FEBS Letters 589 (2015) 2200–2206

journal homepage: www.FEBSLetters.org

Hypothesis

Change in heat capacity accurately predicts vibrational coupling in enzyme catalyzed reactions

Vickery L. Arcus [a], Christopher R. Pudney [b]

[a] School of Science, Faculty of Science and Engineering, University of Waikato, Hamilton 3240, New Zealand
[b] Department of Biology and Biochemistry, Faculty of Science, University of Bath, Bath BA2 7AY, United Kingdom

ARTICLE INFO

Article history:
Received 5 February 2015
Revised 24 June 2015
Accepted 30 June 2015
Available online 11 July 2015

Edited by Paul Bertone

Keywords:
Enzyme dynamics
Vibrational coupling
Promoting motion
Heat capacity
Catalysis

ABSTRACT

The temperature dependence of kinetic isotope effects (KIEs) have been used to infer the vibrational coupling of the protein and or substrate to the reaction coordinate, particularly in enzyme-catalyzed hydrogen transfer reactions. We find that a new model for the temperature dependence of experimentally determined observed rate constants (macromolecular rate theory, MMRT) is able to accurately predict the occurrence of vibrational coupling, even where the temperature dependence of the KIE fails. This model, that incorporates the change in heat capacity for enzyme catalysis, demonstrates remarkable consistency with both experiment and theory and in many respects is more robust than models used at present.

1. Introduction

The contribution of protein dynamics has emerged as one of the key theoretical notions required to understand the activity of enzymes. Much of the research in this area has centred on how the molecular vibration of the enzyme and/or substrate influences catalytic activity [1,2]. Enzyme catalyzed quantum mechanical tunneling (QMT) reactions involving the transfer of hydrogen (H^-, $H^\cdot$, H^+) represent some of the best characterized systems attempting to relate the importance of enzyme dynamics to enzyme turnover and have been extensively studied in the context of so called 'vibronic' models [1,2]. Broadly, these models explicitly recognize the role of vibrational modes of the enzyme and/or substrate coupled to the reaction coordinate, and in some cases, infer that these coupled vibrations can promote the rates of enzyme catalyzed reactions as so called 'promoting vibrations'. There is some experimental evidence that such modes can provide a small rate enhancement to enzyme turnover [3] and computational evidence that altering these modes impacts on the enzyme chemistry [4]. However, there is also computational evidence that these modes are not themselves catalytic [5]. These notions are highly contentious with a broad range of views and little consensus. Despite major effort, providing experimental evidence for the role and significance of protein dynamics remains a significant challenge. The temperature dependence of the kinetic isotope effect (KIE), $\Delta\Delta H^\ddagger$, has been used to infer the importance of protein dynamics coupled to the reaction coordinate in enzyme catalyzed H-transfer reactions. That is, a temperature-dependent KIE [$\Delta\Delta H^\ddagger > \sim 5$ kJ mol^{-1}; larger than the contribution from zero point energy (ZPE) alone] is considered to reflect significant vibrational coupling and a temperature-independent KIE ($\Delta\Delta H^\ddagger = 0$ kJ mol^{-1}) suggests far less significant, or an absence of vibrational coupling [1].

In recent years there has been a drive to augment analysis of the temperature dependence of KIEs with alternative models both to probe the validity of the approach and to offer new insight into the role (if any) of vibrational coupling. Several researchers have postulated that elevated values of $\Delta\Delta H^\ddagger$ result from the presence of multiple reactive states [6–8]. Alternatively, Canepa has attempted to relate the curvature of Arrhenius plots to the coupling of vibration modes [9]. These studies rely on the simple fitting of multi-parameter models to experimental data and do not provide comparative analysis with existing evidence and theory. Here we describe a new model that detects vibrational coupling of enzyme/substrate dynamics to the reaction coordinate in enzyme catalyzed reactions, based on fundamental thermodynamic principles.

Author contributions: VLA and CRP conceived the manuscript, analyzed data and wrote the manuscript.

