Research

CLINICAL INVESTIGATION

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

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.

Keywords: Michaelis-Menten constant • Enzyme concentration • Nzyme-substrate

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Introduction

CIn 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 based on the fact that a biochemical principle contradicts a mathematical principle. The relevance of the proposed theory extends beyond merely reconsidering a fundamental principle in enzyme kinetics. This theory could have profound implications in various fields of biochemistry and pharmacology, particularly in the context of drug design and understanding mechanisms of drug resistance. Modifying the Michaelis-Menten constant (Km) based on enzyme concentration not only challenges the current dogma but also provides a new perspective on how enzymatic reactions can be manipulated to achieve desired therapeutic outcomes. In particular, applying this theory in the development of enzyme inhibitors could lead to new strategies for blocking critical metabolic pathways in cancer cells or autoimmune diseases by manipulating enzyme concentrations to reduce the enzyme's affinity for its natural substrate. This aspect opens new avenues for further research and provides a solid foundation for the development of innovative therapeutic agents, thus underscoring the practical importance of this theory in the life sciences. 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:

When enzyme concentration increase, the fractional change in V is less than the

fractional change in V_{max}.

Km is constant regardless of the enzyme concentration.

According to the first principle, as V_{max} increases up to V_{max}', $V_{max}/2$ will increase to less than $V_{max}'/2$, 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

$$\frac{\frac{V_{max}}{V_{max}}}{a}=\frac{\frac{V_{max}'}{V_{max}'}}{a}\Rightarrow\frac{Km+[S]}{[S]}=\frac{KM+[S]'}{[S]'}=a\Rightarrow Km1=(a-1)[S]\,and\,Km2=(a-1)[S]^{1}$$

Where,

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 (R)

Since when enzyme concentration increse the fractional change in V is less than the fractional change in V_{max}, and V varies hyperbolically with the substrate concentration, it follows that [s]<[s]'. ⇨Km increase with enzyme concentration. (km2/km1=S'/S)

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

Let's consider, for simplicity, an ideal case; a circle containing a solution of enzyme and substrate, simplifying from a sphere to a circle. On each substrate, a force acts that is 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 these are evenly distributed on the surface of a circle, any deviation from the center of the circle in 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 forces from the same side as the deviation. Since the substrate concentration is much higher than the enzyme concentration, it means that the repulsion force dominates. Therefore, as the substrate deviates more from the center of the circle, the electrostatic force that drives it toward the periphery of the circle increases, causing the substrate to collide with an enzyme located closer to the periphery than the substrate. From this, 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.

■ Experimental biochemistry data supporting the theory and analysis of existing literature

- When enzyme concentration increases, the fractional change in v is less than the fractional change in V_{max}! 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.
- The disproportion between the fractional change in V and the fractional change in V_{max} 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 (Figure 1).
- These two experimental pieces of evidence demonstrate the theory through inductive reasoning: the first proves that the phenomenon actually occurs, and the second that the explanation is the proposed theory.

This graph represents the increase in reaction rate with enzyme concentration at a constant substrate concentration.

- From this, it can be postulated that the fraction of increase in V is smaller than the fraction of increase in V_{max} with enzyme concentration.
- From this same graph, it can be concluded that the disproportion between the two fractions decreases with substrate concentration.
- 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 V_{max} 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 V_{max} with enzyme concentration, it implies an error that these are approximately equal.

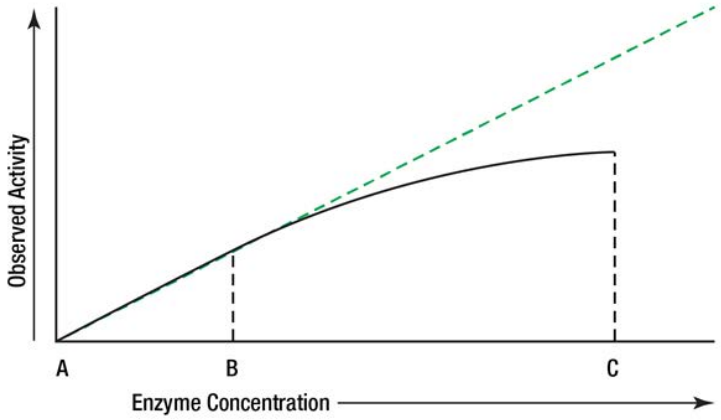

Figure 1: The variation of the reaction rate with enzyme concentration.

