Substrate and enzyme concentration dependence of the Henri–Michaelis–Menten model probed by numerical simulation

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Abstract The use of the classic Henry–Michaelis–Menten (HMM) model (or simply, Michaelis–Menten model) to study the substrate and enzyme concentration dependence of enzyme catalysis is a very important step in understanding many biochemical processes, including microbial growth. Although the HMM model has been extensively studied, the conditions in which the substrate concentration is not in excess have still not been adequately defined mathematically. This lack of definition occurs despite at the cellular and molecular levels most systems generally do not operate in a state of substrate excess. In the present work, we describe an approach for studying enzyme reactions in which substrate concentrations are not in excess. Our results show that the use of extent of reactions and numerical simulation of the velocities of reaction provides an important advance in this field and furnishes results not obtained in previous studies involving these aspects. This approach, in association with knowledge of the rate constants, provides a direct and easy means of examining the single substrate–enzyme profile during product formation at any

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enzyme–substrate ratio. This approach is more direct than previous models that required the use of empirical equations with arbitrary constants.

1 Introduction

The Michaelis–Menten model was proposed in 1913 [1] and was mathematically described by Briggs and Haldane [2] and recently extended to conditions beyond the steady-state [3]. In a recent report, Bajzer and Strehler [4] discussed various aspects of the enzyme–substrate dependence of kinetic processes involving the Henri–Michaelis–Menten (HMM) model of enzyme action. These authors proposed a model of enzyme action based on a quasi-steady-state condition involving a prolonged steady-state that is achieved when the total substrate concentration is near the initial substrate concentration and higher than the initial enzyme concentration. The importance of understanding this system, which has also been highlighted by others [5–7], resides in the fact that such conditions reflect what occurs in normal physiological states. Previous investigations that have addressed this question have generally started with the classic Michaelis–Menten equation obtained for conditions of high substrate concentration [8–10]. In the present study, we propose a more complete explanation of this behavior based on a numerical simulation of the extent of reaction equations that describe this process. This proposal, which is based on a previous approach by our group [3], uses a fourth order Runge–Kutta method and provides very accurate results without the need for empirical constants or functions such as usually employed in kinetic analyses of enzyme–substrate interactions. This solution is more accurate than previous proposals because it does not start with the Michaelis–Menten equation which has limited applicability at a high substrate concentration. Although the results obtained with this approach are similar to those of previous studies, they are generally more accurate. We show that the extent of reactions can be used to obtain individual rate constants, as previously described in detail by our group for high substrate concentrations [3]. These results can then be used to determine the kinetic behavior of enzyme action at any enzyme–substrate ratio based on a numerical simulation using the respective velocities of reaction. Overall, our findings show how the HMM model and knowledge of the rate constants and enzyme–substrate concentrations can be used to study kinetic behavior at high enzyme and low substrate concentrations.

2 Materials and methods

When investigating the kinetic conditions described by the HMM model using our previously described approach [3] we start with the classic representation of an enzymatic reaction as

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} P + E \tag{1}$$

where S, E, ES and P correspond, respectively, to substrate, free enzyme, enzyme–substrate complex and product. The time-dependence of the concentration of each species in solution, $C(t)$, can be expressed by the following series of equations:

$$C_S(t) = c_S - x_1(t) + x_{-1}(t) \tag{2}$$
$$C_E(t) = c_E - x_1(t) + x_{-1}(t) + x_2(t) \tag{3}$$
$$C_{ES}(t) = x_1(t) - x_{-1}(t) - x_2(t) \tag{4}$$
$$C_P(t) = x_2(t) \tag{5}$$

where c represents the initial concentration, x the extent of reaction (mol/L) and the subscript indicates the corresponding direction of the process.

