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Review

Temperature and the catalytic activity of enzymes: A fresh understanding

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ABSTRACT

The discovery of an additional step in the progression of an enzyme from the active to inactive state under the influence of temperature has led to a better match with experimental data for all enzymes that follow Michaelis–Menten kinetics, and to an increased understanding of the process. The new model of the process, the Equilibrium Model, describes an additional mechanism by which temperature affects the activity of enzymes, with implications for ecological, metabolic, structural, and applied studies of enzymes.

1. Introduction

Leonor Michaelis and Maud Menten are often regarded as the founders of modern quantitative enzymology. In their studies on invertase [1], they recognised the necessity of assaying the enzyme under defined and controlled conditions, principally with respect to the pH of the reaction, and the need to measure initial rates, thereby avoiding various complicating factors including the known inhibition of the enzyme by the product of the reaction. Their data then fitted what we now know as the Michaelis–Menten equation, the fundamental equation of enzyme kinetics:

$$\mathcal{V} = \frac{V_{max}[S]}{K_M + [S]} \quad (1)$$

Clearly, in addition to a defined pH, kinetic measurements are made with a constant amount of enzyme in the assay, and at a constant temperature that does not result in loss of catalytic activity during the time course of the assay. The choice of assay temperature is extremely important since the way enzymes respond to temperature is fundamental to many areas of biology. Thus, it is this aspect, the effect of temperature on enzyme catalytic activity, that we wish to explore in the current paper, and provide new insights that are of both theoretical and practical importance.

Where the effect of temperature on enzyme activity has been considered, textbooks have said that higher temperatures increase enzyme activity to a certain point, after which the enzyme denatures, losing its activity irreversibly (e.g., [2–4]). In this model, the change in enzyme activity with increasing temperature is simply the combined result of the effect of temperature increasing k_{cat} and k_{inact} on a simple two step conversion (i.e., $E_{act} \rightarrow X$), then the time-dependent loss of activity, expressed as V_{max}, is described by the following equation:

$$V_{max} = k_{cat} \cdot [E_0] \cdot e^{-k_{inact} \cdot t} \quad (2)$$

where V_{max} = maximum velocity of enzyme; k_{cat} = enzyme's catalytic constant; $[E_0]$ = total concentration of enzyme; k_{inact} = thermal inactivation rate constant; t = assay duration.

The variation of the two rate constants in Eq. (2) with temperature is given by:

$$k_{cat} = \frac{k_B T}{h} e^{-\left(\frac{\Delta G^{\ddagger}_{cat}}{RT}\right)} \quad (3)$$

and

$$k_{inact} = \frac{k_B T}{h} e^{-\left(\frac{\Delta G^{\ddagger}_{inact}}{RT}\right)} \quad (4)$$

where k_B = Boltzmann's constant; R = Gas constant; T = absolute temperature; h = Planck's constant; $\Delta G^{\ddagger}_{cat}$ = activation energy of the catalysed reaction; $\Delta G^{\ddagger}_{inact}$ = activation energy of the thermal inactivation process.

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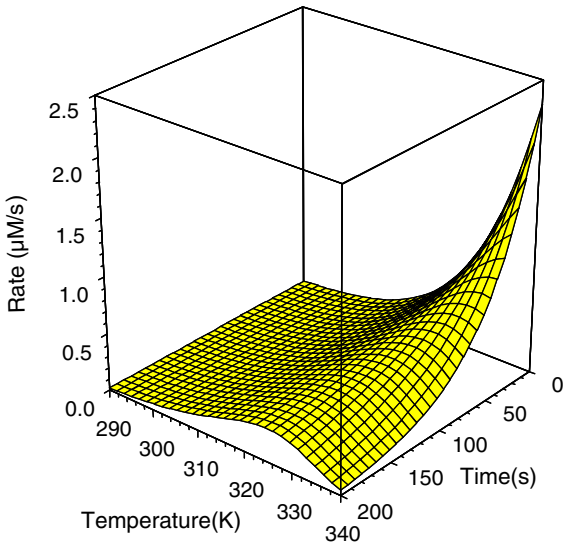

Fig. 1. The Classical theory of the effect of temperature on enzyme activity. The temperature dependence of enzyme activity with time. The data were simulated using Eqs. (2)–(4), with the parameter values: $\Delta G^{\ddagger}_{cat} = 75$ kJ mol^{-1} and $\Delta G^{\ddagger}_{inact} = 95$ kJ mol^{-1}. Note that the apparent temperature optimum decreases with increasing length of the assay. Reproduced with permission from [24].

