Properties of enzyme catalysts

Introduction

Enzymes: Enzymes are single or multiple -chain proteins that act as a biological catalysts with the ability to promote specific chemical rxn under the mild condition that prevail in most living organism.

Over-view of Enzymes catalysts

All reaction in the body are mediated by enzymes, which are protein catalysis that increase the rate of reaction without being changed in overall process. Among the many biologic reaction that are energetic possible, Enzyme selectively channel reactant called substrate into useful pathways. Enzynes thus direct all metabolic events.

Enzyme are Protein Catalyst that increase the velocity of the chemical rxn, and are not consumed during the rxn they catalyse. Some type of RNA act like a Enzyme, RNA with catalytic activity are called Ribozymes. Enzymes are protein catalysts, they influence the kinetics but not the thermodynamics of a reaction

Increase the rate of a chemical reaction

Do not alter the equilibrium

Properties of enzymes

Enzyme molecules contain a special pocket called a active site. The active site contain amino acid side chain that create a three dimention surface complementary to the substrate . the active site bind the substrate , forming an enzyme substrate (ES) complex. The ES is converted to enzyme product(EP), which subsequently dissociated to enzyme and product.

Catalytic efficiency: Most enzyme catalytic rxn are highly efficient , proceeding from 103 to 108 times faster than the uncatalysed rxn. Eacg enzyme molecule is capable of transforming 100 to 1000 substrate molecules into product each sec. The number of molecules of substrate converted to product is called the turnover no.

Characterstic of Enzymes

Certain substance is small amount have unique capacity of speedingup chemical rxn without being alter after the rxn, they acceleration the velocity of the rxn without necessary initially it. Substance that behave in this manner are called catalyst or catalytic agent. For eg hydrogen and oxygen do not combine to any appreciable extent under normal atmospheric condition. However unlike platinum , while is inorganic , enzyme are organic compound produce by living organism. Thus we may define enzyme as organic catalyst produced by a organic cell.

The three distinctive characterstic are 1)specificity. 2)high Catalyst rate 3)high capacity for regulation.

A general model of reaction kinetics of biological systems

Dyanamic mathematical model in biotechnology require beside the information require the stoiciometry ok the biological rxn system.. The identification of a priori unknown reaction kinetics is often a critical task due to the non-linearity and (over-) parameterization of the model equations introduced to account for all the possible modulation phenomena. The contribution of this paper is to propose a general formulation of reaction kinetics, as an extension of the Michaelis-Menten kinetics, which allows

limitation/activation and inhibition effects to be described with a reduced number of parameters.

The dianamic model of a perfectly stirred tank bioreactor is usually derived from a mass balance which lead to a differential eq systwm for the concentration vector

) = c0;

r(c(t))= q(c(t))cx0(t)

The matrix ſ€R^m*n contains the information on the stoichiometry of the reaction system and is usually timeinvariant. The biological reactions r € R ^ m are catalysed by the viable biomass, whose concentration is denoted by cx0(t); and the specific reaction rate vector q €R^m is usually a non-linear function of the concentrations. D€ R is the renewal (or dilution) rate and u € R^ n containsthe reactor input/output conditions. There are a large variety of mathematical descriptions of the reaction kinetics available in the literature. A systematic approach is, therefore, necessary to find the best model structure and the best values ofthe model parameters with respect to some imposed criterion. For instance, in terms of model identification, the optimal structure is characterised by minimal correlations between parameters and maximal identifiability properties. In terms of state estimation and control, however, simplicity and (non-)linearity play important roles.[5]

ENZYME CATALYST

Most of the rxn that occur in living organism are catalyst by molecule called enzyme. Most enzymes are proteins (certain RNA molecules also act as enzyme).

An enzyme is in specific in its action. Many enzymes catalyst only the conversion of a particular reactant to a particular product ; other enzyme catalyst only a certain class of rxn(by ester hydrolysis) . Enzyme speed up rxn rate very substantionally and in their absent most biological rxn occur . The molecule an enzyme act on is called the substrate. the substrate bind to a specific active site on the enzymeso form as enzyme substrate complex. Some physiological poison act by binding to active site of an enzyme, there blocking the action of the enzyme. the structure of an inhibitor may resemble the structure of enzyme substrate . Cyanide act by blocking the enzyme cytochrome oxidase.

