UNIVERSITY of LIFE SCIENCES in Lublin
FACULTY OF VETERINARY MEDICINE

DEPARTMENT OF BIOCHEMISTRY
20-033 Lublin, Akademicka 12
Phone +48 81 445 66 08
www.biochwet.up.lublin.pl

Enzyme kinetics

(theory)

Usually, one molecule of the enzyme can convert 1000 molecules of the substrate within 1 s. Inside the cell – in an environment in which reactions take place – practically, the enzyme molecules can't move, while small-molecule substrates move in a sol at a rate similar to that observed in aqueous solutions. These random molecular movements make the substrates move to the active sites of the enzymes, where they are transformed into products. Therefore, the rate at which the enzyme binds with the substrate depends primarily on the substrate concentration in the cell.

1. Effect of substrate concentration

The velocity of enzymatic reaction (*V*) increases as substrate concentration increases (*c*). At a certain concentration range (in one-substrate reactions), the reaction rate is linearly dependent on the substrate concentration. This dependence is presented in the Michaelis-Menten chart. The curve of dependence V from c is a hyperbola. At high substrate concentration the velocity of the reaction reaches the maximum value (Vmax). Subsequent increase of substrate concentration does not increase the reaction rate.

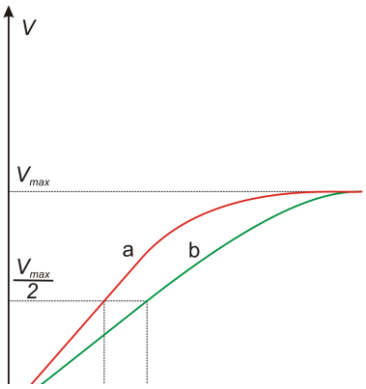

Fig. 1. ***Michaelis-Menten chart showing the dependency of the velocity of the reaction (V) on substrate concentration (c) for the enzyme, which has different affinity to substrates (a and b).***

The concentration of the substrate at which the velocity of the reaction reaches half the maximum speed is called the Michaelis constant, described by the symbol K_M. It is a measure of the affinity of the enzyme for the substrate. It is most often expressed in µmol/dm3.

In many cases, the enzyme can convert several substrates, but has different affinities for each of them (Fig. 1). The individual substrates are easier - or more difficult - to bind in the active site of the enzyme. In case **a**, the reaction reaches the maximum velocity at low substrate

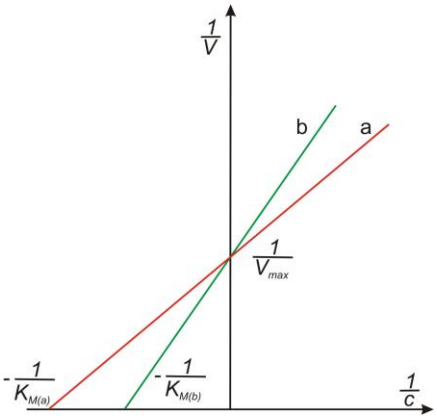

Fig. 2. ***Dependency of the velocity of enzymatic reaction (V) on substrate concentration (c) according to Lineweaver-Burk) for the enzyme, which has different affinity to substrates (a and b).***

concentration. The Michaelis constant is low.

In case **b**, the reaction reaches the maximum speed at high substrate concentration. The K_M is high.

The dependence of the reaction rate on substrate concentration can be presented in the form of a **Lineweaver-Burk** plot (Fig. 2). O the y axis the inverse of the reaction velocity is marked, on the x axis - the substrate concentration. The dependence is linear. The straight line intersects both axes at two characteristic points, the y axis at the point corresponding to the inverse of the V_{max}, and the x axis at the point corresponding to the negative inverse of the Michaelis constant.

Another example of the use of Michaelis constant: there are two enzymes in the mammalian body that catalyze the same reaction. These are: glucokinase characteristic for liver cells and hexokinase, occurring in other tissues. Both enzymes catalyze the conversion of glucose into glucose-6-phosphate, which enables intracellular further glucose conversions. Hexokinase is characterized by a low value of K_M and V_{max}, thanks to which it is active at low glucose concentration, which allows tissues such as muscles to continuously take up and manage glucose. Glucokinase present in the liver is characterized by a high value of K_M and V_{max}. Thanks to this, the enzyme is practically inactive at low glucose concentration, while it activates effectively with the increase of blood glucose, which takes place primarily after a meal full of carbohydrates. Temporary glucose excess is stored in the liver in the form of glycogen. In this way, the body has a mechanism to regulate the level of glucose in the blood independent of hormonal regulation.