The velocities of reaction, expressed in terms of the extent of reaction, can be defined as:

$$v_1(t) = \frac{dx_1(t)}{dt} = k_1 C_S(t) C_E(t)$$
$$v_{-1}(t) = \frac{dx_{-1}(t)}{dt} = k_{-1} C_{ES}(t)$$
$$v_2(t) = \frac{dx_2(t)}{dt} = k_2 C_{ES}(t)$$

From Eqs. (2)–(5) it follows that:

$$v_1(t) = \frac{dx_1(t)}{dt} = k_1(c_S - x_1(t) + x_{-1}(t))(c_E - x_1(t) + x_{-1}(t) + x_2(t)) \tag{6}$$
$$v_{-1}(t) = \frac{dx_{-1}(t)}{dt} = k_{-1}(x_1(t) - x_{-1}(t) - x_2(t)) \tag{7}$$
$$v_2(t) = \frac{dx_2(t)}{dt} = k_2(x_1(t) - x_{-1}(t) - x_2(t)) \tag{8}$$

Equations (6)–(8) furnish a system of differential equations that can be solved by numerical methods such as the fourth order Runge–Kutta method [11,12]. However, this solution requires prior knowledge of the rate constants k_1, k_{-1} and k_2 and of the initial substrate and enzyme concentrations (c_S and c_E, respectively). With this information in hand, we need only define the time interval for the calculation, usually referred to in standard numerical simulation textbooks as h [11,12].

To facilitate comparison with the findings of Bajzer and Steller [4], we initially simulated the kinetic behavior of the HMM model defined by Eqs. (6)–(8) to obtain the time-dependence of Eqs. (2)–(5), assuming that $k_1 = k_{-1} = k_2 = 1$, $c_E = 1$ and c_S varied from 1 to 10 for $h = 2.5 \times 10^{-4}$. In a second simulation, a similar calculation was done for conditions where $c_S = 1$ and c_E varied from 1 to 10, which yielded a substrate/enzyme ratio that ranged from 1 to 0.1.

3 Results and discussion

Figures 1 and 2 show the results for the situation where $c_E = 1$, c_S varied from one to ten and the substrate-enzyme ratio varied from one to ten. Similarly, Figs. 3 and 4 show the results for the situation where $c_S = 1$, c_E varied from 1 to 10 and the substrate-enzyme ratio ranged from 1 to 0.1. The advantages of the present proposal are best understood by comparing Figs. 1–4 with data in the literature. Figures 1a, c and 2a, c show the surface profiles of the species concentrations in Eq. (1) at distinct reaction times and substrate ratios, as well as the contour plots of these surfaces for $c_S = [S_o]$ from 1 to 10 (panels b and d of Figs. 1, 2). These results were similar to those
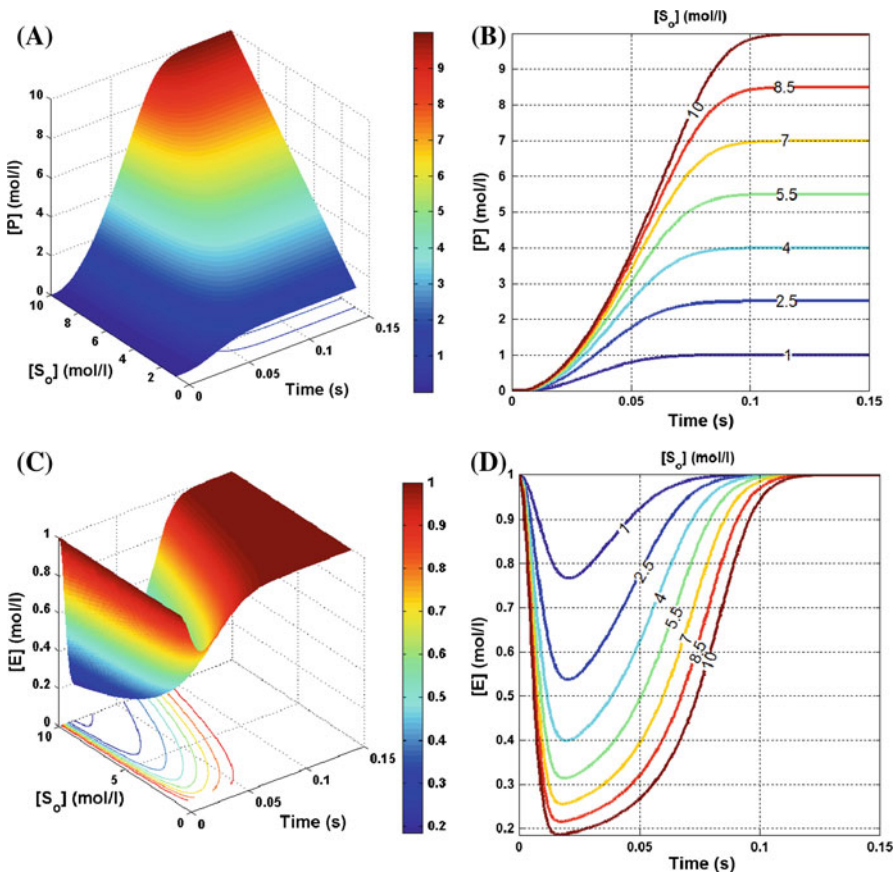