The single called Escherichia coli, a bacterium that flourished in human colons, contain about 2500 different enzymes .[6]

Enzyme Kinetics

1 Michaelis-Menten Kinetics 2. Lineweaver-Burk Kinetics 3. Hanes-Woolf Kinetics

4. Eadie-hofstee 5. Reversible Inhibition[7]

K1 k2

E+S ↔ ES ↔ E+P

k-1 k-2

E is the free enzyme , S is the substrate, ES is the enzyme substrate complex & p is the product. Thp overall rxn is s gives p. The enzyme is assumed in step 1 and rearranged in step 2. Enzymes can catalyze up to several million reactions per second 2. Enzyme rates depend on solution conditions and substrate concentration. Conditions that denature the protein abolish enzyme activity, such as high temperatures, extremes of pH or high salt concentrations, while raising substrate concentration tends to increase activity. To find the maximum speed of an enzymatic reaction, the substrate concentration is increased until a constant rate of product formation is seen. This is shown in the saturation curve on the right. Saturation happens because, as substrate concentration increases, more and more of the free enzyme is converted into the substrate-bound ES form. At the maximum velocity (Vmax) of the enzyme, all the enzyme active sites are bound to substrate, and the amount of ES complex is the same as the total amount of enzyme. However, Vmax is only one kinetic constant of enzymes. The amount of substrate needed to achieve a given rate of reaction is also important. This is given by the Michaelis-Menten constant (Km), which is the substrate concentration required for an enzyme to reach one-half its maximum velocity. Each enzyme has a characteristic Km for a given substrate, and this can show how tight the binding of the substrate is to the enzyme

In most experimental studies on enzymes kinetics, the enzyme concentrationis much less than the substrate concentration; [E]<<[S]. Hence the concentration of the intermediate ES is much less than that of S, and the steady-state approximation can be used for ES:

[ES]-[E][P]

0=([E] -[E][S])(k[E][S]-[P])-(+)[ES]

If [is the initial enzyme concentration than [E]=[E]+[ES]. since the conc is [E]during the rxn is generally not known while [E] is known , we replace [E] by [E]

The const rate is =-

R=[E][S]-[ES]

R=[E][S]-([S]+[ES]

Since the concentration of the intermediate ES is very small, we have

Usually, the rxn is followed only to a few percent completion and the initial rate determined. Setting the product concentration [P]equal to 0 and [S]equal to [S]

We get as the initial rate r

where the Michaelis – Menten const is defined by . The reciprocal of above eq is

1/r= 1/

Equation 2 is the Michaelis Menten eq , and above eq is the Lineweaver Burk eq. One measure r for several [S] values with [E] held fixed. The constant

Since [E] is known . strictly speaking r is not the rate at t= 0 , since there is a short induction period before steady state condition are establish .

Although many exp studies on enzyme kinetic give a rate law in agreement with the Michaelis Menten eq . the mech ‘ a’ is grossly over simplified. For one thing , there is much evidence that , while the substrate is bound to the enzyme , it generally undergoes a chemical change before being released as product . hence a better model is

E + S ES ↔ EP ↔ E+ P

The above model gives a rate law that has the same form as the Michaelis Menten eq but the const
are replaced const with diff significance . Enzyme rxn are quite fast but can be studied using “ classical” methods by keeping [E] and [S]very slow.

Lineweaver- Burk Equation

The method describe for the determination of is someone complex and therefore simpler method have been devised. Two such method are given below:

First method-a convient means of evaluating and is to plot kinetic data as the reciprocals of v and (S) where v velocity and (S) is the total conc of substrate. such a double reciprocal was proposed by Hans Lineweaver and Dean Burk in 1934. If one take the reciprocal of Michaelis Menten eq, the following eq is formed

This is known as Lineweaver- Burk Equation. This eq is the form y= mx+b, if one condition the variable to b and 1/(s). When one plots a graph against these two variable , a straight line is obtained . the slop of this line

corresponds to and the 1/v intercept corresponds to 1/. Since can be determine from the intercept , the can be calculated .

Second method: another graphical method for the measurement of for experimentel data on V as a measure of (S) makes use of the above Lineweaver- Burk Equation . multiplication

on sides of the by (S) gives:

A plot of verses (S) gives a straight line on axis is and the slope is and can be obtained from intercepy of the slope. A lineweaver – burk plots provide a quick test for adhereance to Michaelis Menten eq kinetic and allows easily evaluation of the critical const . it also allow the discrimination between diff kinds of enzyme inhibition and regulation. A disadventure of lineweaver-Burk plot is that a long extrapolation is often require to determine , which corresponds uncertainty in the result. Consequently , other way of plotting the data are sometimes used.

