C, for 90 min, the enzyme activity was reduced to 53%. Km and Vmax were 0.18 mM and 4.26 U/mg respectively.