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Enzyme kinetics

(theory)

Enzyme kinetics describes the influence of different factors(temperature, pH, the presence of catalysts and inhibitors, the concentration of reactants, etc.) on the velocity of enzymatic reaction. The effect of the most important factors on the velocity of enzymatic reaction has been discussed below.

The effect of temperature

The velocity of the enzymatic reaction in a certain temperature range (0-40ºC) increases with the temperature. Usually, the rise of the temperature by 10ºC doubles the velocity of enzymatic reaction and it is characterized by Van't Hoff factor.

Most of the enzymes loses irreversibly the activity above 65ºC, because of the thermal denaturation of enzyme proteins.

The increase of the velocity of the reaction under the influence of the temperature is a result of activation of substrate molecules. This energy - called energy of activation – causes loosening of bonds in reacting molecules and it is used to overcome the intermolecular repulsive forces.

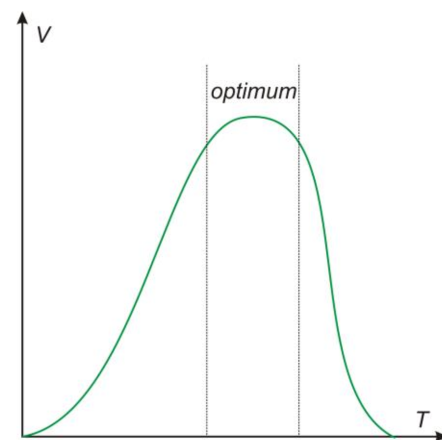
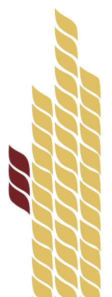


Fig. 1. Dependence of the velocity of enzymatic reaction (V) on temperature (T)

Enzymes decrease the energy of activation and decrease the energy barrier between the reacting molecules. The reduction in energy of activation, even small, leads to significant increase of the velocity of the reaction. For instance, during the breakdown of H_2O_2 to O_2 and H_2O, the energy of activation without the catalyst is 75 kJ/mol, while in the presence of catalase (enzyme) only 23 kJ/mol, and the reaction is faster 3×10^{11} times.

Optimal temperature is dependent on the time of incubation, pH, the concentration of salt, the presence of activators and inhibitors. The activity of the enzyme in optimal temperature is not constant, but it decreases with time as the incubation proceeds.



This phenomenon has several causes:

- increase of velocity of the back reaction caused by the accumulation of products,
- inhibition of activity by products,
- decrease of the concentration of substrate,
- changes in the pH of the environment of the reaction, etc.

✲ Van't Hoff factor indicates how many times the velocity of the reaction increases, while the temperature increases by 10°.

$$Q_{10} = \frac{k_{T+10}}{k_T}$$

where: k_T – reaction rate constant in the initial temperature

k_{T+10} – reaction rate constant in the 10° higher temperature

The effect of pH

Optimal pH for every enzyme is maintained thanks to the presence of buffers. It has been documented, that the composition and the concentration (ionic strength) of the buffer is extremely important during the determination of optimal pH, which may be different within different buffers. Usually, the velocity of enzymatic reaction is inversely proportional to the square of ionic strength. The components of the buffer may activate or inhibit the activity of the enzyme in an indirect or direct way. An indirect effect is associated with the impact of buffer components on the active site of the enzyme, while a direct effect is associated with the ionization of groups outside the active site.

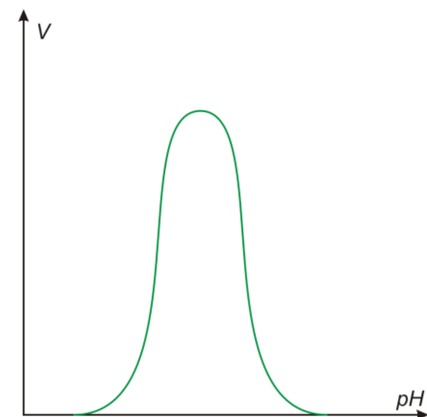
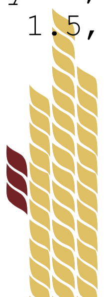


Fig. 2. Dependence of the velocity of enzymatic Reaction (V) on pH

The components of the buffer may also react with cofactors, for example they can bind cations required for the activity of enzymes (e.g. calcium ions binded by orthophosphate derived from phosphate buffer). It is also important to pay attention to the buffer capacity - especially when acidic products are created during a reaction, because these products may lead to exceed the buffer capacity and significantly change the pH.