Alternate plot are based on Hanes-eq: [s]/v

So that v/[s] is plotted against v. The relative merit of the Lineweaver-Burk, Hanes and Edlie-Hoffstee eq for the determination of and are llustrated below in fig. Using the same set of e ixperimental values of v for a series of substrate concentration . it can be seen that the lineweaver-Burk eq gives the unequal distribution of points and greater emphasis to the points at low substrate concentration that are subject to a greater experimental error, whilst the Edlie-Hoffstee eq and a Hans eq gives a better distribution of

points. In the case of the Hans plot , greater emphasis is placed on the experimental data at higher substrate

1)Lineweaver plot 2)Hanes plot 3) Eadie-hofstee plot

Lineweaver burk, Hanes and Eadie hofstee plot the same set of experimental data of the effect of substrate conc. [S] on the initial rate v of the enzyme catalyst rxn.

Reversible Inhibition

Now consider the effect of reversibly-binding inhibitors on an enzyme. If an inhibitor binds reversibly at the same site as the substrate, the inhibition is referred to as competitive. If the inhibitor binds to another site on the enzyme, the binding is described as noncompetitive. These two alternative behaviors may be distinguished by their effects on Lineweaver-Burk or Hanes-Woolf plots. If a reversible inhibitor can bind to the enzyme active site in place of the substrate, it is described as a “ competitive inhibitor.” In pure competitive inhibition, the inhibitor is assumed to bind to the free enzyme but not to the enzyme-substrate (ES) complex. The binding is described as shown below:

Here Ki is the dissociation constant for the EI complex. EI does not react to form E + P, and the enzyme is unable to bind both S and I at the same time. There are several graphical methods for detecting and analyzing competitive inhibition. The Michaelis-Menten, Lineweaver-Burk, and Hanes-Woolf equations can all be modified to include a term that describes the inhibition by I. Choose one of the cases below to consider each of these in more detail: The Michaelis-Menten equation for competitive inhibition is:

The Lineweaver-Burk equation for competitive inhibition is:

The Hanes-Woolf equation for competitive inhibition is:

Noncompetitive Inhibition

If a reversible inhibitor can bind to the enzyme at a site that is distinct from the active site, it is described as a " noncompetitive inhibitor." In pure noncompetitive inhibition, the inhibitor binds with equal affinity to the free enzyme and to the enzyme-substrate (ES) complex. The binding is described as shown below:

Here Ki is the dissociation constant for either the EI complex or the IES complex. Neither of these complexes can react to form E + P.

There are several graphical methods for detecting and analyzing noncompetitive inhibition. The Michaelis-Menten, Lineweaver-Burk, and Hanes-Woolf equations can all be modified to include a term that describes the inhibition by I. Choose one of the cases below to consider each of these in more detail:

The Michaelis-Menten equation for noncompetitive inhibition is:

The Lineweaver-Burk equation for noncompetitive inhibition is:

The Hanes-Woolf equation for noncompetitive inhibition is:

Limiting Kinetics of Enzyme-Catalysed Reactions

At very low concentrations of substrate many enzyme-catalysed reactions display approximately second-order kinetics, with rate given by the following equation:

v = kA [E]0 [A] . . . . . in which the symbol kA (or, in general, kR for a reactant R) is the apparent second-order rate constant or specificity constant and [E]0, which may also be written as [E]t or [E]stoich, is the total or stoichiometric concentration of catalytic centres. The rationale for the subscript 0 is that the total enzyme concentration is normally the concentration at the instant of mixing, i. e. at time zero. Conversely, at very high substrate concentrations the same reactions commonly display approximately first-order kinetics (zero-order with respect to substrate): v = k0 [E]0 . in which k0, which may also be written as kcat is the apparent first-order rate constant. Although these limiting types of behaviour are not universally observed, they are more common than Michaelis-Menten kinetics)and provide a basis for classifying inhibitory and other effects independently of the need for Michaelis-Menten kinetics.

The quantity k0[E]0 is given the symbol V and the name limiting rate. It is particularly useful when k0 cannot be calculated because the total catalytic-centre concentration is unknown, as in studies of enzymes of unknown purity, sub-unit structure and molecular mass. The symbol Vmax and the names maximum rate and maximum velocity are also in widespread use although under normal circumstances there is no finite substrate concentration at which v = V and hence no maximum in the mathematical sense. The form Vmax is convenient in speech as it avoids the need for a cumbersome distinction between ‘ capital V’ and ‘ lower case v’. When a true maximum does occur the symbol vmax (not Vmax) and the name maximum rate may be used for the true maximum value of v but care should be taken to avoid confusion with the limiting rate.

