APPLICATION OF DHURRIN FOR KINETICS AND THERMODYNAMIC CHARACTERIZATION OF LINAMARASE (B-GLUCOSIDASE) GENETICALLY ENGINEERED FROM SACCHAROMYCES CEREVISIAE.

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ABSTRACT:
Recombinant Saccharomyces cerevisiae cells at the stationary phase of growth were recovered, homogenized and centrifuged to obtain crude extracts designated as GELIN0. Carboxy methyl cellulose, diethyl amino-ethyl-sephadex and diethyl amino-ethyl-cellulose were used to purify the crude extracts of GELIN0 resulting in GELIN1, GELIN2 and GELIN3, respectively. The ability of the enzyme extracts and a commercial native linamarase (CNLIN) to hydrolyse cyanogenic glucosides was challenged using dhurrin from sorghum as substrate. Precisely, the actions of commercial native linamarase (CNLIN) and the genetically engineered linamarase (β-glucosidase) from Saccharomyces cerevisiae on dhurrin as influenced by degree of its purification, pH (6.8 ) and temperature(30-45°C) were investigated and the data derived were applied for kinetics and thermodynamic characterization of the enzymes. Enzymic degradation kinetics of the dhurrin were evaluated using a 4 x 6 x 8 B/W design comprising of 4 enzyme types (GELIN0, GELIN1, GELIN2 and GELIN3), 6 temperatures (30, 32, 35, 37, 40, 45 °C) and 8 time intervals( 0-70 min.). Data obtained from the residual HCN with time were fitted into zero, first and second order kinetics models to derive reaction rate constant (Kmin⁻¹) values which were analyzed using the Arrhenius and absolute reaction rate models. Thermodynamic parameters were obtained including; activation energies (Ea), frequency factor(Ko), enthalpy (∆H ) and entropy (∆S) that characterized the reactions on dhurrin catalyzed by commercial native linamarase (CNLIN) and the genetically engineered linamarase (β-glucosidase) from Saccharomyces cerevisiae. The results showed that the best fitted order based on higher coefficient of linear regression (r²) values > 0.998 and linearity of curves was the first order kinetics model and not either zero or second order models. Kmin⁻¹ values ranged from GELIN0 to GELIN3 0.03-0.07 µmol/min while the derived D-values from K-values were in the range of 24-65 min. The frequency factors (Ko) increased with enzyme purity from GELIN0 to GELIN3 corresponding to Ko (min⁻¹) of 22.585 to 56.462. The energy of activation Ea (KJ/mol) generated 60.0995 to150.6900 corresponding to enzymes GELIN0 to GELIN3 followed the same pattern with frequency factor for breaking of bonds in dhurrin molecules. At pH 6.8 CNLIN showed no action on dhurrin. The high correlation coefficient values of (r²= 0.97 to 0.99) indicated the best fit of the Arrhenius and the absolute reaction rate models. The entropy change (∆S) increased with enzyme purity from 0.588 J/mol.deg. to 1.4625 J/mol degree. The enthalpy change KJ/mol followed the same pattern whereby increases influenced by enzyme purity ranged from 1892 KJ/mol to 13104 KJ/mol.

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