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Evidence for a low temperature transition state binding preference in bovine adenosine deaminase

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Abstract

Arrhenius plots of the interactions of bovine adenosine deaminase (ADA) and of coformycin-inhibited ADA with adenosine are non-linear and reveal that coformycin significantly increases the activation energy for reaction only at temperatures well below the normal operating temperature of the enzyme (38.3°C). This apparent enhanced affinity of the enzyme for the transition state analog at low temperature is confirmed from determinations of coformycin binding at 38.3°C ($K_I = 5.3 \times 10^{-11}$ M) and at 21°C ($K_I = 1.1 \times 10^{-11}$ M). It is suggested that these data are inconsistent with a model for general enzyme catalysis that requires an initial transition state complementary active site. Instead, it is suggested that an initial active site transition state complementarity is undesirable and the tendency of the enzyme to exist in this conformer at low temperatures is responsible for its inefficient interaction with adenosine substrate. © 1998 Elsevier Science B.V.

Keywords: Non-linear Arrhenius; Coformycin; Shifting specificity model; Ground state complementarity; Complementarity; Transition state complementarity; Activation energy

1. Introduction

Many enzymes exhibit non-Arrhenius behavior in their interactions with substrates where the activation energy (E_{act}) for the reaction increases below a certain temperature. The temperature of the Arrhenius plot inflection (T_{API}) typically occurs a few degrees below the normal operating temperatures of the enzymes. Historically, there have been two explanations offered to account for these discontinuities [1]: a temperature-dependent change in the rate determining step of the reaction or an alteration of the enzyme global conformation that renders it less active at low temperatures.

There exists strong evidence that for many enzyme/substrate systems the source of non-Arrhenius behavior is a temperature-induced enzyme conformational change. Kumamoto et al. [2] conclude that the sharp breaks observed in Arrhenius plot inflections cannot be due to a change in the rate determining step but instead must be attributable to a temperature-induced phase change of the enzyme. Temperature-dependent changes in enzyme physical properties coinciding with T_{API}s have been observed in D-amino acid oxidase [3], yeast 3-phosphoglycerate kinase [4], urocanate hydratase [5], tobacco rubisco [6], penicillopepsin [7], and rabbit pyruvate kinase

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[8]. Other apparent temperature-induced enzyme conformational changes have also been observed [9–12].

The question naturally arises as to whether the putative conformational change has any functional significance or whether it is only an irrelevant artefact produced by artificially subjecting the enzyme to a temperature where, at least in birds and mammals, it is never required to function in vivo. Recently, a model for general enzyme catalysis was proposed that suggests the temperature-induced conformational change may reflect a crucial aspect of enzyme function [13,14]. This model states that enzymes, at their normal, physiologically relevant temperatures, possess an active site geometry which is not complementary to the transition state of the reaction, as is generally supposed [15–19], but which is complementary to the ground state of the substrate. Interaction of the substrate with the active site via the same noncovalent interactions that define the enzyme interdomain interactions induces a global conformational change in the enzyme. Evolution has selected for a conformational change that transforms the active site from substrate-specific (E_S) to transition state-specific (E_{TS}). The transformation of the enzyme from E_S to E_{TS} is coincidental with the transformation of subtrate from the ground state to the transition state [20]. The model therefore possesses an 'induced fit' aspect [18,19] but is an alteration from a ground state specificity which involves the *entire* enzyme molecule. Furthermore, the conformational energy of E_{TS} is lower than that of E_S. Such a decrease in the conformational energy during the transition state of the reaction could account for some of the rate acceleration over the non-catalyzed reaction. If this last point is correct, then it seems likely that lowering the temperature of the enzyme well below its normal operating temperature may artificially induce E_{TS}. We report here the results of our investigations into the possibility that the non-Arrhenius behavior observed in the adenosine deaminase (ADA)/adenosine system reflects the adoption of an enzyme conformer with an enhanced affinity for the transition state at low temperatures.

ADA is a well-studied enzyme that catalyzes the hydration of adenosine or deoxyadenosine to inosine or deoxyinosine and is strongly inhibited by coformycin [21–23]. Coformycin is thought to be a transition state analog (TSA) because of the similarity of the sp^3 hybridized C-8 to the tetrahedral geometry that occurs at the C-6 position of adenosine during the transition state [24,25]. It is found in all human tissues though it tends to concentrate in T lymphocytes. Absence or diminished activity of the enzyme may result in Severe Combined Immunodeficiency Disease (SCID).