Most of the enzymes are inactive below pH 4 and above pH 10 (extremely high or low pH values generally result in complete loss of activity for most enzymes because of the denaturation of the protein). Optimal pH for enzymes derived from tissue extracts is in the environment close to neutral. There are also enzymes, which have extreme optimal pH, for example: pepsin – pH 1.5,



alkaline phosphatase – pH 9.5.

The effect of activators

Different kind of activators, that do not participate in the catalytic reaction, make enzymes active or increase their activity. They may be divided into 3 groups:

1. Ions of some metals, which are embedded in apoenzyme molecule.
2. Macromolecules, like proteins, which acts via exposure of active groups.
3. Small-molecule organic compounds that remove the effect of inhibitors.

Many trace elements are essential nutrients for organisms, because they play role as activators of individual enzymes.

Usually, enzymes are activated by following ions:

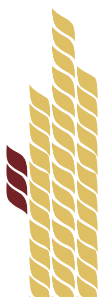
1. Mg^{2+} (phosphatases, phosphorylases, phosphokinases, synthetases);
2. Zn^{2+} (carbonic anhydrase, lactate dehydrogenase, alcohol dehydrogenase, proteases);
3. Mn^{2+} (peptidases, arginase);
4. Ca^{2+} (lipase);
5. Cu^{2+} (oxidase);
6. Sometimes: Fe^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Na^{+}, K^{+}. Cations of heavy metals have an inhibitory effect.

Anions have a small influence on the activity of enzymes. Chloride ions (Cl^{-}) are an exception – they are an activators for amylase.

Sometimes, enzymes are secreted in inactive form, which does not have catalytic properties – in the form of precursors (proenzymes). For example, trypsinogen turns into an active form, trypsin, under the influence of activator – proteolytic enzyme enteropeptidase (enterokinase).

The activity of the plenty of enzymes undergoes the inhibition process in an easy way. The agents that inhibit an enzymatic reaction are called inhibitors, and they are divided into 3 groups:

1. Competitive inhibitors
2. Noncompetitive inhibitors
3. Uncompetitive inhibitors



Competitive inhibitors (Fig. 3-4) are characterized by the structure similar to substrate. Thanks to this they can compete with the substrate for place in active site of the enzyme and combine only in this place. As the consequence of these properties is the dependence of the degree of inhibition on the inhibitor concentration and the reversibility of such inhibition.

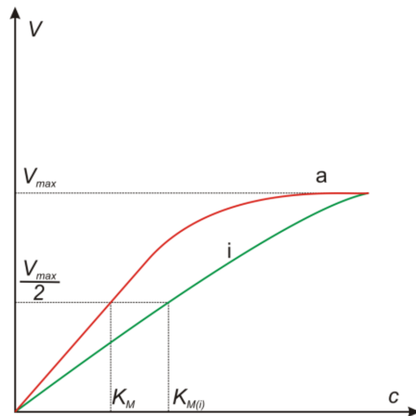


Fig. 3. Michaelis-Menten chart in the presence of competitive inhibitor (i) and the lack of inhibitor (a)

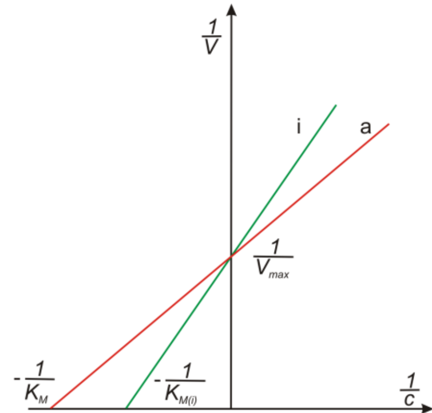


Fig. 4. Lineweaver-Burk chart in the presence of competitive inhibitor (i) and the lack of inhibitor (a)

K_M value increases, V_{max} does not change, and K_M/V_{max} ratio increases.

Noncompetitive inhibitors (Fig.5-6) may have different structure. They connect not only with the enzyme, but also with the enzyme-substrate complex, which blocks its transformation and breakdown. In this case K_M value does not change, V_{max} decreases, and K_M/V_{max} ratio increases.

