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The Equilibrium Model for the effect of temperature on enzymes

Insights and implications

ABSTRACT

A new, experimentally-validated "Equilibrium Model" describes the effect of temperature on enzymes, and provides a new mechanism for the reversible loss of enzyme activity with temperature. It incorporates two new, fundamental parameters that allow a complete description of the effect of temperature on enzyme activity: ΔH_{eq} and T_{eq}. ΔH_{eq} emerges as an intrinsic and quantitative measure of enzyme eurythermal adaptation, while T_{eq}, the equilibrium temperature, has fundamental and technological significance for our understanding of the effect of temperature on enzymatic reactions. For biotechnological purposes, these parameters need to be considered when enzymes are applied or engineered for activity at high temperatures.

INTRODUCTION

It is now established that the effect of temperature on enzyme activity cannot be described by a simple, or "Classical", two-state model arising from the temperature dependence of enzyme activity on the catalytic reaction (defined by k_{cat}) and irreversible thermal inactivation (defined by k_{inact}), described by $\Delta G^{\ddagger}_{cat}$, and $\Delta G^{\ddagger}_{inact}$ respectively (1, 2). The assumption of a two-state model, namely

$$E_{act} \rightarrow X \qquad \text{(eqn 1)}$$

can now, on the basis of experimental evidence, be replaced by a new model (the Equilibrium Model) (1) that assumes that the active enzyme (E_{act}) is in equilibrium with a reversibly inactive form (E_{inact}) and that the inactive form undergoes irreversible thermal inactivation to a denatured state (X). The equilibrium is described by an equilibrium constant (K_{eq}).

$$E_{act} \underset{}{\overset{K_{eq}}{\rightleftharpoons}} E_{inact} \xrightarrow{k_{inact}} X \qquad \text{(eqn 2)}$$

In the Equilibrium Model, two new parameters are introduced to describe the effect of temperature on the equilibrium between active and inactive forms of a protein: ΔH_{eq} is the enthalpy of the equilibrium, while T_{eq} is the temperature at which the concentration of E_{act} equals the concentration of E_{inact}.
The variation of K_{eq} with temperature can be described as:

$$\ln\left(K_{eq}\right) = \frac{\Delta H_{eq}}{R}\left(\frac{1}{T_{eq}} - \frac{1}{T}\right) \qquad \text{(eqn 3)}$$

At T_{eq}, $[E_{act}] = [E_{inact}]$; therefore, $K_{eq} = 1$, $\Delta G^{\ddagger}_{eq} = 0$, and ΔHeq = Teq·ΔS_{eq}, where ΔS_{eq} is the change in entropy associated with the equilibrium. In this context, T_{eq} can be regarded as the thermal equivalent of K_M since it is the temperature at which half of the enzyme is active. Overall, the quantitative expression of the dependence of apparent catalytic rate on temperature (T) and time (t) is given by the equation:

$$V_{max} = \frac{k_B T e^{-\left(\frac{\Delta G^{\ddagger}_{cat}}{RT}\right)} E_0 e^{-\frac{k_B T e^{-\left(\frac{\Delta G^{\ddagger}_{inact}}{RT}\right)} e^{\left(\frac{\Delta H_{eq}\left(\frac{1}{T_{eq}}-\frac{1}{T}\right)}{R}\right)} t}{h\left(1+e^{\left(\frac{\Delta H_{eq}\left(\frac{1}{T_{eq}}-\frac{1}{T}\right)}{R}\right)}\right)}}}{h\left(1+e^{\left(\frac{\Delta H_{eq}\left(\frac{1}{T_{eq}}-\frac{1}{T}\right)}{R}\right)}\right)} \qquad \text{(eqn 4)}$$