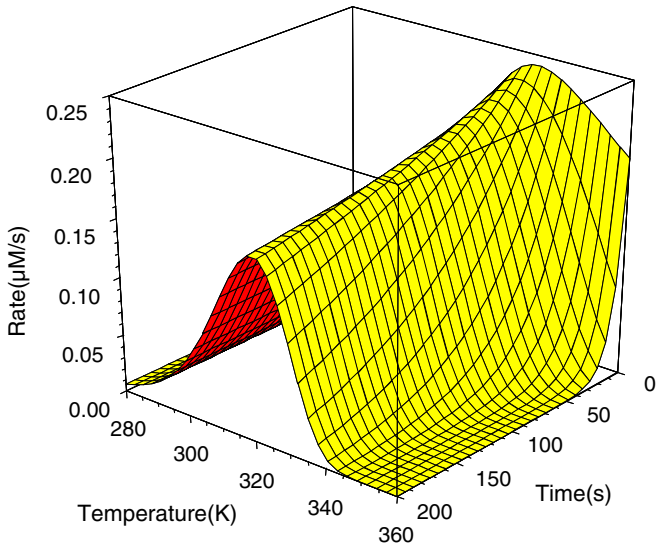

Fig. 2. The Equilibrium Model for the effect of temperature on enzyme activity. The temperature dependence of enzyme activity with time. The data were simulated using Eqs. (3)–(6), with the parameter values: $\Delta G^{\ddagger}_{cat} = 75$ kJ mol^{-1}, $\Delta G^{\ddagger}_{inact} = 95$ kJ mol^{-1}, $\Delta H_{eq} =$ kJ mol^{-1} and $T_{eq} = 320$ K. Reproduced with permission from [24].

Using these equations, and inserting plausible values for $\Delta G^{\ddagger}_{cat}$ and $\Delta G^{\ddagger}_{inact}$, a plot of activity against temperature and time can be constructed (Fig. 1). At zero time there is no denaturation, defined here as the time-dependent, irreversible loss of activity, and initial rates will therefore rise continuously with temperature; the expected reaction progress with time at various temperatures can be seen later in Fig. 5C. However, despite this model being commonly used to describe the effect of temperature on enzyme activity, it is now clear that no enzymes actually exhibit this behaviour; careful measurements show that initial rates decline at high temperatures independent of irreversible inactivation. A re-examination of the effect of temperature on enzyme catalytic activity has therefore led to the proposal of a new model [5,6], the Equilibrium Model, described below.

2. The Equilibrium Model

The new model for the temperature-dependent behaviour of enzymes, the Equilibrium Model, introduces an intermediate inactive (but not denatured) form of the enzyme that is in rapid equilibrium with the active form, and it is the inactive form that undergoes irreversible thermal inactivation to the denatured (irreversibly inactive) state (X):

$$E_{act} \overset{K_{eq}}{\rightleftharpoons} E_{inact} \overset{k_{inact}}{\rightarrow} X$$

where E_{act} is the active form of the enzyme, which is in equilibrium with the inactive form, E_{inact}. K_{eq} is the equilibrium constant describing the ratio of E_{inact}/E_{act}; k_{inact} is the rate constant for the E_{inact} to X reaction; and X is the irreversibly-inactivated form of the enzyme. Within this model, there is no implication that the conversion of E_{inact} to X occurs in a single step, or that X is a single species, only that they can be considered as such since all species beyond E_{inact} are irreversibly inactivated.

Using the Equilibrium Model, the variation of enzyme activity with temperature can be expressed by:

$$V_{max} = \frac{k_{cat} E_0 e^{-\frac{k_{inact} K_{eq} t}{1+K_{eq}}}}{1 + K_{eq}} \quad (5)$$

where

$$K_{eq} = e^{\frac{\Delta H_{eq}}{R}\left(\frac{1}{T_{eq}} - \frac{1}{T}\right)} \quad (6)$$

T_{eq} is the temperature at which the E_{act}/E_{inact} equilibrium is at its mid-point ($K_{eq} = 1$, $\Delta G_{eq} = 0$, and therefore $T_{eq} = \Delta H_{eq}/\Delta S_{eq}$), where ΔH_{eq} is the change in enthalpy for the E_{act}/E_{inact} transition. In this case, a plot of rate vs temperature vs time (Fig. 2) does have an optimum for initial rates (i.e., at zero time) because the E_{act}/E_{inact} equilibration is rapid. This is known from the experimental observation that enzyme initial rates do in fact decline at high temperatures (see Fig. 6 for an example), and at all temperatures the E_{act}/E_{inact} equilibration is faster than the time needed to start the reaction in a stirred spectrophotometer cuvette (of the order of 1–3 s), and the line of product vs time extrapolates back to zero [7–10].

So far, all enzymes we have studied that obey Michaelis–Menten kinetics follow this Model [7–9], irrespective of mechanism or structure (e.g., Table 1), and all have a temperature optimum for initial rates, as expected from the Model. Nevertheless, many enzymes do not follow ideal kinetics, when the enzyme is substrate or product inhibited for example, and the Model must be regarded as describing an ideal, and a degree of departure may occur after significant reaction progress, as is the case for all models of enzyme behaviour.

3. Mechanism of the Equilibrium Model

The discovery of a new and apparently universal mechanism by which enzymes lose activity as the temperature rises is of considerable interest in itself, and has a number of interesting implications. Although the Equilibrium Model does not of itself offer an explanation of the molecular basis of the E_{act}/E_{inact} transition, this interconversion can be clearly differentiated from denaturation. Compared with denaturation, it operates over much shorter timescales [6,7,9,11,12], structural changes are imperceptible [9], and the associated ΔH_{eq} is an order of magnitude smaller than ΔH_{unfold} [9,13–15]. Moreover, changes of T_{eq} can be independent of changes in stability [9]. Thus, the evidence indicates that the E_{act}/E_{inact} transition involves only a small conformational change and there is

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