Study the Behavior of Activity and Express for Enzymes

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Abstract

Enzymes are be defined as soluble, colloidal, organic catalysts which are produced by living cells. Enzymes are biologic catalysts to accelerate the rate of biochemical reactions.

in this study Quantification of the Enzyme Activity**,** Where: E = enzyme concentration; S = substrate concentration; ES = enzyme-substrate complex concentration; k1, k3= 1st order reaction rate constants (t-1); P = product concentration; k2= 2nd reaction rate constant (M-1.t-1).At first, it must be stressed that the formation of ES (enzyme-substrate complex) is an obligatory step to any type of enzyme-catalyzed reaction. The ES, depending on the reaction conditions, can form the product (forward reaction) or not (reward reaction)**.** The immobilization technique allowed a more frequent use of biomaterials in industry, therapeutics (confection of subcutaneous capsules for controlled delivering of hormones), and lab analysis (automatic dosing equipments, enzyme electrode, biosensors and enzyme immunoassays). In industry, the use of glucose isomerase for the conversion of glucose into fructose (high fructose corn syrup), the aminoacylase for the separation of racemic mixture of amino acids, microbial lipase in triglyceride hydrolysis, and the lactase for removing lactose from milk and/or whey are stressed**.**

Key word : *enzyme , activity , immobilization, Specificity, factors, thyromodynamic*

Introduction

Enzymes are be defined as soluble, colloidal, organic catalysts which are produced by living cells. Enzymes are biologic catalysts to accelerate the rate of biochemical reactions. All enzymes are protein in nature; they are water soluble, precipitated by usual reagents like alcohols, ammonium sulfate and alkaloid reagents.

Most enzymes are present in cells at much higher concentrations than in plasma.

Normal plasma levels reflect the balance between the synthesis and release of enzymes during ordinary cell turnover and their clearance from the circulation, therefore, enzymes can be used as markers to detect and localize cell damage proliferation. The enzyme (holo-enzyme) composed of inactive protein (apo-enzyme) with cofactor which may be organic (coenzyme) or inorganic (elements like Mn, Mg, Fe). If the cofactor linked forced to the protein portion of enzyme then it called prosthetic group (1).

Enzyme unit: the amount of enzyme required for transformation one micromole of substrate (reactant molecules) in one minute. Turnover number: the number of substrate molecules transformed per one minute by a single enzyme molecule

Specificity

Enzymes catalyze a reaction accepting only one substance as substrate. When it accepts two or more substrates, one ofthem is always preferred .The enzyme specificity is determined by the active site, a particular domain of the peptide molecule. There are two main theories seekingto explain the enzyme-substrate interaction. One –proposed by Fischer in the beginning of the 20th century –states that the substrate-enzyme interaction would resemble a key-lock mechanism, in which the key (substrate) fits into the lock (active site), since two conditions are satisfied: the substrate and the active site have complementary structures and compatible polarity and size(2). The other –proposed by Koshland in the 1960–states that the substrate nearsthe enzyme molecule induces on it some structural modifications thatfavorthe enzyme-substrate fitting

Nowadays, it is accepted that the active site can be subdivided into two particular regions, one called bond site (responsible for enzyme specificity) and the other called catalytic site (in which a sequence of well-defined 1st and 2nd order reactions occur transforming the substrate into the product or vice-versa) (3).

Enzyme Actıvıty

When enzymes are used in industrial processes and analytical procedures, the precise evaluation of the enzyme activitybecomes important. Focusing on the industrial scale, the decision to use or not an enzyme in a process must be based on issues such as1) the amount of enzyme required for carrying out the process properly, 2)the duration of the reaction, 3)the amount of substrate to be converted, 4)the conditions under which the reaction occurs, and 5)the overall cost of the process.Once the decision for using an enzyme is taken, and considering that the enzyme has a cost, the impactsof this cost on the overall process must be evaluated (4). Thereby, the increase in product yield and/or the value added to the final product, as well as the reduction of the overall energy consumption,could minimize the effectsof enzyme cost on the overall process cost.Thereby, it is clear that the success of the enzyme process depends on the optimization of three factors, i.e., amount of enzyme needed, operation conditions (pH, temperature, agitation etc.),and reaction yield. Theoretically, as the enzymes are catalysts not consumed during the reaction,a small amount could transform into huge amount of substrate. However, an optimized and finite correlation among the amount of catalyst, its initial activity and the amount of substrate to be converted ntoproduct must be found. Moreover, the duration of industrial processes can take one hour (such as the mashing of malted barley in brewing), three hours (such as the saccharification of the liquefied starch with glucoamylase in syrup production),or about twenty hours (such as the hydrolysis of lactose by lactase inmilk or whey(5).Of course, the enzyme technology aims to perform quick reactions at a low cost, albeit respecting the restrictions imposed by the operational conditions and the scale of the process.

