Turkish Journal of Chemistry

Turkish Journal

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Chemistry

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Purification and Characterization of Glucose 6-Phosphate Dehydrogenase from Sheep Liver

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Abstract: Glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate: $NADP^+$ oxidoreductase, EC 1.1.1.49; G6PD) was purified from sheep liver by a simple and rapid method. The purification process consisted of two steps: preparation of the homogenate, and 2', 5'-adenosine diphosphate (ADP) Sepharose 4B affinity chromatography. Through the use of these two consecutive steps, the enzyme was purified with a yield of 35.6% and 1,920 fold, having the specific activity of 11.2 enzyme units (EU/mg protein). A K_M of 0.176 mM and a $V_{\max}$ of 0.0179 EU/ml were obtained for G6-P, and 0.0194 mM and 0.0223 EU/ml for $NADP^+$. Enzymatic activity was measured spectrophotometrically according to Beutler's method at 340 nm and optimal pH and assay temperature were determined. By means of a Lineweaver-Burk plot, the inhibitor constant for NADPH was determined to be K_i, 4.707 \pm 0.49 μ M and it was shown to inhibit the enzyme in a non-competitive manner. The purification of enzyme was monitored by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE showed a single band at \sim 55 kDa for the enzyme and gel filtration chromatography indicated it to be a dimer.

Key Words: Glucose 6-phosphate dehydrogenase, purification, sheep, liver

Turk. J. Chem., **27**, (2003), 395-402.
Full text: pdf
Other articles published in the same issue: Turk. J. Chem.,vol.27,iss.3.