By linearizing the plateau phase starting from a substrate concentration that is as high as possible, we neglect the fact that the slope is steeper in the case of higher enzyme concentrations (We can conclude that it is more pronounced from the fact that fraction change V is less than the fractional change in V_{max} and the disproportion decrease with substrate concentration). The lower the substrate concentration I attribute to the velocity from which v was linearized, the more I will neglect the variation of Km with enzyme concentration. Since the only mathematical factor that varies when using a higher value of v is Km, and because its influence is very minimal at high substrate concentrations, any small increase in V will lead to a slight decrease in Km. Additionally, since these increases in V are made in such a way that the disproportion between the fractional increase in v and the fractional increase in V_{max} is approximately neglected, this error in data interpretation will result in approximately constant Km values. Therefore, by eliminating this error from the data interpretation, Km will increase with the enzyme concentration each time (Figure 2).

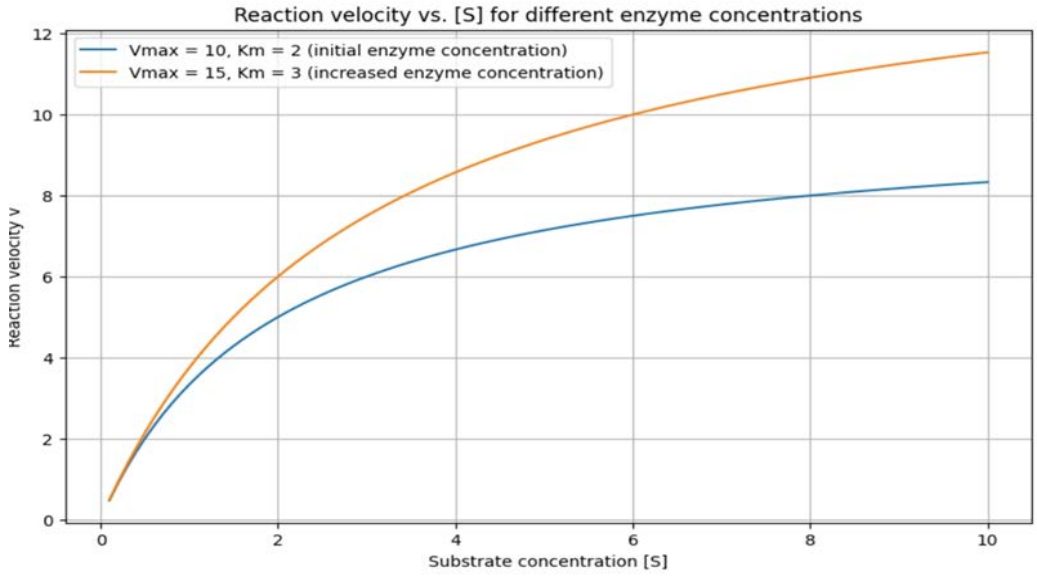

Figure 2: The variation of Km with enzyme concentration.

This image illustrates two hypothetical graphs for different enzyme concentrations so that the results are consistent with the graph in figure 1.

- It is observed that for the graphs to be consistent with the graph in figure 1, it is necessary for Km to increase with enzyme concentration since the slope of the hyperbola is given by Km.
- Linearizing the plateau phase leads to ignoring the difference between the fraction of increase in v and the fraction of increase in V_{max} with enzyme concentration.

Results

Km increase with enzyme concentration: In a study done by Hanson, S. M., & Schnell, S in 2008 [2]. and in another one done by Schnell, S. in 2013, it was demonstrated that QSSA (Quasi Steady State Approximation) does not necessarily include RSA (Reactant Stationary Assumption) and they are different approximations [3]. Thus, we can conclude that for k1[E][S]≈k2[ES] (RSA valid), considering that [ES] is constant since QSSA is applied, it is assumed that k1[E][S]>k2[ES] when only QSSA is valid. Additionally, the concentration of the substrate must increase from QSSA in order to also achieve RSA, which means that this necessarily implies variations in the values of k1 and k2 witch is in accordance with the theory.

- considering the plateau phase as linear will lead to significant errors in the calculation of Km
- as the concentration of the enzyme increases, the force with which the substrate moves towards the enzyme decreases.

Discussions

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 and the fact that the way in which Km varies with the enzyme concentration depends on the specific characteristics of each enzyme-substrate pair, it is unrealistic to believe that we can create a mathematical model for predictions, and it will require numerous practical experiments.

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

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