Quantification of the Enzyme Activity

Let us consider the simplest model for an enzyme-catalyzed reaction:(6)

E+SDES®E+P

k1 k2k3

Where: E = enzyme concentration; S = substrate concentration; ES = enzyme-substrate complex concentration; k1, k3= 1st order reaction rate constants (t-1); P = product concentration; k2= 2nd reaction rate constant (M-1.t-1).At first, it must be stressed that the formation of ES (enzyme-substrate complex) is an obligatory step to any type of enzyme-catalyzed reaction. The ES, depending on the reaction conditions, can form the product (forward reaction) or not (reward reaction). An enzymatic reaction can be divided in three phases (Figure 1). During phase I(reaction onset),there is the accumulation of the enzyme-substrate complex (ES) without product formation and significant substrate consumption. The existence of the ES was predicted by Brown in 1892, reinforced by Henry in 1902 and experimentally demonstrated in 1936 (3).The presumption of ESexistence allowed Michaelis and Menten (1913)to postulate the first mathematicalmodel for quantifying enzyme activity.Briggs and Haldane improved itin 1926. Phase IIbegins as soon as the ES concentration reaches a maximum value,remaining invariable for a period. The duration of the steady stateregarding the ES concentration depends on the relative concentrations between the enzyme and the substrate. Along phase II,the substrate is consumed and the product accumulates in the reaction medium. Along the phase III–when the ES concentration is no longer constant –,the substrate consumption and product formation occur slowly. The quantification of the enzyme activity considersthe conditions observed in phasesII and III

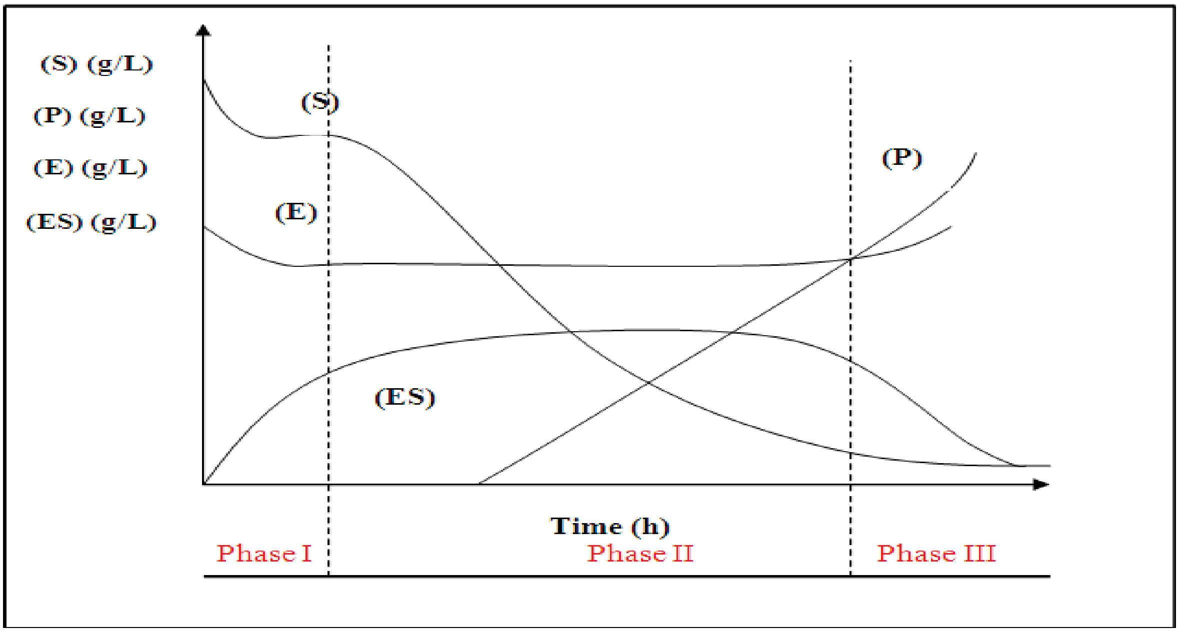

Figure 1: Variation of enzyme (E), substrate (S), product (P) and enzyme-substrate complex (ES) concentrations in relation to reaction time