Fig. 1 Time dependence of species concentration in the HMM model given by numerical simulation using the 4th order Runge–Kutta method applied to Eqs. (6)–(8) to obtain the time dependence of Eqs. (2)–(5). In this simulation, $k_1 = k_{-1} = k_2 = 1$, $c_E = 1$ and c_S (initial substrate concentration, $[S_o]$) varied from 1 to 10 for $h = 2.5 \times 10^{-4}$. **a** Surface results for the time and $[S_o]$ dependence of product formation ($[P]$). **b** Contour plots of the time dependence of product formation at distinct $[S_o]$ values. **c** Surface results for the time and $[S_o]$ dependence of free enzyme (E) concentration. **d** Contour plots of the time dependence of free enzyme concentration at distinct $[S_o]$ values
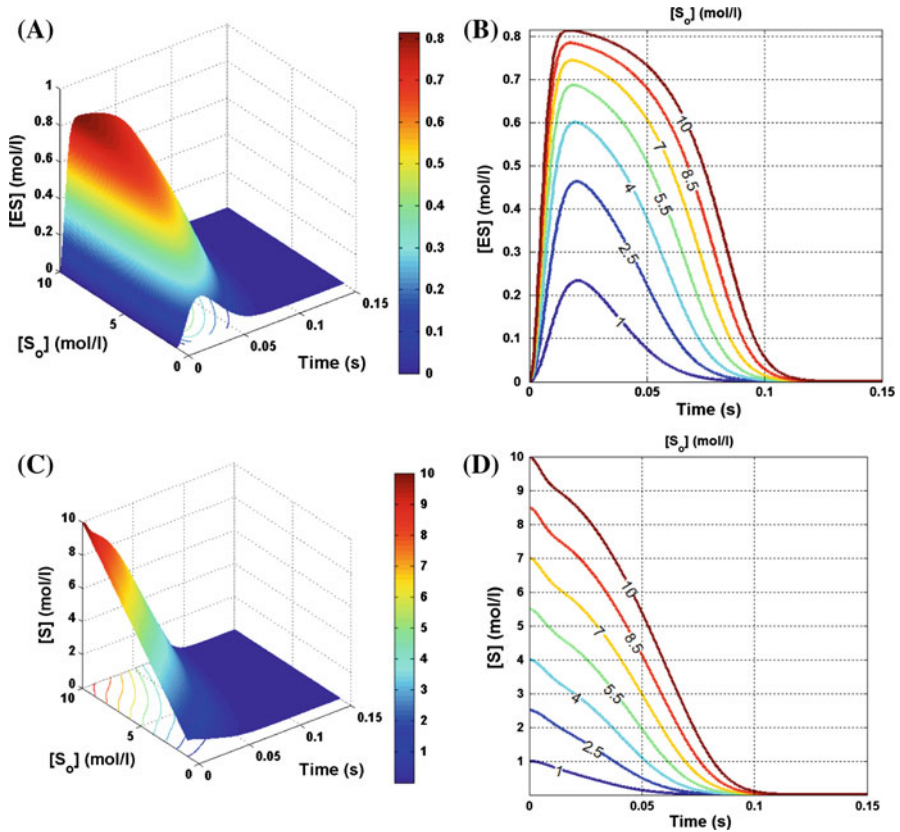
Fig. 2 Time dependence of species concentration in the HMM model obtained for the conditions described in Fig. 1. **a** Surface results for the time and $[S_o]$ dependence of complex concentration ($[ES]$). **b** Contour plots of the time dependence of complex concentration at distinct $[S_o]$ values. **c** Surface results for the time and $[S_o]$ dependence of substrate concentration ($[S]$). **d** Contour plots of the time dependence of substrate concentration at distinct $[S_o]$ values

