Purification, partial kinetic characterization and reactive sulfhydryl groups of the phosphoenolpyruvate carboxykinase from Perumytilus purpuratus adductor muscle

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Phosphoenolpyruvate carboxykinase (PEPCK) from the adductor muscle of Perumytilus purpuratus was purified to homogeneity, as determined by SDS-polyacrylamide gel electrophoresis (PAGE). The purification consisted of a three-step procedure: ammonium sulphate precipitation, ion exchange chromatography on phosphocellulose and affinity chromatography on GTP-agarose. The enzyme presented a native molecular mass of 85 kDa, appearing as an active monomer. Under denaturing conditions (SDS-PAGE), the enzyme showed a relative molecular mass of 74 kDa. The specific activity of homogeneous PEPCK in the presence of 2.3 mM Mn2+ was 13.0 U/mg at 25°C. Apparent Km values at pH 7 and in the presence of 2.3 MM Mn2+ were 0.55, 2.4 and 0.045 mM for phosphoenolpyruvate, HCO3 and inosine 5?-diphosphate (IDP), respectively. Apparent Km for GDP was < 0.01 mM. ADP was not a substrate of the enzyme. Inosine 5'-triphosphate (ITP) inhibited the PEPCK activity (IC50 = 1.7 mM), and this inhibition was not reverted