The starting point for establishing the enzyme kinetic equation is the determination of the variation in the initial concentration of substrate in relation to the reaction time at a fixed amount of enzyme (E0). This is made by measuring the amount of substrate consumed along the period, followed by plotting the data in a (S) =f (t) graph (Figure 2). The inclination of the linear part of each curve related to a determined initial substrate concentration (S1, S2, S3,……Sn) represents the initial reaction rate of the enzymatic reaction (v1, v2, v3,…..vn). By plotting v versus S, the result is a hyperbola, whose curvature tends asymptotically to a maximum v (Vmax), i.e., the reaction rate becomes invariable in relation to the substrate concentration (Figure 3). Under controlled reaction conditions, the Vmax becomes a kinetic constant, which describes the enzyme catalysis quantitatively. The phenomenon of saturation is seldom observed in non-enzymatic reactions. The asymptotic zone of the hyperbola would correspond to the condition in which the amount of substrate is enough to saturate the enzyme molecules present into the reaction medium completely, i.e., the ES concentration into the reaction medium is constant.

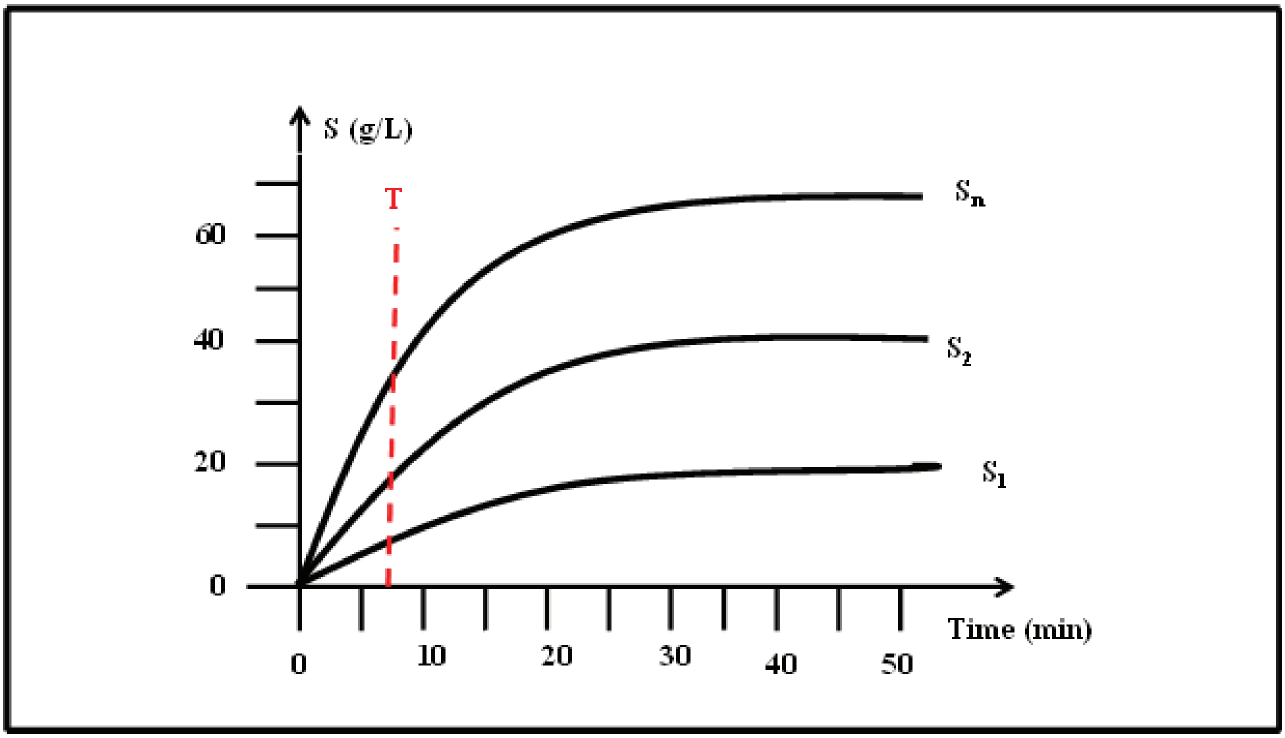

Figure 2: Variation of the substrate concentration *versus* time. The initial enzyme concentration (E0) was constant. At the interval T, the substrate consumption varies linearly with time, i.e., the reaction rate is constant. Therefore, the initial substrate concentration (S1, S2….Sn) was enough to saturate all the enzyme (E0).

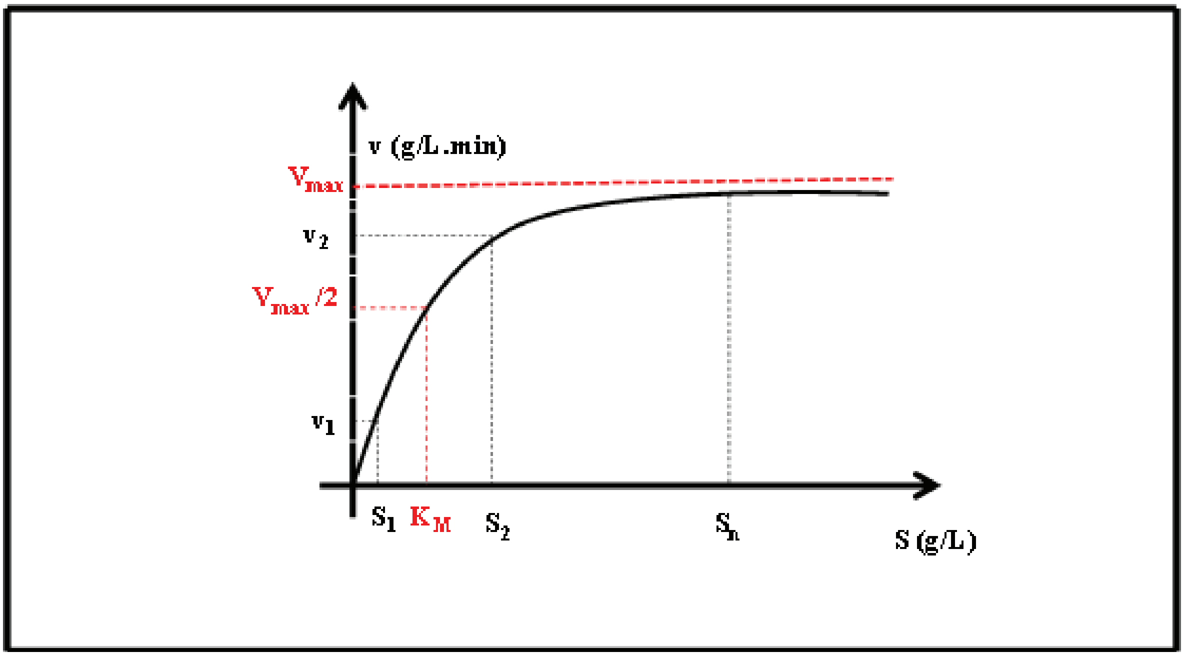

Figure 3: Reaction rate (v) *versus* initial substrate concentration. At the substrate concentration over S2, the curve tends to a maximum value of reaction rate (Vmax), which indicates that all enzyme (E0) is saturated with substrate

Considering (ES → E + P) as the limiting step of the overall reaction, then

$(- dS/dt) = v = k3.(ES)$ (1)

However, remembering that at any time along the reaction, the whole enzyme is distributed as

$E0 = (E) + (ES)$ (2)

Where E0 = total amount of enzyme in the reaction medium; E = amount of enzyme not bound to the enzyme-substrate complex (ES).