To investigate the possibility that low temperature favors the formation of an E_{TS} structure in ADA we have constructed Arrhenius plots of the free and coformycin-inhibited enzyme for the purpose of comparing the temperature-dependence of the E_{act}s for the conversion of adenosine substrate to inosine. We reason that any observed increase in the E_{act} for the inhibited enzyme over that of the uninhibited enzyme reflects the increased E_{act} associated with the unfavorable dissociation of the tight-binding inhibitor from the active site. The magnitude of this increase is therefore a reflection of the affinity of the active site for the TSA. Accordingly, we have also determined the temperature-dependence of K_I for coformycin binding to the ADA active site.

Our findings are that coformycin increases the apparent E_{act} at all temperatures but most significantly only at temperatures well below the normal operating temperature of the enzyme. We also find that coformycin binds to bovine ADA approximately five-fold more strongly at 21°C than at 38.3°C, the normal body temperature of the cow. These data are consistent with a significantly increased affinity of the ADA active site for coformycin, and by inference, for the true transition state of the adenosine deamination reaction, at low, non-physiologic temperatures.

2. Materials and methods

2.1. Reagents

Adenosine and ADA from calf spleen as an ammonium sulfate suspension were obtained from Sigma Chemical (St. Louis, MO). Coformycin was a gift from Parke-Davis (Ann Arbor, MI). All reactions were performed in 10 mM phosphate buffer at pH = 6.3, the pH optimum of the enzyme.

2.2. Instrumentation

Data were obtained on an Applied Photophysics SX-17MV stopped flow reaction analyzer equipped with a Neslab RTE-111 circulating water bath. Rates of reaction were determined by monitoring the decrease in adenosine absorbance at 260 nm. Temperatures were measured at the time of mixing from the water reservoir which bathed the sample syringes and reaction cell and are good to within 0.1°C.

2.3. Arrhenius plots

Kinetic data of the turnover of adenosine by free and coformycin-inhibited ADA were determined from 20 to 40°C in 2°C increments. For the uninhibited reactions, initial concentrations of 5 nM ADA and 100 μM adenosine were mixed. For the inhibited reactions, 5 nM ADA was combined with 100 μM adenosine and 25 nM coformycin.

Pseudo-first order rate constants describing the conversion of adenosine to inosine were obtained using a $\Delta\varepsilon_{260}$ of 7930 M^{-1} cm^{-1} for the reaction [26]. For the uninhibited reaction, rates were obtained from linear fits to the earliest time portions of the traces. For the inhibited reactions, it was observed that no significant inhibition of the enzyme occurred for several seconds, in accord with the slow binding kinetics of the inhibitor [21] (Fig. 1). The entire time courses of these traces were best fit by two exponentials. Rates from the reaction catalyzed by the inhibited enzyme were determined by fitting the entire time traces to two exponentials with the constraints that the large-amplitude exponential was equal to that obtained from an analogous fit of a single exponential to the early time portions of the traces from the uninhibited enzyme and that the final absorbance was equal to that observed from the uninhibited reaction. Rate constants were then obtained from the lower-amplitude exponential fits to the data after correcting for the effective initial concentrations of adenosine. All rate constants are expressed as μmoles of adenosine consumed per second.

2.4. Temperature dependence of K_I for coformycin inhibition of ADA activity

Determinations of K_I were made using $I_t/(1 - v_i/v_0) = E_t + K_I(1 + S/K_M)v_0/v_i$, where I_t is the total inhibitor concentration (13.5 nM), E_t is the total enzyme concentration (4.5 nM), v_i is the initial reaction velocity in the presence of inhibitor, v_0 is the initial reaction velocity in the absence of inhibitor, K_I is the inhibition constant, K_M is the Michaelis constant, and S is the substrate concentration (100 μM) [21,27].

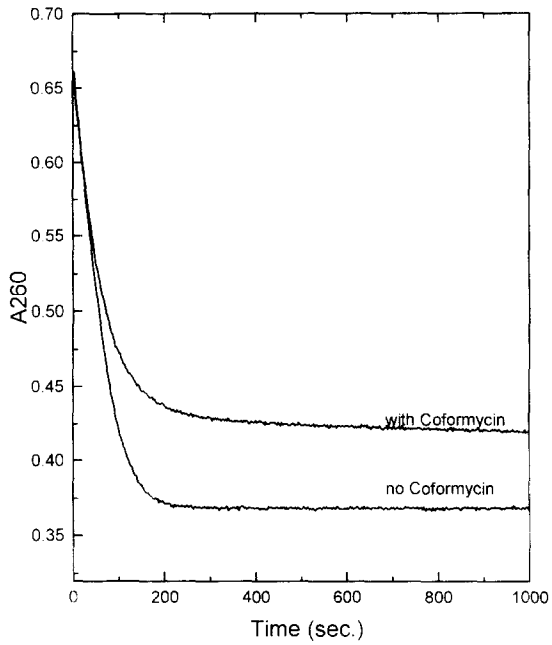

Fig. 1. Representative kinetic traces (at 40°C) of the interaction of ADA with adenosine (5 nM ADA + 100 μM adenosine) and of coformycin-inhibited ADA with adenosine (5 nM ADA + 25 nM coformycin and 100 μM adenosine) at pH = 6.3.