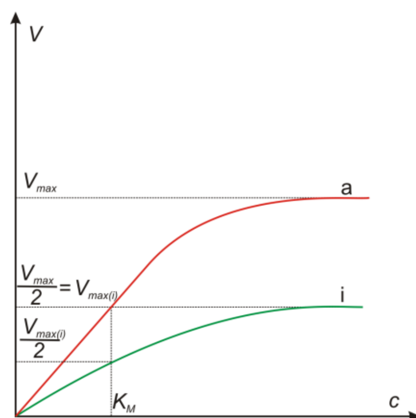


Fig. 5. Michaelis-Menten chart in the presence of noncompetitive inhibitor (i) and the lack of inhibitor (a)

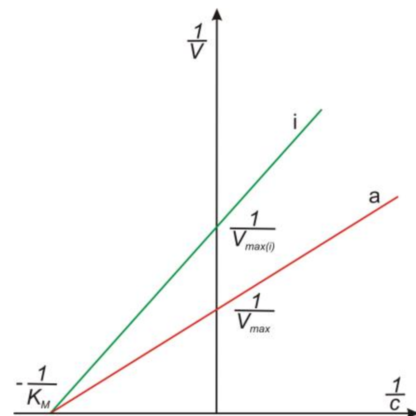
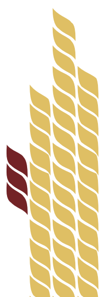


Fig. 6. Lineweaver-Burk chart in the presence of noncompetitive inhibitor (i) and the lack of inhibitor (a)



Uncompetitive inhibitors combine only with the enzyme-substrate complex. K_M value decreases, V_{max} decreases, and K_M/V_{max} ratio does not change.

✲ Practically, many enzymes exhibit intermediate properties lying between the competitive and noncompetitive inhibition. This phenomenon is called mixed inhibition.

✲ The type of inhibition (competitive or noncompetitive) may also be specified using Dixon's method. The velocity of enzymatic reaction at different concentration of the inhibitor is determined at constant substrate concentration. The dependence of $1/V$ on the inhibitor concentration (c_i) is plotted repeating the measurements for other substrate concentration (c_s) (or a few). The intersection of the curves of different concentrations determines the constant of the reaction of combining the inhibitor with the enzyme (creating inhibitor-enzyme complex).

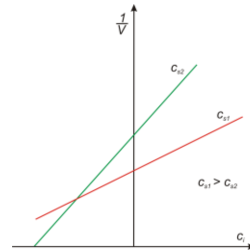


Fig. 7. Dixon's chart for a competitive inhibitor

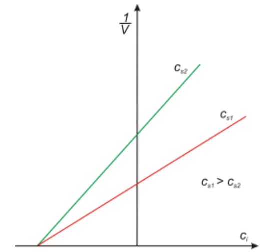
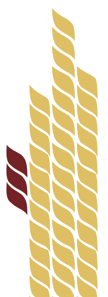


Fig. 8. Dixon's chart for a noncompetitive inhibitor

The effect of the concentration of the enzyme and time

It is most convenient to observe the dependence of the velocity of enzymatic reaction on the enzyme concentration at high concentrations of the substrate. Then, the velocity is independent from the substrate concentration („0"-order reaction) and it is proportional to the enzyme concentration (at not high enzyme concentration).

In the reaction mixture under defined conditions, optimal for the enzymatic reaction, the activity of enzymes only in a certain time range has a constant value (the velocity of the reaction is a function of a straight line) and then it decreases. As a result of this, the concentration of the processed substrate (created product) is not proportional to enzyme concentration anymore. Therefore, it is important to conduct the measurements at the period of time, when the velocity of the reaction has constant value (the reaction has „0"-order kinetic) or accomplish the measurements of so-called "initial velocity of the reaction". The initial velocity is independent from the order of the reaction.



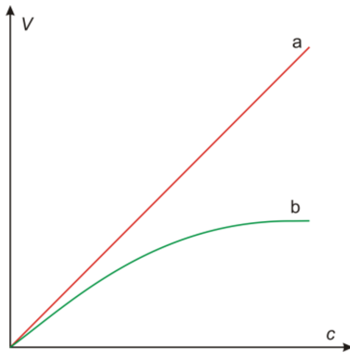


Fig. 9. Dependence of the velocity of enzymatic Reaction (V) on the concentration of the enzyme (c) *at 2 different concentrations of substrate (enzyme saturated (a) and unsaturated (b) with a substrate)*

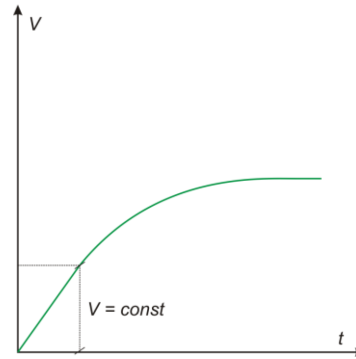


Fig. 10. Dependence of the velocity of enzymatic Reaction (V) on time (t)

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