E-mail addresses: varcus@waikato.ac.nz (V.L. Arcus), c.r.pudney@bath.ac.uk (C.R. Pudney)

http://dx.doi.org/10.1016/j.febslet.2015.06.042
0014-5793/

Recently, Hobbs et al. have established a model for the temperature dependence of enzyme catalyzed rates that relates the change in heat capacity, $\Delta C_P^{\ddagger}$, to the temperature optima for a range of enzyme catalyzed reactions [10] (termed macromolecular rate theory, MMRT [11]). Hobbs et al. found that changes to $\Delta C_P^{\ddagger}$ are sufficient to alter the enzyme T_{opt}. The fundamental thermodynamic parameter $\Delta C_P^{\ddagger}$, quantifies the temperature-dependence of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ and hence $\Delta G^{\ddagger}$ (E_a). The temperature dependence of the reaction rate is given by:

$$k = (k_B T/h)e^{-\Delta G_{\ddagger}/RT} \quad (1)$$

$$ln(k) = ln\left(\frac{k_B T}{h}\right) - \frac{[\Delta H^{\ddagger}_{T_0} + \Delta C_P^{\ddagger}(T - T_0)]}{RT} + \frac{[\Delta S^{\ddagger}_{T_0} + \Delta C_P^{\ddagger} ln(T/T_0)]}{R} \quad (2)$$

where T_0 is an arbitrary reference temperature, and $\Delta H^{\ddagger}_{T_0}$ and $\Delta S^{\ddagger}_{T_0}$ are the difference in enthalpy and entropy between the ground state and the transition state, respectively, at T_0. $\Delta C_P^{\ddagger}$ determines the change in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ with temperature and defines the temperature-dependence of the Gibbs free energy between the ground state and the transition state ($\Delta G^{\ddagger}$). If $\Delta C_P^{\ddagger} \neq 0$ the relationship between rate and temperature will show marked curvature in a plot of ln(k) versus T. Typically the temperature dependence of the rate is fit to either the Eyring or Arrhenius equation. For the Eyring equation the data are fit to:

$$ln\left(\frac{k}{T}\right) = ln\frac{k_B}{h} + \frac{\Delta S^{\ddagger}}{R} - \frac{\Delta H^{\ddagger}}{RT} \quad (3)$$

The greatest contribution to the heat capacity of a folded protein is the number of populated vibrational and rotational modes associated with covalent bonds (i.e., protein dynamics). These modes contribute an estimated 85% of the C_p term with the hydration term contributing the remaining 15% for globular proteins [12,13]. For an enzyme catalyzed reaction, a negative $\Delta C_P^{\ddagger}$ arises from tight binding of the transition state and a reduction in the population of conformational states (vibrational modes) at the transition state compared to the ground state.

Hobbs et al. have suggested that changes in protein dynamics (vibrational modes of the protein) may be the dominant source of changes to $\Delta C_P^{\ddagger}$ for enzyme catalysis with a far smaller contribution from the hydration term, e.g. desolvation of the active site. To be clear, the hydration term is the dominant source of changes in $\Delta C_P^{\ddagger}$ for protein folding [10]. Hobbs et al. find that Eq. (2) can fully account for Arrhenius plot curvature for a range of enzymes, independent of protein denaturation and suggest this provides compelling evidence that differences in protein dynamics may be the source of a negative $\Delta C_P^{\ddagger}$ [10].

Based on this model, we hypothesize that Eq. (2) may be a powerful method to identify the coupling of vibrational modes of the protein and or substrate to the reaction coordinate at the transition state or tunneling-ready configuration (TRC) in the case of QMT reactions. Physically we would expect this relationship to arise since where vibrational coupling is present, it should disappear or be dramatically reduced at or near the TS (as observed in the case of HLADH [14]), giving a large difference in C_p between the reactive state and TS. From Eq. (2), by measuring the temperature dependence of the rate of H-transfer, we therefore hypothesize that curvature (-ve $\Delta C_P^{\ddagger}$) in plots of the temperature dependence of the rate (ln$k$) will reflect the occurrence of significant vibrational coupling of the enzyme–substrate complex to the reaction coordinate.