Enzyme Mechanism:

Enzyme kinetic studies, together with the other typeof investigation have led to inside into the way in which enzyme exert their catalytic action. aspects of this are of special interest . This specificityof enzymes is explained in terms of the an elaborating of Fischer's " lock and key" which is concerned with the way in which an enzyme and the substrate fixed together in forming a Enzyme substrate complex and in undergo subsequent rxn . the second aspect is the very high effectiveness of enzyme in comparison with other catalyst. the high effectiveness of catalyst almost always is associate with a low energy of activation for the rxn. in some cases the effect has been attributed to the fact that the enzyme is acting as a bifunctional catalyst, in tha two catalytic group are presentside by side at the active centre.

Transition state : When a chemical reaction occurs, the energy content of the reacting molecule or atom increases. This is why most chemical reactions, whether they release heat or absorb heat, happen faster as the temperature is raised. The high-energy state of the reactants is called the transition state. For example, in a bond-breaking reaction, the transition state may be one where the reacting bond, although not completely broken, is vibrating at a frequency high enough that it is equally likely to split apart as to reform. Forming reactants or products results in the loss of energy from the transition state. This principle is shown in Figure 1 , where the increased energy of the transition state is represented as a hill or barrier on the energy diagram. Catalysts reduce the height of the barrier for achieving the transition state.

General Catalytic Mechanism

Catalysed reaction occur by a wide variety of mechanism. There is however one pattern that applies to a no of single – substrate rxns catalysted by surfaces, enzymes, acids and bases. It is useful to consider this schmene of rxn first show as to appreciate the similarities that exist between certain rxn that are catalysted by different type od catalyst.

The rxn schme:

$C + S \leftrightarrow X + Y$

$X + W \leftrightarrow P + Z$

Here C represent the catalyst and s is the substrate; X and Y are intermediate , the first of which undergoes a second rxn with a species W to give final product or products P together with the addition substance Z. This scheme shows only the kinetically significance reactions; the substate Y and Z undergo other process that do not have any effect on yhe kinetic behaviour. To simplify the treatment it is assumed that the second rxn does not in the reverse direction ; this can b ensured if the product P is removed as far as it is formed.

In surface catalysis X is an adsoption complex, Y and W are non exitance. The const in this case are first order rate const, while is the second order const. In catalysis by acid and base however Y and W play important role. Thus i c is an acid catalyst, rxn 1 involces the transfer of a proton to S, so that Y is the base conjugate to the acid C. In acid catalyst the intermediate X is the protonated substrete SH+ and a rxn 2 is proton is transferred to a

species W. The species W therefore has basic properties and it may b molecule of a solvent and a solute. For eg It may be the species Y formed in 1 step . we will see that the kinetic behaviour depends in an important way on wheather the intermediate X transfers its proton to solvent molecule or to a solute molecule.

Conversely in base catalyst Y is the acid conjugate to the base C. the intermediate X is the substrate Molecule minus a proton., and in rxn 2 it accepts a proton from W. again we have a solvent molecule or a solute molecule. in some situations a rate with which a intermediate X undergoes rxn 2 may be sufficiently slow that the first rxn may b regarded as being at equilibrium. the exact condition for this is [W][X][Y]. since this case corresponds to Arrhenius concept of an intermediate in eq with the reactants, such intermediate have being called Arrhenius intermediates.

The converse case is that the condition is [X][Y]the concentration of X is small and the steady state treatment may be appliedto it. Intermediate of this kind have been called Van't Hoff intermediates. If neither of this of this extreme condition applies , the kinetic situation is more complicated , and the appropriate differential eq have to be solved. Only the equolibium and steady-state treatments are considered here.

Equilibrium Treatment : Arrhenius intermediates

In this case the equation :

Applies. However , the concentration of C and S do not correspond necessary to yhe initial concentration [C] and [S]. Since appreciable amount of C and S have been used to form a intermediate X. These initial concentration may be expressed as

[C]=[C] + [S]

[S]= [S] + [X]

As long as attention is confined to initial rates. Eq a become

This is quadratic in [X] and can be solve for [X]. Yhen the expression for rate equal to k[X][W] , can be written down. However it os, more useful to consider two special cases.

Case 1: if the initial conc of the substrate is much greayer than that of yhe catalyst, that is, if [S][C], it follows that [S] – [X] is very close to [S], since [X] cannot exceed [C]. Eq b there fore reduces to :

And thus

The rate of rxn therefore

V=[X][W]=

This rate eq correspond toa variation of rate of all type represent . At lower substrate conc , when K[S][Y], the rate become independent of [S], as long as the condition [S]>>[C] holds, however the rate varies linear with [C].

This type of behaviour is characteristic or single-substrate rxn on surfaces and of enzyme rxn.

For both of these the species Y and W are nonexistence and eq c become:

The eq is also eq to the Michaelis Menten eq for enzyme rxn. This eq usually written

Where is the Michaelis const , is equal to 1/k in the present treatment.