reported by Bajzer and Steller [4] (Fig. 1 of their report) for these conditions. However, inspection of panels b and d of Figs. 1 and 2 for $c_S = 10$ indicated that these results were more coherent with the data obtained by using the omega function proposed by Schnell and Mendoza [6] (Fig. 1). With respect to the time-dependence and shape of the corresponding curves the procedure described by Schnell and Mendoza [6] is more precise than previous proposals [4–8], although the approach of Bajzer and Steller [4] is easier to apply. The time span used in these simulations is particularly suitable for the time interval h in these numerical simulations and increasing this time interval leads to an increase in the time of reaction at which the species behavior shown in Figs. 1–4 are observed.

In this view, despite the time interval considered, it is possible to conclude that the present results are more precise than those reported previously because instead of starting the simulations with the kinetic equations provided by the Michaelis–Menten equation, as is usually done, we started by using Eq. (2), introduced into
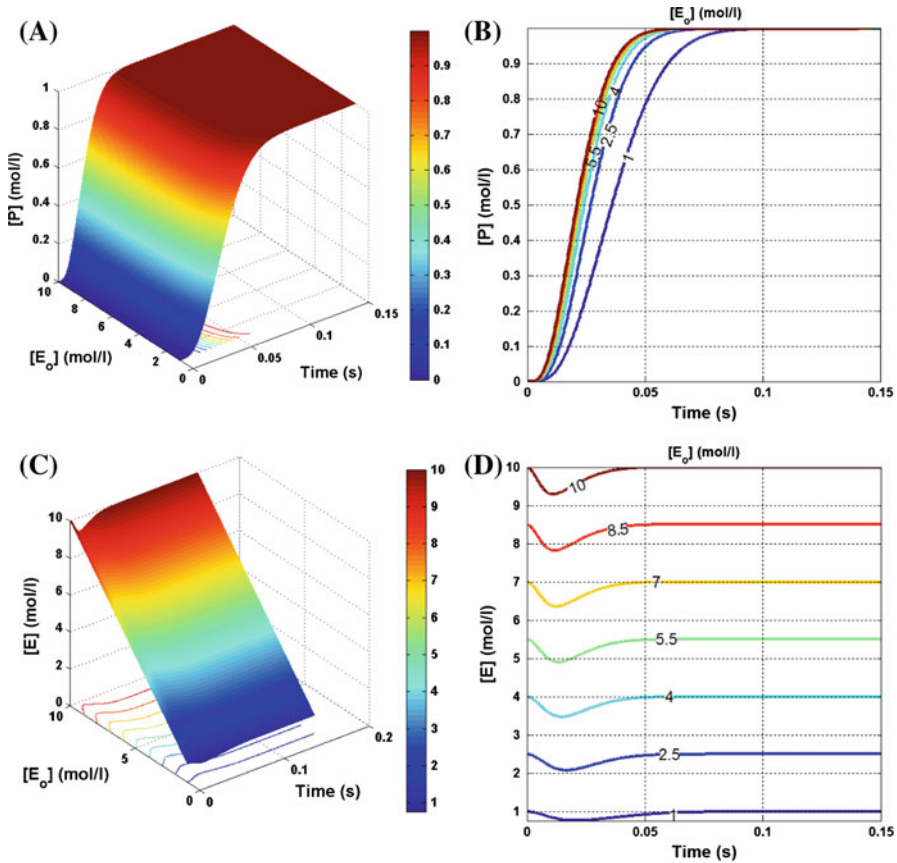
Fig. 3 Time dependence of species concentration in the HMM model given by numerical simulation using the 4th order Runge–Kutta method applied to Eqs. (6)–(8) to obtain the time dependence of Eqs. (2)–(5). In this simulation, $k_1 = k_{-1} = k_2 = 1$, $c_S = 1$ and c_E (initial enzyme concentration, $[E_o]$) varied from 1 to 10 for $h = 2.5 \times 10^{-4}$. **a** Surface results for the time and $[E_o]$ dependence of product formation ($[P]$). **b** Contour plots of the time dependence of product formation at distinct $[E_o]$ values. **c** Surface results for the time and $[E_o]$ dependence of free enzyme concentration ($[E]$). **d** Contour plots of the time dependence of free enzyme concentration at distinct $[E_o]$ values