✲ Enzymatic reactions are complex cascades of several indirect reactions of different velocity. In 1913 Michaelis and Menten implied the followed equation:

$$E + S \underset{v2}{\overset{v1}{\rightleftharpoons}} ES \underset{v4}{\overset{v3}{\rightleftharpoons}} E + P$$

[E] – enzyme concentration, [S] – substrate concentration, [P] – product concentration and [ES] – enzyme-substrate complex concentration, v_1 – velocity of the formation of enzyme-substrate complex, v_2 – velocity of the dissociation of enzyme-substrate complex, v_3 – velocity of the dissociation of enzyme-product complex, v_4 – velocity of the formation of enzyme-product complex.

Corresponding velocities of the reactions can be shown thus:

$$v_1 = k_1[E][S];\quad v_2 = k_2[ES];\quad v_3 = k_3[ES];\quad v_4 = k_4[E][P]$$

k_1 – reaction rate constant E + S → ES, k_2 – reaction rate constant ES → E + S, k_3 – reaction rate constant ES → E + P, k_4 – reaction rate constant E + P → ES.

ES complex is forming with the velocity $v_1 + v_2$, and it dissociates with the velocity $v_2 + v_3$. In the equilibrium state one can assume that both sums are equal: v1 + v2 = v2 + v3. The substitution leads to:

$$k_1[E][S] + k_4[E][P] = k_2[ES] + k_3[ES]$$

$$[E](k_1[S] + k_4[P]) = [ES](k_2 + k_3)$$

$$\frac{[ES]}{[E]} = \frac{k_1[S] + k_4[P]}{k_2 + k_3} = \frac{k_1[S]}{k_2 + k_3} + \frac{k_1[P]}{k_2 + k_3}$$

Initial concentration of the product [P] is very low, so you can omit it:

$$\frac{[ES]}{[E]} = \frac{k_1[S]}{k_2+k_3}$$

$$\frac{[ES]}{[E][S]} = \frac{k_1}{k_2+k_3}$$

The velocity of enzymatic reaction is dependent on the enzyme-substrate complex concentration [ES]. Reaction rate constant can be shown as:

$$K = \frac{k_2 + k3}{k_1} = \frac{[E][S]}{[ES]}$$

This is Michaelis constant with its corresponding symbol: K_M.

In the equilibrium enzyme concentration [E] is equal to free (not bound) enzyme concentration in the complex [ES], [E] = [E]-[ES], therefore:

$$K_M = \frac{([E]-[ES])[S]}{[ES]}$$

After transformation you get:

$$[ES] = \frac{[E][S]}{K_M+[S]}$$

Dividing by [S] results in:

$$[ES] = \frac{[E]}{\frac{K_M}{[S]}+1}$$

The velocity of enzymatic reaction mainly determines enzyme concentration and speed of transformation of enzyme-substrate complex [ES] into a product. This is described by the following equation: $v = v_3 = k_3[ES]$. Comparing the above equations, you get:

$$v = k_3 \frac{[E]}{\frac{K_M}{[S]}+1}$$

When substrate concentration [S] is significantly greater than K_M, you get:

$$v = k_3[E]$$

This equation describes the maximum reaction rate, V_{max}. Therefore the equation rearranges to:

$$v = \frac{V_{max}}{\frac{K_M}{[S]}+1}$$

The transformation of above equation leads to:

$$(V_{max}-v)(+[S]) = (V_{max}K_M)$$

This relationship is called Michaelis-Menten equation and has the same form as the equation for a rectangular hyperbola, which aims to achieve the V_{max} value.

When substrate concentration [S] is equal to K_M, the Michaelisa-Menten equation is:

$$v = \frac{V_{max}}{2}$$

This leads to graphic description of Michaelis constant K_M. Unfortunatelly, this method is inconvenient, because it does not give confidence, wheter V_{max} was achieved. To help the determination of the K_M, let's transform the Michaelis-Menten equation into a linear form:

$$\frac{1}{v} = \frac{\frac{K_M}{[S]}+1}{V_{max}}$$

$$\frac{1}{v} = \left(\frac{K_M}{[S]}+1\right)\frac{1}{V_{max}}$$

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} + \frac{1}{[S]}$$

The above equation is called the Lineweaver-Burk equation.

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