In rrn catalysed by acid and base it will be seen that the rate remain linear with the substrate concentration ; this is because of the special type of equilibrium that are rapidly established in the sol.

Case 2: on the other hand , if the catalyst is greatly in excess of the substrate, that is [C]>>[S] eq a reduces to

And the rate of rxn is

The rate now varies linearily with the concentration of substrate , but the variation with the catalyst concentration .

(b)The rate of reaction as a function of substrate conc for the case in which [S]>>[C]
(a) rate of rxn as a function of catalyst concentration for the case[C]>>[S]

Steady-state Treatment: Van, t hoff intermediate

If the condition [W]>> applies, the concentration of X is small and the steady – state treatment is available. The steady-state is

substitution of [C] -[X] for [C] and of [S]-[X] for [S} gives

since [X] is very small the term in can be neglected ; with its approximation above eq gives

the rate is therefore

this eq again indicates that at low conc of either catalyst or substrate the rate is proportional to either [C] or [S] ; at a higher concentration of either the rate become independent of that concentration .

in catalyst by surface and enzymes , W and Y are nonexistence and the rate eq become

An eq of essentially this form was first derived by Briggs and Haldane for enzyme reaction

Catalyst By Enzyme

Catalysis by enzyme , the biological catalyst , is much more specific than that by acids and bases. soe enzyme shows absolute specificity ; an eg is urease, which only the catalyzed of Urea.

A lower degree of specificity is shown by such enzyme as the proteolytic enzymes, which catalyst the hydrolysis of the peptide linkage provided that certain structural condition are specified in the neighbourhood of the linkage.; this is known as group specificity. many enzyme exhibit stereochemical specificity, in that the catalyze the rxn of one stereochemical form and not the other. the proteolytic enzyme. The enzymes are protein but may be associated with non protein substance that essential to the action of enzyme. the action of enzyme shows some resemblance to the catalytic action of acids and bases but is more complicated. the present treatment of enzyme kinetics is confined to the influence concentration, ph, and temperature and to some brief comments about enzymes mechanisms.

Measurement of the kinetics of biological systems at elevated temperatures utilizing flow techniques

Continuous flow-type reactors have been used to study the kinetics of biological systems for quite some time. For continuous media sterilization, tubular flow reactors are particularly useful being simple in character and easy to control. However, one aspect quite often neglected in sterilization calculations is the residence time distribution of the reactor system. Serious errors in estimating the degree of bacterial destruction can be encountered if the residence time distribution is neglected; especially when a high degree of destruction is desired. This paper reports a study made to characterize and use the residence time distribution of a tubular reactor in the interpretation of high-temperature, short exposure time data for inactivation of Bacillus stearothermophilus spores. Mathematical models accounting for the residence time distribution of the tubular reactor have been proposed and employed to obtain high-temperature death-rate data.[14]

Result

Since enzymatic reactions are so important to biological chemical reactions, it is of great interest to be able to model them. By use of the study of chemical kinetics, it is possible derive rate equations for the steps involved in an enzymatic reaction. These rate equations are differential equations and can be used to model theconcentrations of each compound in the system. However, this system of differential equations is hard to determine experimentally because of the difficulty of determining the rate equations into theMichaelis-Menten enzyme equation. Many benefits stem from this transition. One benefit is the fact that it is now easy to determine the constants related to the enzyme equations. However, how do we know the

Quasi-Steady-State Assumption is valid? It seems reasonable from a physical argument. Byuse of dimensional analysis, we can give a more rigorous mathematical argument for the Quasi-Steady-State Assumption. The Michaelis-Menten enzyme equation is very important in the study of cellular systems by allowing a model that can be easily derived through experimentation.

Summary

Enzymes are single or multiple -chain proteins that act as a biological catalysts with the ability to promote specific chemical rxn under the mild condition that prevail in most living organism. All reaction in the body are mediated by enzymes, which are protein catalysis that increase the rate of reaction without being changed in overall process . than properties of catalyst in which Enzyme molecules contain a special pocket called a active site. Than the characterstic of enzymes where enzyme are organic compound produce by living organism. Thus we may define enzyme as organic catalyst produced by a organic cell. Then we studied the Enzyme Kinetics where studied the five equation: 1 Michaelis-Menten Kinetics 2. Lineweaver-Burk Kinetics 3. Hanes-Woolf Kinetics 4. Eadie-hofstee 5. Reversible Inhibition. Than we studied the enzyme mechanism where studied two equations theSteady-state Treatment: Van, t hoff intermediate and the Equilibrium Treatment : Arrhenius intermediates.