

enzyme

by Enzyme Enzyme

Submission date: 20-Apr-2021 12:23PM (UTC-0400)

Submission ID: 1564783734

File name: Enzymes.edited_1.docx (12.22K)

Word count: 1013

Character count: 5188

Introduction

An enzyme is a protein that acts as a catalyst in the body of human body, and they have some specific reactions. Enzymes provide alternative reactions with the activation energy, which is lower, enabling the response to occur at a high rate. Enzymes only increase the rate, but they do not change the reaction of the equilibria. The summary will show the understanding of the experimental design of enzyme kinetics and the Michaelis-Menten model of enzyme kinetics. The summary will also indicate that the enzymes can be modified by the genetic mutations and inhibition of the kinetics using different types of the inhibitors techniques and the spectrophotometer data analysis of the enzyme measurements of the kinetics.

Lab Theory

Enzymes are essential in the human body as they are required to perform some specific metabolic reactions. A characteristic of the more important enzymes is that they are not used up when the response is taking place. The enzymes enable the catalyzed reaction to be ready to convert another set of substrates. The enzyme that first catalyzes the metabolism of the alcohol in human is the ADH, the Alcohol Dehydrogenase. It catalyzes the oxidation of the substrates like ethanol, whereby the alcohol is converted into acetaldehyde, a toxic substance. The Alcohol Flush Syndrome is a condition experienced by individuals who possess ADH1B*2 isozyme. This condition leads to symptoms incorporated with the hangovers after consuming a few alcohol amounts. The elevated or increased level of acetaldehyde in the blood level. This occurs because the ADH1B*2 activity is higher compared to the ADH1B*1

Enzyme Kinetics

The enzyme kinetics is the study of the mechanism of the enzyme, which is carried out by determining the reaction rates under good conditions. The reaction rate depends on factors like the concentration of the enzyme and that of the substrates, the pH, temperature, and the present inhibitors are some of these factors.

Michaelis-Menten

The Michaelis-Menten is a model which is simple of an enzymatic reaction that was developed in 1913 by Leonor Michaelis and Maud Menten. The model has been based on two assumptions: an enzymatic reaction only proceeds in two steps: that complex enzyme-substrate is formed, ES and enzyme dissociation, and the product. Another hypothesis is that after only a short period, the ES complex concentration reaches a steady state whereby the rate of ES formation equals the speed of its consumption. Michaelis-Menten first assumption implies that four reactions that are different make up the enzymatic reactions. E and S lead to the formation of the ES, the

dissociation of the ES into E and S. The third reaction is the dissociation of ES from E and P.² The reaction rate is measured when the response begins because no significant amount of P has been formed which leads the formation of E and P from ES is ignored.

⁴ The Michaelis Menten equation is $k_m = (k_{-1} + k_2) / k_1$, whereby the v_0 suggests that the equation is only usable in describing the initial rates showing that no amount of product has been formed. This reaction applies to many enzymatic reactions. The equation describes the substrate enzyme reaction, which is catalyzed. The reaction of V shows the concentration change which occurs over time. This rate is expressed as a rate of formation of the products (P) or even as the consumption rate of the reactants. The rate of this reaction is expressed as $V = d[P]/dt = -d[S]/dt$. The k_2 is also called the turnover number. It is also important to measure the reaction rate, which is initial, as it is the reaction rate at the beginning. When the enzymatic reaction occurs during the initial reaction rate, S decreases while there is an increase in P, which leads to a decrease in the reaction rate, which happens overall.

Analyze spectrophotometer data and calculate Km and Vmax.

The K_m is a parameter that is present in the Michaelis –Menten equation. Which equals the concentration of the substrate where the rate of the reaction corresponds to $\frac{1}{2} \cdot V_{max}$. The enzyme that archives the half-maximal velocity has a low K_m at a low substrate concentration. In contrast, a substance with a high K_m requires a high substrate concentration that is high to achieve velocity. The Michaelis-Menten equation showed that the reaction rate, which is initial V_0 , increases when the substrate S is also increased. The maximum speed of V_{max} is always reached when the response is saturated. V_{max} is never fully reached because the enzyme is never saturated fully. The V_{max} can be determined by using the Lineweaver –Burk equation.

Inhibitors

The enzyme inhibitors are the molecules that reduce the activity of the enzyme. They are either reversible or cannot be irreversible. The irreversible inhibitors are responsible for decreasing the enzymes' activity by using various mechanisms in destroying the enzyme. The enzymes are kept functional by the reversible enzymes. The enzyme inhibition is classified into 3 groups: the Competitive inhibitors, the uncompetitive inhibitor, and the mixed inhibitors. The equation for the mixed inhibition is $V_0 = V_{max} \cdot [S] / K_m \cdot \alpha + [S] \cdot \alpha$.

where $\alpha = 1 + [I]/K_I$ and $\alpha' = 1 + [I]/K$ and can also be rearranged.

⁶ The equation for the competitive inhibitor is $1/V_0 = 1/V_{max} + K_m \cdot \alpha / V_{max} \cdot 1/[S]$ where $\alpha = 1 + [I]/K_I$ for the concentration to be calculated, the inhibitor constant, different concentrations must be conducted. The steps of calculating the kinetic parameter when using the competitive inhibitor include preparing the Lineweaver Burk plots using the linear regression method.

The spectrophotometer is the instrument that is used in providing information regarding the intensity of the energy which is radiated. It determines the ratio between the power of the emitted light from a source that is internal and through a presented solution. The concentration of the

molecules which has dissolved ios obtained from the ratio gathered. The absorbency ¹ is calculated as the $\log(I_0/I_t)$, where I_0 represents the intensity of the incident light. In contrast, It is the intensity of the light which passes through the solution, which goes through the detector, which is photovoltaic.

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