where k_B is Boltzmann's constant, R is the gas constant, and h is Planck's constant (2). The Equilibrium Model parameters of an enzyme can be obtained by fitting experimental data of enzyme time-course assays to the mathematical model (3): so far, all the enzymes (>30) characterized in our laboratories follow the behaviour described by the Equilibrium Model, and none fit the Classical Model (1, 2, 4, 6). A comparison of the Equilibrium and Classical models can be seen in Figure 1.
Figure 1A shows a set of experimental data obtained by measuring the rate of phenylalanine ammonia lyase with time over a range of temperatures, assayed as described by Lee et al (4). If the experimental data are fitted to equation 4, one can then derive the four Equilibrium Model parameters for the enzyme ($\Delta G^{\ddagger}_{cat}$ = 80 kJ mol^{-1}, $\Delta G^{\ddagger}_{inac}$ = 97 kJ mol^{-1}, T_{eq} = 330 K and ΔH_{eq} = 181 kJ mol^{-1}). Re-inserting these values into the Equilibrium Model results in a simulated plot that matches the experimental data (Figure 1B). The experimental data cannot be fitted to the two-state Classical model, but if the values obtained for $\Delta G^{\ddagger}_{cat}$ and $\Delta G^{\ddagger}_{inact}$ are inserted into the Classical model, the result is as expected, with no optimum at zero time since denaturation is time-dependent (Figure 1C). This figure illustrates well the limitations of the Classical model (see also refs 1 and 5). One effect of incorporating an additional inactive species of the enzyme, in reversible equilibrium with the active form, is the appearance of a temperature optimum (T_{opt}) at zero time in the Equilibrium Model, which is absent in the Classical Model. The Equilibrium Model thus implies the existence of a new mechanism for the reduction in activity as temperature increases, since denaturation is time-dependent (1). More than 30 enzymes have been studied so far (2, 4, 6) and all show the "tent" type graph with a temperature optimum for activity at zero time, at temperatures where no significant denaturation could have occurred, and all fitted the Equilibrium Model.

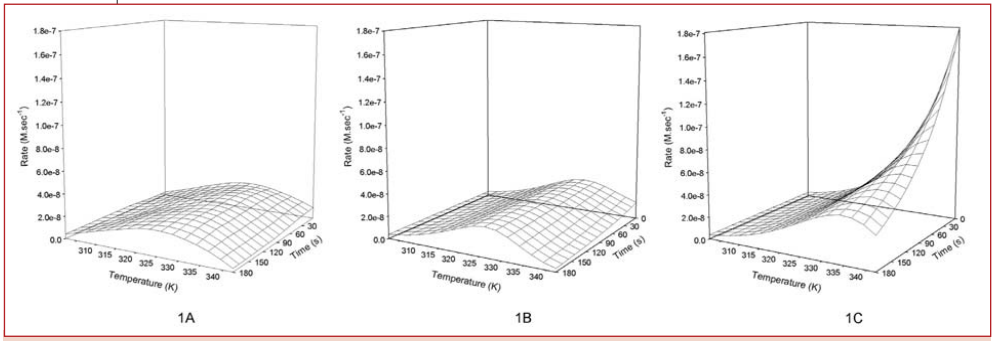

Figure 1. The temperature dependence of enzyme activity. The plots of rate (M·s^{-1}) versus temperature (K) versus time (s) illustrate the effect of temperature on enzymatic activity. 1A Experimental data plot, 1B simulated data plot (Equilibrium Model), 1C simulated data plot ("Classical" Model).

EVOLUTIONARY AND ENVIRONMENTAL IMPLICATIONS (TEMPERATURE ADAPTATION)

The adaptation of enzymes to temperature has been studied by a variety of methods, including variation of k_{cat}/K_M in organisms adapted to different temperatures. It has recently been demonstrated that an enzyme having a higher catalytic efficiency can, at certain substrate concentrations, catalyze an

identical reaction at lower rates than an enzyme with a lower k_{cat}/K_M. This term is, therefore, an inadequate measure of catalytic effectiveness of enzymes and is not recommended as an index for comparing enzymes as catalysts or conducting enzyme temperature adaptation comparisons (7). Enzyme thermal stability, while generally correlated with growth temperature of the source organism (e.g. ref 4), is usually determined in the absence of substrate, while in the presence of substrate such studies give rise to a temperature optimum that is dependent on assay duration. The Equilibrium Model can provide a solution to these problems, since its parameters are derived under substrate-saturating conditions and are time independent; they are an intrinsic property of all enzymes and therefore can be used to compare enzymes of diverse origins and nature (4). Empirical evidence obtained through the characterization of twenty-one enzymes showed that ΔH_{eq} is a valid and universal measure of enzyme eurythermalism. The same set of experimental data also provided validation of the Equilibrium Model as a tool for studying temperature adaptation. Specifically, a strong link between T_{eq} and the optimal temperature of growth of the source organism was found (3). Statistical analysis indicated that T_{eq} correlated better with optimal growth temperature than enzyme stability ($\Delta G^{\ddagger}_{inact}$), and that ΔH_{eq} and T_{eq} are independent of $\Delta G^{\ddagger}_{cat}$ and $\Delta G^{\ddagger}_{inact}$ (3). Experimental data based on the Equilibrium Model also provide potential evidence in support of the thermophilic origin of life. The difference between T_{eq} and the optimal growth temperature decreases as optimal growth temperature increases (4), which seems to support the theory that microbial evolution has proceeded down-temperature from a hyper-thermophilic common ancestor (8). In conclusion, ΔH_{eq}, the enthalpic change associated with the conversion of E_{act} to E_{inact}, emerges as a new intrinsic and quantitative measure of enzyme eurythermalism, while T_{eq}, the temperature of the mid point of the E_{act}/E_{inact} transition, has fundamental and technological significance, enabling improvement in our understanding of the effect of temperature on enzymatic reactions within the cell, and of enzyme evolution in response to temperature, and establishing the Equilibrium Model as a valid tool for assessing enzyme eurythermalism and thermophily.

