Journal of Mathematical Chemistry, Vol. 42, No. 4, November 2007 (© 2006)
DOI: 10.1007/s10910-006-9142-5

An alternative analysis of enzyme systems based on the whole reaction time: evaluation of the kinetic parameters and initial enzyme concentration

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Received 27 April 2006; revised 16 May 2006

This work presents an alternative analysis of the integrated rate equations corresponding to the simple Michaelis-Menten mechanism without product inhibition. The suggested new results are reached under a minimal set of assumptions and include, as a particular case, the classical integrated Michaelis–Menten equation. Experimental designs and a kinetic data analysis are suggested to the estimation of the maximum steady-state rate, V_{max}, the Michaelis–Menten constant, K_m, the initial enzyme

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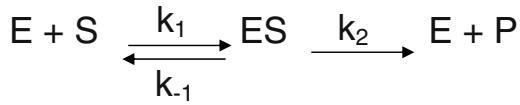
concentration, $[E]_0$, and the catalytic constant, k_2. The goodness of the analysis is tested with simulated time progress curves obtained by numerical integration.

KEY WORDS: Enzyme kinetics, Michaelis–Menten, integrated equation, product rate, substrate rate, numerical integration

1. Introduction

The simple Michaelis–Menten reaction mechanism shown in scheme 1 (see below) is the most widely used in enzyme kinetics analysis, in spite of only few enzyme reactions evolve according to this mechanism. In scheme 1, E denotes the free enzyme, S the substrate, ES the complex enzyme–substrate, and P is the product of the reaction and k_1, k_{-1} and k_2 the rate constants corresponding to the elementary reaction steps. The steady-state parameters describing scheme 1 are the maximum initial rate, $V_{\max}$ (i.e. $k_2[E]_0, [E]_0$ being the initial enzyme concentration), the catalytic constant, k_{cat} (i.e. k_2) and the Michaelis–Menten constant K_{m} [i.e. $(k_{-1} + k_2)/k_1$]. The reason for the wide use of scheme 1 is that most of the enzyme systems can be apparently described by means of the same equations corresponding this scheme, but using apparent maximum initial, apparent catalytic constant and apparent Michaelis–Menten constant, which are composed of algebraic combinations of the individual rate constants.



Scheme 1.

The most frequently used equation related with reaction mechanisms fitting scheme 1 is the called Michaelis–Menten equation which gives the initial steady-state rate, v, of product formation, P, at the steady-state of the reaction as a function of the initial enzyme and substrate concentrations, the rate constant k_2 and the global kinetic parameter K_{m} [1,2]. By deriving this equation, it is assumed that the initial substrate concentration remains approximately constant during the reaction time assayed. To reach experimentally this condition it is necessary and sufficient that the initial substrate concentration, $[S]_0$, is much higher than that one of the free enzyme, $[E]_0$, and that the reaction time assayed is such that the product concentration at this time, $[P]$ is much less than the initial substrate concentration, e.g. $[P] = 0.05[S]_0$.

But under some experimental situations can be not advisable to restrict the reaction extension to a small reaction progress. Some examples of these situations are: (1) K_{m} is very small. In this case, the initial concentration of the substrate used must be also very small in order to the product rate is $[S]_0$-dependent and thus can use the steady-state rate equation. But if $[S]_0$ is very small, then, the concentration of the formed product is also very small and difficult to measure and, moreover, the substrate concentration diminishes rapidly and