Influence of enzyme concentration on the Michaelis-Menten constant: A new theory

Yassin Fadel

yassinfadel44@gmail.com

ABSTRACT:

This study proposes a novel theory that challenges the traditional view of the Michaelis-Menten constant (Km) as a fixed value for a given enzyme-substrate pair. We explore the possibility that Km can be modulated by enzyme concentration.

By analyzing the relationship between initial reaction velocity and substrate concentration at different enzyme concentrations, we observed a change in the apparent affinity constant (designated as Km1 and Km2). This seemingly contradicts the established theory , and we have proposed a new theory to explain this variation.

INTRODUCTION:

In the context of enzyme kinetics, it has traditionally been argued that the Michaelis-Menten constant (Km) is independent of enzyme concentration. This paradigm was based on the idea that, under standard conditions, Km represents a specific constant for an enzyme, reflecting its affinity for the substrate. Thus, it was assumed that, regardless of changes in enzyme concentration, the value of Km remains constant, representing an intrinsic characteristic of the enzyme-substrate relationship. (1)

In this communication, I will propose a theory suggesting that enzyme concentration influences the value of Km.

This theory has the potential to open new avenues for research that could explain the Cytotoxicity of medication in more detail. In terms of applicability to allosteric enzymes, it allows us to consider the possibility of creating metabolic pathway blockers by increasing the enzyme concentration sufficiently so that [S] cannot reach Km.

Methodology :

This paper is based on a contradiction between two fundamental principles of biochemistry, namely:

1. When enzyme concentration increase increases, the fractional change in v is less than the fractional change in Vmax. (2 p. 9)
2. Km is constant regardless of the enzyme concentration.

According to the first principle, as Vmax increases up to Vmax', Vmax/2 will increase to less than Vmax'/2, which means that the value of Km increases with the enzyme concentration.

In this paper, we will attempt to explain why when the first principle is valid, the second one is not.

THEORY:

$$\frac{VMAX}{\frac{VMAX}{a}} = \frac{VMAX'}{\frac{VMAX'}{a}} = a \Rightarrow \frac{Km+[S]}{[S]} = \frac{KM+[S]'}{[S]'} = a \Rightarrow Km1 = (a-1)[S] \text{ and } Km2 = (a-1)[S]'$$

- V_{MAX} represents the maximum velocity before the enzyme concentration increase.
- $V_{MAX'}$ represents the maximum velocity after the enzyme concentration increase.
- $[S]$ represents the substrate concentration at V_{MAX}/a,
- $'[S]'$ represents the substrate concentration at $V_{MAX'}/a$,
- a is a number greater than 1, belonging to the set of real numbers ($\mathbb{R}$)

Since when enzyme concentration increse the fractional change in v is less than the fractional change in vmax , and v varies hyperbolically with the substrate concentration, it follows that [s]<[s]'. ⇨Km increase with enzyme concentration.

For this mathematical model, I would like to propose this theory.

Let's consider, for simplicity, an ideal case: a sphere containing a solution of enzyme and substrate. On each substrate, a force acts equal to the sum of the attraction forces between each enzyme and its respective substrate, plus the sum of the repulsion forces between each substrate and its respective substrate. Since the solution is spherical, any deviation from the center of the sphere of the substrate's position will result in an increase in the repulsion and attraction forces acting on the substrate from the opposite side of the deviation, and a decrease in the repulsion and attraction forces from the same side with the deviation. Since the substrate concentration is much higher than the enzyme concentration, it means that the repulsion force dominates. Therefore, it follows that as the substrate deviates more from the center of the sphere, the electrostatic force that drives it towards the periphery of the sphere increases, so the substrate will collide with an enzyme located closer to the periphery than the substrate. From here, we can conclude that with an increase in enzyme concentration, the forces opposing the substrate's trajectory increase, thus decreasing the enzyme's affinity for the substrate.

Fundamental biochemistry data supporting the theory:

1. When enzyme concentration increases, the fractional change in v is less than the fractional change in Vmax! This statement is consistent with the theory as it confirms that the activity of each enzyme decreases with an increase in enzyme concentration for the same substrate concentration.

2. The disproportion between the fractional change in v and the fractional change in Vmax decreases with an increase in substrate concentration! This is another statement consistent with the theory as it confirms the decreasing relevance of the attraction forces between each enzyme and substrate, compared to the repulsion forces between each substrate and its reference substrate with substrate concentration.

3. According to this theory, this variation of Km with the enzyme concentration is difficult to identify as long as the rule [S]>>[E] is respected, because the relevance of the factors determining this variation is reduced since they are reflected in the very small increase in reaction rate in the plateau phase, and this increase is ignored as is commonly done when measuring Vmax and Km, the increase in Km with enzyme concentration is also ignored since this approximation neglects the small difference between the fractional change in v at that substrate concentration and the fractional change in Vmax with enzyme concentration, it implies an error that these are equal.It is also well known that if this rule is not respected, the results indicate that Km increases with the enzyme concentration .

Results and Discussions

This study, taking into account the fact that the final force with which the enzyme and the substrate attract each other is not strictly related to the attraction force between the enzyme and substrate but also to other forces determined by the rest of the enzymes and substrates in the environment on each individual substrate, demonstrates through mathematical modeling as well as deductions not considered in fundamental practical biochemistry experiments that enzyme concentration affects the enzyme's affinity for the substrate. It provides a theoretical explanation in line with experimental data from the literature on how this phenomenon occurs.

This theory can be used for the further development of a new class of drugs by increasing the concentration of the allosteric enzyme (for example, through strong agonization of a receptor) until the concentration of the physiological substrate no longer exceeds Km, resulting in metabolic pathway blockage. These drugs could be useful in cases of cancer or autoimmune diseases, for example.

The limitations of this theory are that due to the numerous factors to be considered, it is unrealistic to believe that we can create a mathematical model for predictions, and it will require numerous practical experiments. The only mathematical relationship we have is that $Km2/Km1=S'/S$.

CONCLUSION:

This study demonstrates an unexpected relationship between enzyme concentration and Km**.** Our observations suggest that Km is not a fixed constant, but rather can vary depending on the amount of enzyme present.

This finding has significant implications for the interpretation of enzyme kinetic data**.** Traditionally, Km has been considered an intrinsic characteristic of an enzyme, reflecting its affinity for its substrate. However, our findings indicate that Km can be influenced by enzyme concentration, which must be taken into account when analyzing and modeling enzyme reactions

REFERENCES:

Bibliography

1. *The original Michaelis constant: translation of the 1913 Michaelis-Menten paper.* **Michaelis L, Menten ML, Johnson KA, Goody RS.** 2011, ACS Publication, p. 34.

2. **Worthington, Charles C., Worthington, Von and Andrew Worthington, Ph.D.** *introduction to enzyme.* s.l. : Worthington Biochemical Corporation.

.