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Enzymatic activity assay of D-hydantoinase by isothermal titration calorimetry. Determination of the thermodynamic activation parameters for the hydrolysis of several substrates

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Abstract

Isothermal titration calorimetry (ITC) has been applied to the determination of the activity of D-hydantoinase (EC 3.5.2.2) with several substrates by monitoring the heat released during the reaction. The method is based on the proportionality between the reaction rate and the thermal power (heat/time) generated. Microcalorimetric assays carried out at different temperatures provided the dependence of the catalytic rate constant on temperature. We show that ITC assay is a nondestructive method that allows the determination of the catalytic rate constant (k_{cat}), Michaelis constant (K_M), activation energy and activation Gibbs energy, enthalpy and entropy of this reaction.

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Keywords: D-hydantoinase; Isothermal titration calorimetry; Activity assay; Activation parameters

Abbreviations: ITC, Isothermal titration calorimetry; k_{cat} , catalytic rate constant; K_M , Michaelis constant; E_a , activation energy; LB, Luria Bertani; ΔH_m , molar enthalpy.

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