Eq. (6), which accounts for substrate consumption based on the extent of reaction of binding and release of the complex (x_1 and x_{-1} in Eq. (2)). The other results in panels b and d of Figs. 1 and 2 for $c_S \neq 10$ indicate that increasing the substrate-enzyme ratio from 1 to 10 reduced the time required to reach steady-state and the time required for maximum complex formation (Fig. 2b). In addition, a low substrate-enzyme ratio meant that complete enzyme complex (ES) formation in which almost all the enzyme was bound to substrate was not reached. This in turn meant that the maximum velocities of product formation were not reached. The characteristic sigmoid profile of product formation (Fig. 1b) reflected the rate constants and complex formation and influenced the reciprocal behavior of the time dependence of substrate concentration that was seen as nuances in the curves (Fig. 2d).
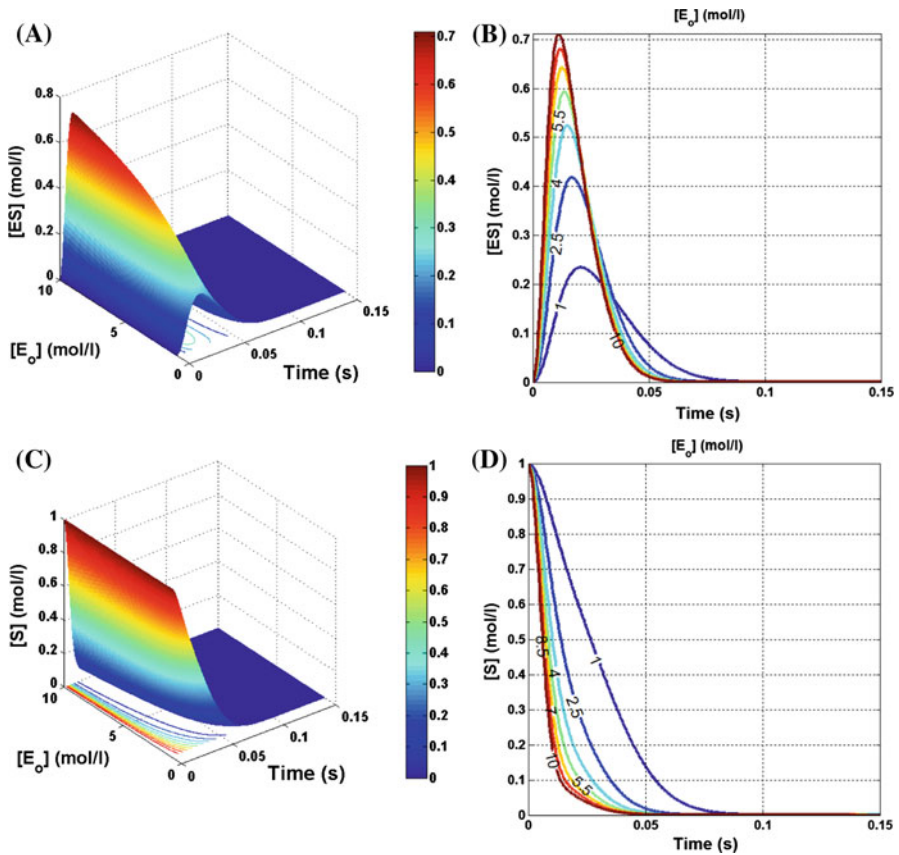
Fig. 4 Time dependence of species concentration in the HMM model obtained for the conditions described in Fig. 3. **a** Surface results for the time and $[E_o]$ dependence of complex concentration ($[ES]$). **b** Contour plots of the time dependence of complex concentration at distinct $[E_o]$ values. **c** Surface results for the time and $[E_o]$ dependence of substrate concentration ($[S]$). **d** Contour plots of the time dependence of substrate concentration at distinct $[E_o]$ values

