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Review

A century of enzyme kinetic analysis, 1913 to 2013

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ABSTRACT

This review traces the history and logical progression of methods for quantitative analysis of enzyme kinetics from the 1913 Michaelis and Menten paper to the application of modern computational methods today. Following a brief review of methods for fitting steady state kinetic data, modern methods are highlighted for fitting full progress curve kinetics based upon numerical integration of rate equations, including a re-analysis of the original Michaelis–Menten full time course kinetic data. Finally, several illustrations of modern transient state kinetic methods of analysis are shown which enable the elucidation of reactions occurring at the active sites of enzymes in order to relate structure and function.

1. Introduction

In their 1913 paper Leonor Michaelis and Maud Menten sought to achieve "the final aim of kinetic research, namely to obtain knowledge of the nature of the reaction from a study of its progress" [1]. The challenge of the day was to account for the full time course of product formation in testing the postulate that the rate of an enzyme-catalyzed reaction was proportional to the concentration of enzyme–substrate complex. They did so without knowing the concentration or even the chemical nature of enzymes—a tribute to the power of quantitative kinetic analysis. Today, the important questions have advanced to asking how enzymes achieve such extraordinary efficiency and specificity, while structural and spectroscopic studies have provided a powerful complement to kinetic analysis to greatly expand our understanding of enzyme catalysis. While the techniques for data collection and analysis have advanced to meet the sophistication of the questions that are being addressed, kinetic analysis has remained as a cornerstone of enzymology because studies of the rate of reaction allow alternative pathways to be distinguished. Here, I will briefly review the methods of kinetic analysis developed by Michaelis and Menten that go beyond the simple initial velocity methods for which they are known, and contrast their analysis with modern computer-based global data fitting methods.

Roger Goody and I recently published a complete translation of the 1913 Michaelis–Menten paper originally written in German [2,3]. We were surprised to learn that Michaelis and Menten performed what can be considered as the first global data analysis of full progress curves, going far beyond the simple steady state kinetic studies for which they are commonly recognized.

As the foundation of their analysis, Michaelis and Menten devised the now popular initial velocity measurements, but they also derived equations for competitive product inhibition and measured the dissociation constant (K_d) for each product. They studied the enzyme, invertase (EC 3.2.1.26, β-D-fructofuranosidase), named for the resulting inversion of optical rotation observed upon conversion of sucrose to glucose plus fructose. Interestingly, the crystal structure of invertase from *Saccharomyces* was solved for the first time this year [4]. Michaelis and Menten chose to study invertase because the change in optical rotation provided a convenient signal to monitor the hydrolysis of sucrose and thereby test the theory that the rate of reaction was proportional to the concentration of the enzyme–substrate complex. They are most noted for the Michaelis–Menten equation, which was first derived by Henri [5], although his experiments failed to support the theory because of shortcomings in his experimental design; namely, the failure to control pH and to account for mutarotation of glucose [1,2]. This provides an important example that is still pertinent today. Testing a scientific theory requires careful measurement and accurate

Abbreviations: EPSP, 5-enoylpyruvoylshikimate-3-phosphate; HIVRT, HIV reverse transciptase; MDCC, 7-diethylamino-3-[([(2-maleimidyl)ethyl]amino)carbonyl]coumarin; S3P, shikimate 3-phosphate

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quantitative analysis. Because of their attention to detail in the laboratory and their careful, quantitative analysis, the names of Michaelis and Menten are indelibly linked to the simple equation relating the rate of an enzyme-catalyzed reaction to the concentration of substrate:

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad (1)$$

Measurement of the binding affinity for an active enzyme–substrate complex was a landmark discovery of the day. Although it is now widely accepted that the Michaelis constant, K_m, is not generally equal to the enzyme–substrate dissociation constant, for invertase the K_m probably is equal to the K_d given the weak apparent binding affinity (16.7 mM). The more general derivation of the Michaelis–Menten equation that is presented in most textbooks is based upon the steady state approximation, as derived 12 years later in 1925 by Briggs and Haldane [6].

Finding a method for fitting the concentration dependence of the initial velocity was problematic for Michaelis and Menten. Estimation of K_m could be obtained from the velocity at half of V_{max}, but extrapolation to estimate the velocity at infinite substrate concentration presented an obstacle. They devised a complicated analysis based upon the logarithm of the rate and derived an equation analogous to the Henderson–Hasselbalch equation for pH dependence, which was published 4 years later [7]. They normalized their data based upon the expected slope of a semi-log plot at the midpoint of the transition, thereby affording an estimation of the rate at infinite substrate concentration and hence, the K_m. It is indeed surprising that in spite of the complexities of this analysis, it was not until 20 years later that Lineweaver and Burk devised the simple reciprocal plot [8]. As a tribute to the popularity of this simple algebraic transformation, their paper went onto become the most cited in the history of the *Journal of the American Chemical Society*.

The Lineweaver–Burk reciprocal plot presents some problems due to the unequal weighting of errors as illustrated in Fig. 1. Fig. 1A–C show the same data set fit by nonlinear regression to a hyperbola (Fig. 1A) compared to fits derived by linear regression using a Lineweaver–Burk plot (Fig. 1B) and an Eadie–Hofstee plot (Fig. 1C). In the reciprocal plot, the least accurate data, obtained at the lowest substrate concentrations, alter the slope of the line because of the long lever arm effect on the reciprocal plot, leading to overestimation of k_{cat} and K_m. Of course, this data set was selected to illustrate the problems and proper weighting of errors based upon the measured standard deviation can rectify the unequal weighting of errors in the reciprocal plot, but that is rarely done. These considerations led to the generation of another transform of the Michaelis–Menten equation, known as the Eadie–Hofstee plot as shown in Fig. 1C [9]. Arguments have tended to favor the reciprocal plot because it separates the two primary kinetic constants, k_{cat}/K_m and k_{cat} as 1/slope and intercept, respectively. Although the Eadie–Hofstee plot produces more reliable estimates [10], the presence of the dependent variable, v, in both axes makes rigorous error analysis difficult. Fortunately, now with the advent of fast personal computers and readily available software for nonlinear regression, these arguments can be relegated to history. Today, there is no reason for fitting data using either linear transformation of the Michaelis–Menten equation in analyzing the concentration dependence of the initial velocity.

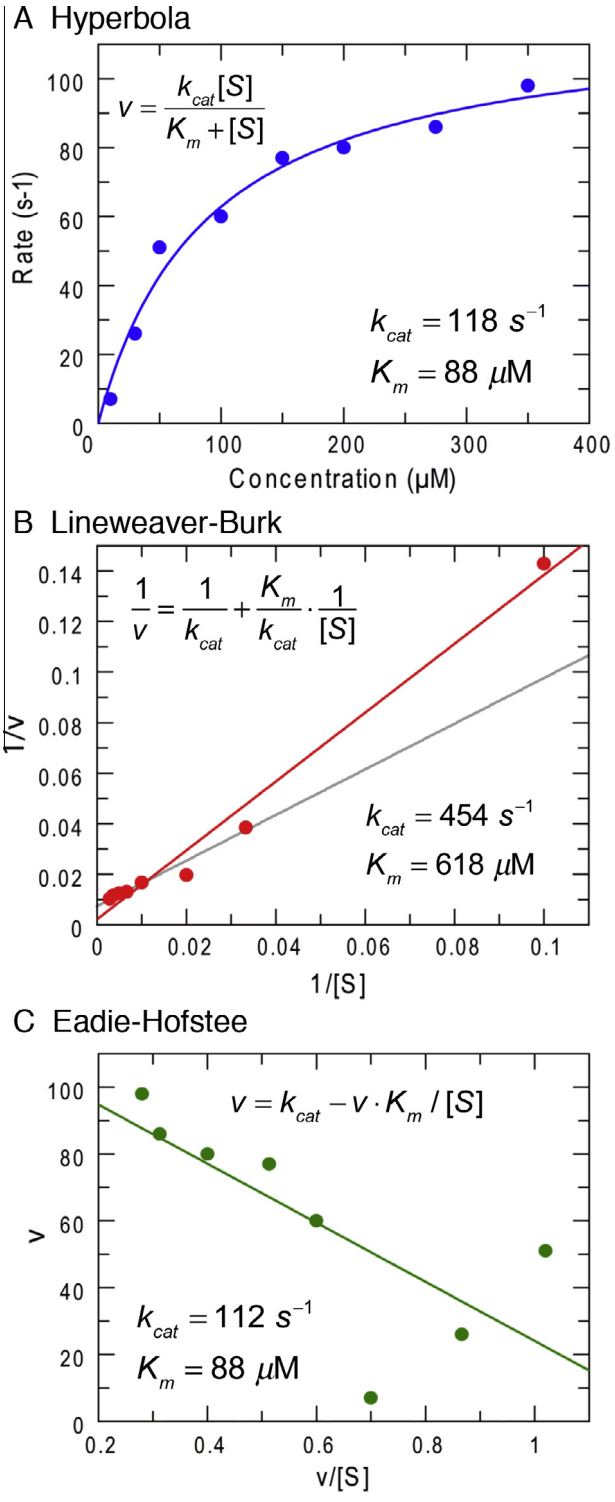

Fig. 1. Comparison of three methods of fitting data to the Michaelis–Menten equation. (A) Data fit by nonlinear regression to a hyperbola. (B) Data fit to a Lineweaver–Burk reciprocal plot. The gray line shows the fit obtained after omitting the point at the lowest substrate concentration. (C) Data fit using the Eadie–Hofstee equation. In each figure, the equation and the resulting k_{cat} and K_m values are displayed.

2. Michaelis–Menten progress curve Kinetics

Although largely forgotten in the past century, Michaelis and Menten were the first to fit full time course kinetic data and compute a fitted parameter by averaging over all of the data to provide a kind of global analysis. They derived an equation that predicted a constant term that could be calculated from the product formed at each time point as the reaction progressed toward completion, including data obtained at several starting sucrose concentrations and accounting for product inhibition.