3. Results and discussion

Fig. 1 shows representative kinetic traces of the interaction of ADA and coformycin-inhibited ADA with adenosine. The slow onset of inhibition by coformycin is obvious, with no detectable alteration in the rate for several seconds. A similar kinetic trace of coformycin-inhibited ADA has previously been reported [21].

Fig. 2 shows the Arrhenius plots for the ADA-catalyzed and the coformycin-inhibited ADA-catalyzed deamination of adenosine. When coformycin is

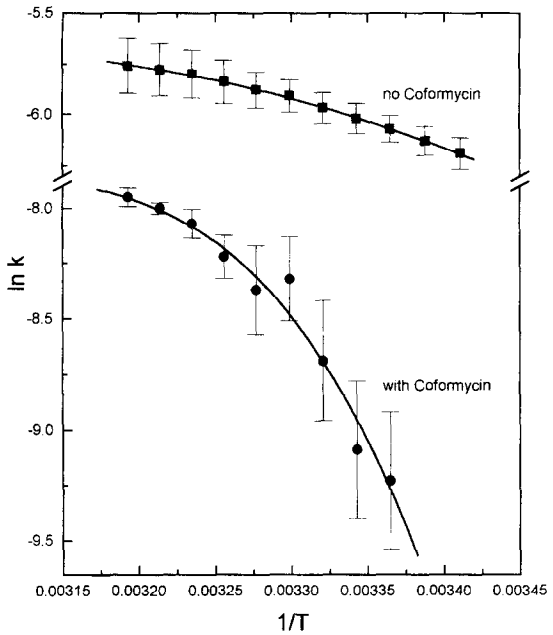

Fig. 2. Arrhenius plots for the deamination of adenosine to inosine by ADA and by coformycin-inhibited ADA. Rates are pseudo-first order rate constants expressed in μmoles of adenosine consumed per second. Curves represent the results of 8 runs for ADA and of 5 runs for coformycin-inhibited ADA. Error bars in terms of single standard deviations are shown.

included in the reaction mixture, the enzyme is $> 99.5\%$ inhibited at all temperatures.

The plot for the uninhibited reaction does not exhibit a sharp break as is observed in many enzyme/substrate systems but it is clearly nonlinear and is well described by a second order polynomial. The rates increase through the bovine normal body temperature (38.3°C, $1/T = 0.00321°C^{-1}$) but soon decrease (data not shown) as a consequence of thermal denaturation of the enzyme. This is reflected in the larger error bars with increasing temperature. The enzyme, not surprisingly, seems optimized for catalysis near the body temperature of its organismal source: the E_{act} at 38.3°C is 10 kJ/mole vs. 23 kJ/mole at 21°C (Fig. 3). If the enzyme exists in different conformations at 21°C and 38.3°C then it is clear that the high temperature conformation is better optimized for catalysis.

Inclusion of the transition state analog coformycin inhibits the reaction at all temperatures, but the effect is most dramatic at low temperatures (Fig. 2). The large error bars accompanying the data at low temperature are a consequence of the greatly diminished rates in the presence of the inhibitor. These data are best described by a third order polynomial. A second order polynomial fit does not encompass all points due to the rapid decrease in the rates that occurs at approximately 30°C ($1/T \approx 0.00330°C^{-1}$). In fact, it is not unreasonable to infer a sharp break in the plot at 30°C.

The temperature dependence of the ability of coformycin to inhibit the reaction is perhaps best illustrated by comparing E_{act}s at temperatures corresponding to the bovine normal body temperature and 21°C, the temperature of maximum affinity of ADA for coformycin (see below) (Fig. 3). At 38.3°C, the E_{act} increases from 10 kJ/mole in the uninhibited enzyme to 19 kJ/mole in the inhibited enzyme (for a 1.9-fold increase), but at 21°C the E_{act} increases from 23 to 162 kJ/mole (a 7.0-fold increase). Thus, coformycin effectively inhibits the enzyme at all