Figures 3 and 4 show the substrate-enzyme profile at higher enzyme concentrations, where $c_S = 1$ and c_E (or initial enzyme concentration, $[E_o]$) ranged from 1 to 10, yielding substrate-enzyme ratios of 1 to 0.1. For $[E_o] = 10$ (panels b and d of Figs. 3, 4) the profile for species concentrations was very similar to that reported by Bajzer and Steller [4], indicating agreement between these two approaches, but differed markedly from the method proposed by Kargi [5]. Another recent report to address this point [7] provides an additional approach to this problem but is more complex than the present proposal because it involves some considerations and equations that are not necessary when working with extents of reaction. The proposals of Tzafriri [8] and Schnell and Maini [9] yield results compatible with those obtained here but, like other studies, they start with the Michaelis–Menten equation and require additional equations to correct the errors of approximation associated with the original equation. With respect to the other substrate-enzyme ratios analyzed in panels b and d of Figs. 3 and 4, an increase in this ratio from 0.1 to 1 ($[E_o]$ from 10 to 1) decreased the amount of ES complex formed

and increased the time required for maximum complex formation (Fig. 4b). Overall, these results show that increasing the enzyme concentration enhances the rate of complex formation and the amount of complex formed. Based on the analysis described here, we believe that the use of extents of reaction (which is usually not described in standard physicochemical textbooks) deserves more attention as an approach for studying enzyme kinetics because of its ability to provide a complete description of any kinetic system by numerical simulations. In addition, under constrained conditions this approach provides suitable analytical solutions of complex processes, including situations beyond the steady-state condition [3]. In this case, the HMM model should be applied to enzymatic processes involving single substrate binding, as previously proposed [3], and the experimental kinetic rate constants obtained can then be used to determine the time dependence of the species concentration under any condition, as shown here for the reaction velocities.

4 Conclusion

The main contribution of this study in relation to previous reports is the way in which the species concentrations and velocities are described. Specifically, the use of extents of reaction should provide a powerful tool for analyzing such kinetic systems since it provides prompt access to the velocities of reaction that include the time dependence of the species concentrations in Eq. (2). Numerical simulation can be easily done without the need for other equations to account for additional numerical approximations. This point is particularly important because the Michaelis–Menten equation, which has generally been the starting point in previous studies of this question, is inadequate since it is constrained to high substrate concentrations. In other words, the Michaelis–Menten equation is limited by the fact that it cannot provide the velocity of product formation as the substrate concentration changes with time (Eq. (2)); this can be clearly demonstrated by introducing time dependence into the Briggs and Haldane model [2]. Although the results described here were similar to those reported in previous studies that used different approaches, our findings provide a more accurate assessment of the enzyme system involved. The use of extents of reaction can provide a more incisive analysis of the events involved, particularly since there is no need for empirical numerical equations.

An adequate description of the kinetic behavior of enzymatic processes under normal intracellular conditions, with a substrate concentration comparable to the enzyme concentration, is fundamental for a better understanding of biological processes. The usual approach for addressing this important topic is to use empirical equations and arbitrary rate constants that simulate the enzyme–substrate concentration ratios under any conditions. Since the parameters provided by classic kinetics generally include the maximum velocity of reaction and the Michaelis–Menten constant (V_M and K_M, respectively), the rate constants are not usually available. In contrast, the approach that we have recently developed [3] allows these specific rate constants for single substrate-enzyme binding to be obtained. Thus, for the first time, it is possible to have direct, non-empirical access to the enzyme kinetics at a low substrate-enzyme ratio that more realistically reflects normal physiological conditions.

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