To test this hypothesis and to explore the use of Eq. (2) as a general model for inferring vibrational coupling, we analyze a range of examples of enzyme catalyzed H-transfer reactions using Eq. (2). We have selected paradigmatic systems with a range of reaction chemistry, cofactor and substrate usage, and size. Namely, pentaerythritol tetranitrate reductase (PETNR, NADH/NADPH), morphinone reductase (MR, NADH) soybean lipoxygenase (SLO-1, linoleic acid), *Escherichia coli* dihydrofolate reductase (*ec*DHFR, NADPH), methylamine dehydrogenase (MADH, methylamine), aromatic amine dehydrogenase (AADH, tryptamine [$AADH_{TA}$]) and horse liver alcohol dehydrogenase (HLADH, benzyl alcohol). These systems represent excellent test cases, since the vibrational coupling in these systems has been intensively studied using a range of approaches. Crucially each of these model systems (i) have experimental kinetic data that reflect as near as is possible the H-transfer step given the limitations of experimental tractability, (ii) have corresponding data for deuterium transfer, (iii) are structurally non-perturbed across the temperature range and (iv) are well characterized in the context of the role of QMT and vibrational coupling, with evidence beyond the magnitude of $\Delta\Delta H^{\ddagger}$ alone.

2. Results and discussion

2.1. Curvature in the temperature-dependence of the rate correlates with vibrational coupling

Fig. 1 shows the fit of Eqs. (2) and (3) to the experimentally determined temperature dependence of observed rate constants for protium transfer for each of the model systems identified above. Table 1 compares the extracted value of $\Delta C_P^{\ddagger}$ with $\Delta\Delta H^{\ddagger}$, grouped into measurably temperature-(in)dependent values based on the magnitude of $\Delta\Delta H^{\ddagger}$. These combined data are shown in Fig. 2. The $\Delta H^{\ddagger}$ and $\Delta\Delta H^{\ddagger}$, values extracted from fitting to both Eqs. (2) and (3) are given in Supporting Information (SI) Table S1. The values are similar within error except in the case of AADH where fitting to Eq. (2) gives a very large value of $\Delta\Delta H^{\ddagger}$ (20.1 ± 7.4 kJ mol^{-1}) compared to fitting to Eq. (3) (−3 ± 0.2 kJ mol^{-1}). We discuss the case of AADH in more detail below.

Our analysis is based on the curvature observed in plots of the temperature dependence of enzyme rate constants (ln$k$). Typically one fits such data sets to either the Eyring or Arrhenius equations to extract (at least) the magnitude of $\Delta H^{\ddagger}$ or the activation energy E_a, respectively. All of the data we present in Fig. 1 and Table 1 have been treated in this way previously and therefore by inference are not considered to show obvious curvature in the temperature dependence of ln$k$. Even so, we expect that it may be possible to extract meaningful data by fitting to Eq. (2) if there is some microscopic curvature that is not readily apparent by visual inspection. Arrhenius plots are expected to show curvature due to the temperature dependence of the pre-exponential factor and so detecting curvature is best achieved from Eyring plots (ln$k$/T versus 1/T) and fitting to Eq. (3). Fig. 1B and D shows the Eyring plots for the data examined in this study corresponding to Fig. 1A and C, respectively. The fits to Eq. (3) are shown as a solid fitted line. To explore if there is some microscopic curvature that Eq. (2) might fruitfully capture, we have fit the Eyring equation to the high and low temperature half of each data set independently (shown as dash lines). A truly linear data set will therefore display no difference between the fitted lines. We find that in many cases the fitted lines do not overlay well, with a smaller magnitude of $\Delta H^{\ddagger}$ and a larger magnitude of $\Delta H^{\ddagger}$ by fitting to the higher and lower temperature half of the data sets, respectively (Table S1). That is, these data suggest the ln$k$ data deviate from linearity outside of error and give rise to some weak curvature for a number of the systems studied. From Table S1, the only data sets that give the same $\Delta H^{\ddagger}$ values within error are MADH and HLADH. As such we would expect that fitting