Time behaviour of the modifier involved in the general mechanism of Botts and Morales assuming rapid equilibrium in the modifier bindings

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To date, the classification as activator or inhibitor of a modifier involved in an enzyme catalysed reaction is established according to its kinetic behaviour at the steady state. Inhibitors and activators are defined as modifiers which decrease or increase, respectively, the steady state rate of an enzyme-catalysed reaction. At this state, in some cases, a modifier always acts as an activator or as an inhibitor for all its possible concentration values. In other cases the action of a modifier as activator or inhibitor depends on its concentration. In this paper we extend the analysis of the kinetic behaviour of a modifier as inhibitor or nonessential activator to the transient phase of the reaction, i.e. to the whole course of the reaction, including both the transient phase and the steady state. Moreover, concerning to the behaviour of a modifier at the transient phase, we suggest its classification as activator or inhibitor based on the concentration and activator or inhibitor based on the rate. We have studied the behaviour of the modifier involved in the general modifier mechanisms of Botts and Morales in which the reversible bindings of the modifier to the enzyme forms are assumed in rapid equilibrium. The result is that depending on the values of the rate constants, equilibrium constants and the initial concentrations of both the involved substrate and modifier, the latter can act during the whole reaction course only as an activator, only as an inhibitor, first as an activator and then, from a determined reaction time, as inhibitor, or vice versa. Therefore, it is possible that a modifier showing an activating behaviour at the steady state behaves as an inhibitor in the transient phase, or vice versa. Novel indices pointing to the conditions under which the modifier can show any of the behaviours indicated above are suggested. The goodness of the analytical results is tested by comparison with the simulated curves obtained by numerical integration. From these results, those corresponding to several reaction mechanisms involving a modifier, and which can be regarded as particular cases of the general case analysed here, can be directly and easily obtained.

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1. Introduction

Reversible modifiers (inhibitors or activators) represent a useful tool for studying enzyme mechanisms and metabolic routes [1–7]. Moreover, they have applications in pharmacology, toxicology, and agriculture [8–10]. To date, inhibitors and activators are defined as modifiers that decrease or increase, respectively, the steady state rate of an enzyme-catalysed reaction [11,12].

As is known, modifiers are best used according to their kinetic characterisation through an evaluation of their corresponding kinetic parameters. Most enzyme reaction mechanisms involving a modifier reversibly acting on Michaelis type enzymes can be considered as particular cases of the general modifier mechanism of Botts and Morales depicted in scheme 1, whose steady state and transient phase kinetics have been widely discussed in the literature [12–24].

Scheme 1.

\[
E + S \quad \underset{k_{-1}}{\overset{k_{1}}{\rightleftharpoons}} ES \quad \underset{k_{-2}}{\overset{k_{2}}{\rightarrow}} E + P
\]

More recently, kinetic analyses of the transient phase have been made of simple enzyme reaction mechanisms involving a competitive [25] or uncompetitive [26] inhibitor acting reversibly on Michaelis type enzymes, whose substrate is substricidal. Nevertheless, these simple mechanisms can be considered as particular cases of a more general enzyme mechanism involving a general modifier mechanism of the model of the mechanism depicted in scheme 1. The results of the mechanism described in scheme 1 have been discussed in detail in previous works, with reference to an effect on the product rate by Segel and Martin [15].

The action of the modifier involved in scheme 1 as activator or inhibitor has only been discussed, as far as we know, with relation to its effect on the product rate at the steady state [11,12,15,27]. Segel [11] and Segel and Martin [15] studied fully the steady-state rate of the general unireactant modifier mechanism described by scheme 1. This author yielded a velocity equation of second degree in both [S] and [M] which was later

\[
V = \frac{V_{max} \left[ S \right]}{K_m + [S] + K_m^* + [M]^*}
\]