When all enzyme molecules are bound to substrate molecules, then (E) = 0, and the equation (2) becomes

$E0 = (ES)$ (3)

Replacing (3) in (1):

$(-dS/dt) = v = k3.E0$ (4)

Equation (4) leads to the conclusion that when the enzyme is saturated with substrate, the reaction follows (4)a "pseudo-zero order" kinetic, i.e., apparently the reaction rate is independent from the substrate concentration. In other words, the reaction rate is directly proportional to the whole enzyme concentration. Under this condition, the k3 is called "turnover number" (kcat), which indicates the number of substrate molecules transformed per unit of time by a single enzyme molecule when the enzyme is the rate-limiting factor(5). Under the saturation condition, kcat.E0 represents the maximum rate of the reaction catalyzed by the enzyme (Vmax). Therefore,

$(-dS/dt) = v = Vmax$ (5)

Integrating Eq. (5):

$(S) = (S0) – Vmax.t$ (6)

Therefore, when the enzyme is saturated by the substrate, the substrate consumption decreases linearly along the period, i.e., the reaction occurs at a constant and maximum rate(6).

Figure 3 – at low substrate concentration (0 →S1) - clearly shows that v varies linearly in relation to S, or in algebraic terms:

$(-dS/dt) = v = k'.(S)$ (7)

Where k' is a 1st order rate constant.

Integrating Eq. (7):

$LnS = LnS0 – k'.t$ (8)

Therefore, Eq. (8) shows that the substrate concentration decreases exponentially with time, when the reaction becomes under unsaturated and substrate concentration decreases (7).

Along phase II, the (ES) remains invariable for a time interval (Figure1). This means that

$d(ES)/dt = 0$ (9)

Consequently,

$k1.(E).(S) = k2.(ES) + k3.(ES)$ (10)

Rearranging Eq. (10):

$(E) = [\mathbf{KM}.(ES)]\ \ (S)$ (11)

Where **KM** = (k2 + k3)/k1.

Replacing Eq. (11) in Eq. (2) [this is valid insofar as the conditions under which the reaction is carried out preserve the full enzyme catalytic activity]:

$(ES) = \{[(S).E0]\ \ [(S) + \mathbf{KM}]\}$ (12)

Replacing Eq. (12) in Eq. (1):

$v = Vmax.(S)/[(S) + \mathbf{KM}]$ (13)

Eq. (13) describes completely the hyperbolic curve shown in Figure 3. The terms Vmax and KM – the so called kinetic constants – characterize an enzyme(8), when the catalysis is carried out under defined conditions (pH, temperature, agitation etc.). These constants are often calculated by the Eq. (14):

$1/v = (1/Vmax) + (1/S).(\mathbf{KM}/Vmax)$ (14)

Equation (14) represents a straight line from which the kinetic constants are calculated.

Regarding KM, three aspects must be stressed: 1) from Eq. (13), when KM = (S), then v = 0.5Vmax; 2) it is considered a referential for setting the operational substrate concentration. When (S) is at least 100 times lower than KM, the reaction occurs in an unsaturated substrate concentration, whereas when (S) is at least 100 times higher than KM, the reaction occurs in substrate saturation; 3) it is considered a characteristic of an enzyme when the catalysis occurs under defined conditions(9).

Along phase III, the (ES) is not constant, but the substrate continues to be transformed into product, although at a lower rate (Figure 1). To evaluate this phase, Eq. (13) must be integrated, resulting in

t = [(S0 – S) – **KM**.Ln(S/S0)] Vmax (15)

Defining substrate conversion (Y) as:

Y = (S0 – S)/S0 (16)

Replacing Eq. 16 in Eq. 15:

t = [Y.S0 – **KM**.Ln (1 – Y)] Vmax (17)

The practical aspect of Eq. (17) is that the duration of the reaction can be estimated from a desired substrate conversion.

Expression of the Enzyme Activity

The enzyme activity can be expressed by several manners. For example, the hydrolysis of sucrose by invertase is expressed as total-reducing sugars formed per minute, the collagen hydrolysis by collagenase is expressed as the viscosity diminution of a standard collagen solution per minute, and the amylase activity expressed as a SKB unit(10),(11),(12). However, it is recommended to express the enzyme activity using International Unit (U), which is defined as the amount of enzyme that catalyzes the formation of 1 mole of (13,14) product per minute in fixed conditions. The specific activity is defined as U/mg protein (if the enzyme preparation is impure), or U/mol enzyme (for pure enzymes) (15).

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Ethical standard: The study was formally approved the research plan by the ethical committee board at the Babylon health directorate.

Informed consent was taken from all the participant patients before being enrolled in the study

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