IMPLICATIONS FOR BIOTECHNOLOGY

There are biotechnological implications of the Equilibrium Model in the areas of enzyme engineering, selection or screening of useful enzymes from the environment, and bioreactor operation. Stable enzymes are valuable for biotechnology as they exhibit prolonged active life, are stable in storage, resist organic solvents and proteolytic attack, and can be used in processes that take place under extreme conditions (9). Although hyper-stable enzymes have been isolated from organisms growing in thermal environments, it is frequently more desirable to improve the stability of existing commercial enzymes. However, the Equilibrium Model introduces additional factors to be considered when enzymes are engineered for enhanced activity at high temperatures. It shows that raising enzyme stability (i.e., increasing $\Delta G^{\ddagger}_{inact}$), will not necessarily improve activity at high temperatures. This is because, if $\Delta G^{\ddagger}_{inact}$ is increased but T_{eq} is unchanged or lowered, then as the temperature is raised activity will still be lost by the transition of the active form of the enzyme to the inactive form, independent of denaturation. Furthermore, if T_{eq} is unaffected or lowered by mutagenesis, and the thermostability is assessed in a high-temperature enzyme assay, increased stability might not be detected when the mutants are screened (10). Thus, when screening protein libraries for useful mutants, it will be important to determine the effect of the mutations on the T_{eq} as well as on thermostability, since both of these will influence activity at high temperatures. The molecular basis of the E_{act}/E_{inact} equilibrium described by T_{eq} has not been defined, although there is evidence that it may involve a local conformational change at the active site (2, 10, 5). If this is so, mutation to shift T_{eq} towards higher temperatures might prove difficult without affecting k_{cat} or K_M. Apart from enzyme engineering, there is still a potential for thermostable enzyme selection from sources that grow at high temperatures, since enzyme thermal stability is correlated to some degree with the growth temperature of the source organism (e.g. 3). However, in this case, it is important to be able to make a clear distinction between the activity at high temperatures and thermal stability, i.e., to determine to what extent high temperature activity depends upon stability, and to what extent it depends upon a high T_{eq}. In order to maximize the output of enzyme bioreactors, it is important to balance the effects of temperature upon enzyme stability and upon activity carefully. However, simulations of reactor performance under different conditions will give quite different predictions of the output of product with time and temperature if the Classical Model is employed as opposed to when the Equilibrium Model is used. Intuitively, it would seem that when an enzyme is used for chemical synthesis in a batch reactor, the higher the operating temperature, the faster the catalysed reaction and the less stable the enzyme. The Equilibrium Model shows that, this is only true if T_{eq} is higher than the operating temperature. The reverse is true when T_{eq} is lower than the operating temperature (10).

CONCLUSION

The Equilibrium Model proposes a new mechanism, additional to denaturation, by which enzymes can (reversibly) lose activity as the temperature rises (1). The experimental validation of this model (2) indicates that the parameters introduced by the model are essential for describing the effect of temperature on enzyme activity and for measuring the temperature adaptation of enzymes (4). The model can provide an improved understanding of the evolution of enzymes and has major implications for enzymology and biotechnology.

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Any researcher wishing to fit their data to the Equilibrium Model can obtain a free stand-alone application based on MATLAB (The MathWorks, Inc.) on a compact disc from Prof. Roy Daniel (r.daniel@waikato.ac.nz) or Dr. Charles K. Lee (cklee@waikato.ac.nz). The application enables facile derivation of the Equilibrium. Model parameters from a Microsoft Office Excel file of experimental progress curves.

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