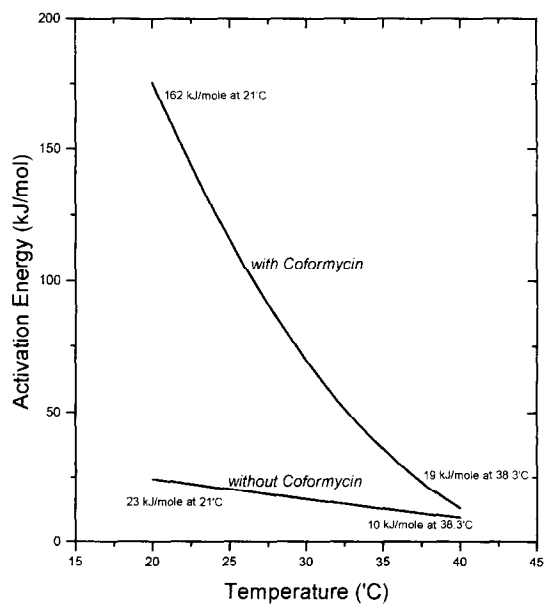

Fig. 3. Temperature dependences of the E_{act}s for ADA- and coformycin-inhibited ADA-catalyzed deamination of adenosine. E_{act}s were calculated from the derivatives of the best polynomial fits to the Arrhenius data from Fig. 2 (a second order polynomial for ADA and a third order polynomial for coformycin-inhibited ADA).

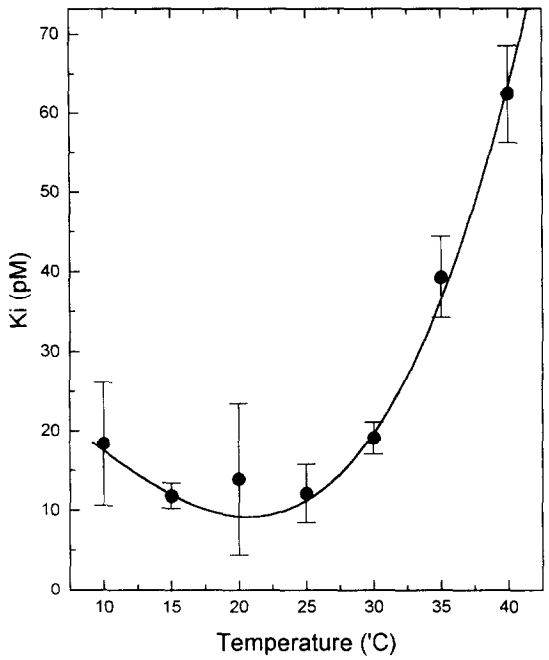

Fig. 4. Temperature dependence of K_I for coformycin inhibition of the ADA-catalyzed deamination of adenosine. Data are the results of 6 measurements. Error bars express uncertainties of values in single standard deviations. Curve is a third order polynomial fit to the data.

temperatures studied, but the effect is most pronounced only at low, non-physiologic temperatures. Since the enzyme active site must of course be free of the competitive inhibitor in order to react with the adenosine substrate, the data indicate that the activation energy for coformycin dissociation is much greater at low, non-physiologic temperatures. This presumably is a reflection of an enhanced affinity for the TSA at low temperature.

The kinetic data suggest an enhanced affinity for coformycin by ADA at low temperatures. Fig. 4 shows the results of our investigations into the T-dependence of inhibition of ADA by coformycin. K_I minimizes at approximately 1.1×10^{-11} M at 21°C. Frieden et al. [21] found K_I to be 2.2×10^{-10} M at pH = 7.0 and 20°C from calf intestinal ADA. We determine K_I at 38.3 to be 5.3×10^{-11} M. Coformycin therefore displays an almost five-fold enhancement for ADA at 21°C compared to 38.3°C.

4. Conclusion

Both the kinetic data, which compare the effect of the transition state analog coformycin on the activation energy of the ADA-catalyzed deamination of adenosine, and the thermodynamic data, which gives the temperature-dependence of coformycin binding to the ADA active site, indicate that the enzyme at low, non-physiologic temperatures possesses a markedly enhanced affinity for coformycin over the enzyme as it exists at its physiological temperature. This increased affinity for the transition state analog is presumably a reflection of the enzyme's increased affinity for the true transition state of the reaction [24,25]. A model for enzyme catalysis that requires an exclusive transition state complementarity of the active site for optimum catalysis seems incongruent with these findings as it would imply that bovine ADA has evolved to possess the greatest affinity for the transition state at a temperature the in vivo enzyme never encounters. Instead, the data are more consistent with the idea that a conformation change from a substrate-specificity at physiologic temperatures to a transition state specificity may be induced by lowering the temperature only a few degrees below the normal operating temperature of ADA. We would also like to suggest that perhaps the wealth of X-ray and NMR structures of enzyme/TSA complexes, which have tended to reinforce the idea that enzymes have evolved active sites which are complementary to transition states, results from the examination of the low temperature (non-physiologic) form of the enzyme, which, according to the Shifting Specificity model for enzyme catalysis [13,14] is the E_{TS}. A more relevant approach to understanding the structure of the physiological molecule may be to determine the structures at their physiologic temperatures.

Acknowledgements

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