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Review

The origins of enzyme kinetics

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ABSTRACT

The equation commonly called the Michaelis–Menten equation is sometimes attributed to other authors. However, although Victor Henri had derived the equation from the correct mechanism, and Adrian Brown before him had proposed the idea of enzyme saturation, it was Leonor Michaelis and Maud Menten who showed that this mechanism could also be deduced on the basis of an experimental approach that paid proper attention to pH and spontaneous changes in the product after formation in the enzyme-catalysed reaction. By using initial rates of reaction they avoided the complications due to substrate depletion, product accumulation and progressive inactivation of the enzyme that had made attempts to analyse complete time courses very difficult. Their methodology has remained the standard approach to steady-state enzyme kinetics ever since.

1. Introduction

Michaelis and Menten are by far the best known of the scientists who created the subject of enzyme kinetics, but what was their real contribution? Have they simply received the credit for work already published by Brown [1] and Henri [2,3] before their paper of 1913 [4] (Fig. 1), as some authors [5,6] have suggested? Here I shall argue that although earlier authors, especially Henri, made important advances they lacked Michaelis and Menten's insight of realizing that an analysis in terms of initial rates would eliminate the complications that had plagued their predecessors' efforts to interpret time courses.

2. The basic contribution of Michaelis and Menten

In common with numerous researchers of their time Michaelis and Menten studied the inversion of sucrose catalysed by invertase. (The word *inversion* refers to the use of a polarimetric method to follow the reaction, the sign of the optical rotation of "invert sugar", or the mixture of glucose and fructose produced in the reaction, being opposite from that of sucrose.) They expressed the rate v of the reaction in the following way:

$$v = C \cdot \Phi \frac{[S]}{[S] + k} \tag{1}$$

defining Φ as the total molar concentration of invertase, $[S]$ as the sucrose concentration (noting, incidentally, that there is no practical difference between the free and total concentrations of substrate when its concentration is very large compared with that of the enzyme), k as the dissociation constant of the enzyme–substrate complex, and C as a constant of proportionality. At the time they were writing, the convention that capital letters are used for equilibrium constants and lower-case letters for rate constants did not exist, so k here is not a rate constant, whereas K_3 in Eqs. (4) and (5) below is a rate constant.

The equation is nowadays usually written in a form resembling the following:

$$v = \frac{Va}{K_m + a} \tag{2}$$

in which v is the initial rate observed at a total substrate concentration of a, and V, the *limiting rate*, and K_m, the *Michaelis constant*, are constants. As any modern textbook will show, the steady-state mechanism introduced by Briggs and Haldane [7] is now taken as the starting point for interpreting this equation:

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA \xrightarrow{k_2} E + P \tag{3}$$

in which E is the enzyme, A is the substrate, P is the product, EA is an enzyme–substrate complex, k_1, k_{-1} and k_2 are rate constants, and the reaction is assumed to be in a steady state in which the rate of production of EA is balanced by the rate of its conversion to

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Die Kinetik der Invertinwirkung.

Von

L. Michaelis und Miß Maud L. Menten.

(Eingegangen am 4. Februar 1913.)

Fig. 1. Title of Michaelis and Menten's paper. Notice the unusual way the English word "Miss" is spelt.

products. With this interpretation $V = k_2 e_0$, where e_0 is the total enzyme concentration, and $K_m = (k_{-1} + k_2)/k_1$. (Briggs and Haldane did not use the symbol K_m, which appeared, however, possibly for the first time, in Haldane's book [8].) This interpretation did not come immediately, and Henri [2,3] and Michaelis and Menten [4] both assumed that K_m was the equilibrium dissociation constant of EA, which would be k_{-1}/k_1 in the symbols used here. (The more usual symbol today for the dissociation constant k_{-1}/k_1 would be K_s rather than K_m.) The question now to be asked is whether Eq. (2) is more appropriately called the Henri–Michaelis–Menten equation or the Michaelis–Menten equation.

Of course, no discovery appears from nowhere — other, perhaps, than Newton's study of colours [9] — and not only did Brown and Henri contribute, but numerous other developments of the preceding century were also important, including general ideas of chemical kinetics [10], the law of mass action [11], the discovery of a papain–substrate complex [12], and earlier studies of invertase [13,14]. Nonetheless, Michaelis and Menten's paper [4] represented a major turning point in the history of our understanding of enzyme catalysis, and its effects are still relevant 100 years later, because they defined how kinetic experiments need to be done if useful information is to be obtained from them: they were the first to understand the importance of controlling the pH, and the first to recognize that initial rates are easier to interpret than time courses. Their third contribution — taking account of the effects of spontaneous mutarotation on the kinetics observed by polarimetric methods — was important for the study of invertase, but does not have a wider importance beyond the obvious point that if the products of a reaction undergo spontaneous changes that affect the method of assay this needs to be taken into account. Not only did they define how experiments should be done, but they also carried them out rigorously, and obtained results with a precision that can stand comparison with that obtainable today; almost as important, they described what they had done with sufficient clarity and completeness for Johnson and Goody [15] to be able to repeat them and check them nearly a century later. Unfortunately, not all enzyme kinetic experiments are described so clearly today, and that is why the guidelines proposed by the STRENDA Commission of the Beilstein-Institut [16,17] have become necessary.

Two modern translations of Michaelis and Menten's paper are available: one, by Boyde [18], is included in this Special Issue of *FEBS Letters*, and is based on an earlier one by the same author [19]. The other is a downloadable supplement to the recent paper of Johnson and Goody [15]. Boyde [19] also includes translations of some relevant publications of Henri [2,3], Sørensen [20] and others. Various of these (but not Michaelis and Menten's paper) have been translated by Friedmann [21].

3. Advances made by other early authors: the enzyme–substrate complex

3.1. Brown and Henri

At the beginning of the 20th century the nature of enzyme catalysis and kinetics was of widespread interest and was studied by several different authors, most notably Brown [1] and Henri [2,3]. Of these, Brown [1] was probably the first to realize that a mechanism that required passage through an enzyme–substrate complex implied an upper limit on the rate of an enzyme-catalysed reaction, and he can be credited with introducing the first model of enzyme saturation. However, his interpretation was qualitative, unsupported by any algebra. Henri [2,3] criticized it for its completely unrealistic assumption that the enzyme–substrate complex had a fixed lifetime, and derived an equation for the instantaneous rate of a reaction subject to product inhibition:

$$\frac{dx}{dt} = \frac{K_3(a-x)}{1 + m(a-x) + nx} \tag{4}$$

in which a is the total amount of sucrose, x is the amount of product at time t, K_3 is a constant proportional to the amount of enzyme, and m and n are also constants. In his thesis [3], but not in his paper [2], he went on to note that if $x = 0$ when $t = 0$ then this can be simplified to

$$\text{initial rate} = \frac{K_3 a}{1 + ma} \tag{5}$$

which is just the Michaelis–Menten equation in unfamiliar symbols, other than the fact that it expresses the rate at which the amount (not the concentration) of product changes, i.e. the *rate of conversion* [22], whereas today a kinetic equation usually expresses the rate at which the concentration changes. However, apart from noting that this gave a good account of the experimental observations with invertase, Henri took the matter no further: he did not point out that this simpler equation could form the basis of an experimental approach that would allow a far easier analysis than the attempts to use the time course that had long dominated efforts to understand the kinetics of enzyme-catalysed reactions.

Most of the early discussion of the enzyme–substrate complex incorporated two assumptions: that it must necessarily participate as an intermediate in the reaction mechanism; and that it was maintained at equilibrium with the free enzyme and substrate. Although Henri [2,3] thought that its participation as an intermediate was the most likely interpretation, he also considered an alternative possibility, and found that if the complex existed only as a "nuisance complex" in a side reaction the kinetic behaviour would be indistinguishable from that given by assuming it to be an intermediate. That is true so far as the steady state is concerned, but transient-state measurements allow the two possibilities to be distinguished [23,24]. Non-productive complexes can certainly exist, and can complicate the interpretation of data for enzymes that act in nature on large polymers when studied with small synthetic substrates [25], but no examples are known for which Henri's alternative mechanism is the whole explanation of enzyme saturation.

3.2. Van Slyke and Cullen

Van Slyke and Cullen [26], who were studying urease at about the same time as Michaelis and Menten's work, did not assume that the enzyme–substrate complex was at equilibrium with the free components; instead they assumed that it would be formed in an irreversible reaction and broken down in a second irreversible reaction to regenerate the free enzyme. They treated the time required for a complete catalytic cycle as the sum of the times required for the two steps, and the steady-state assumption was implicit in their treatment. Processes occurring in series can always be analysed in terms of additive times, but Van Slyke and Cullen's approach has not often been used explicitly in later work. However, it can be very useful, for example, for considering the steps in metabolic processes [27]. Assigning rate constants k_1 and k_2 to Van Slyke and Cullen's two processes allowed the rate equation to be written as follows: