Journal of Biochemistry and Molecular Biology, Vol. 39, No. 2, March 2006, pp. 167-170

JOURNAL OF Biochemistry and Molecular Biology

Transition State Characterization of the Low- to Physiological-Temperature Nondenaturational Conformational Change in Bovine Adenosine Deaminase by Slow Scan Rate Differential Scanning Calorimetry

Melissa A. Bodnar and B. Mark Britt*

Department of Chemistry and Physics, Texas Woman's University, Denton, TX 76204 USA

Received 8 November 2005, Accepted 9 December 2005

Bovine adenosine deaminase undergoes a nondenaturational conformational change at 29°C upon heating which is characterized by a large increase in heat capacity. We have determined the transition state thermodynamics of the conformational change using a novel application of differential scanning calorimetry (DSC) which employs very slow scan rates. DSC scans at the conventional, and arbitrary, scan rate of 1°C/min show no evidence of the transition. Scan rates from 0.030 to 0.20°C/min reveal the transition indicating it is under kinetic control. The transition temperature T_t and the transition temperature interval ΔT increase with scan rate. A first order rate constant k_1 is calculated at each T_t from $k_1 = r_{scan}/\Delta T$, where r_{scan} is the scan rate, and an Arrhenius plot is constructed. Standard transition state analysis reveals an activation free energy $\Delta G^{\neq}$ of 88.1 kJ/mole and suggests that the conformational change has an unfolding quality that appears to be on the direct path to the physiological-temperature conformer.

Keywords: Enzyme conformational change, Slow scan rate differential scanning calorimetry, Transition state thermodynamics, Unfolding

Introduction

We have previously demonstrated that bovine adenosine deaminase (ADA) undergoes a reversible, nondenaturational conformational change at 29°C (Fig. 1) (Anderson and Britt, 2002). Complete thermodynamic analysis of the two conformations suggest that the change from the low-temperature conformer to the physiological-temperature conformer (the bovine normal body temperature is 38.3°C) involves an opening up of the structure (Anderson and Britt, 2002). We also presented evidence from a fluorescence investigation that the low- to physiological-temperature conformational change may be under kinetic control. As the unfolding data indicate the heat capacity change associated with the conformational change is quite large it ocurred to us that differential scanning calorimetry (DSC) may be an excellent probe of the transition if it is realized that the transition may be under kinetic control. We report here the results of this investigation. In the process we discover that DSC may be used to obtain direct information on rate constants for transitions with high activation energies if slow scan rates are employed.

*To whom correspondence should be addressed.
Tel: 940-898-2566; Fax: 940-898-2548
E-mail: mbritt@twu.edu

Experimental Procedures

ADA was obtained from Worthington Biochemical Corporation. All other chemicals were obtained from Sigma Chemical Company. All solutions were made in 150 mM phosphate (pH = 7.0) in deionized water. Graphical analysis was performed either with the calorimeter software or with Microcal Origin.

A stock solution of ADA was dialyzed against a thousand-fold excess of buffer for at least 36 hours. The concentration of the resulting solution was determined from absorbance spectroscopy using $\varepsilon_{280} = 42{,}680\ M^{-1}\ cm^{-1}$ (Gill and von Hippel, 1989). ADA solutions were 2.0 mg/mL and prepared from dilution with dialysate. DSC scans were performed on a Calorimetry Sciences Nano Series III instrument at 1.0 atm and were baseline corrected with a run against dialysate. Scan rates were 0.030, 0.040, 0.075, 0.125, and 0.20°C/min.

Analysis. The conformational change from the low- to physiological-temperature conformer is indicated by a sharp increase in heat capacity. First order rate constants for the conversion of the low-temperature conformer to the physiological-temperature conformer were calculated as follows. Consider a

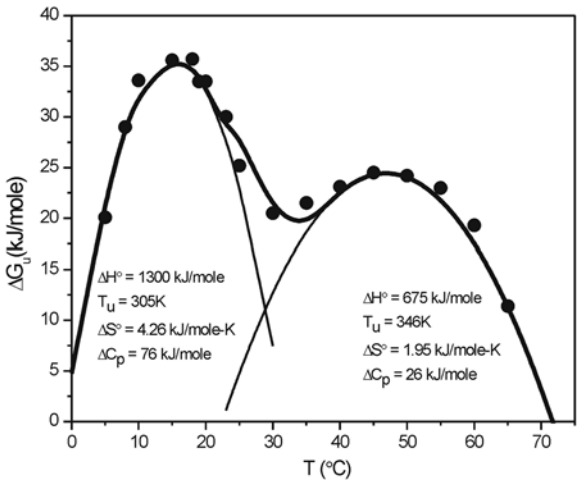

Fig. 1. The ADA stability curve showing how the enzyme undergoes an abrupt conformational change at 29°C. The thin lines are two applications of the Gibbs-Helmholtz equation, with the thermodynamic values indicated, to the lower and upper temperature regimes. The lines cross at 29°C indicating the temperature of the conformational change. The thick line is the sum of the two curves. (See Anderson and Britt, 2002 for details. Note that the thermodynamic values here differ slightly from the original work. In the original work we modelled the data as a phase change. Here we consider that the two conformations may coexist over a narrow temperature range).

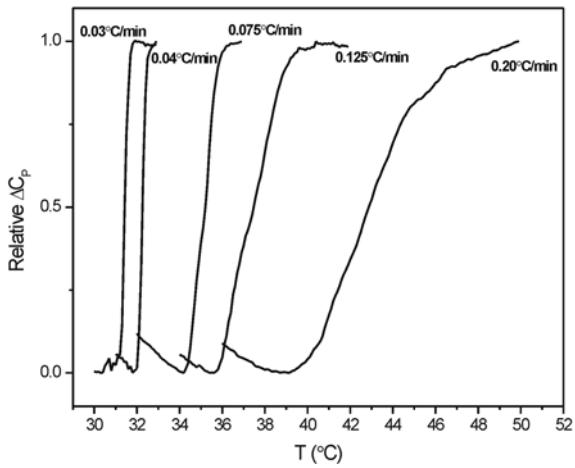

Fig. 2. DSC scans showing the relative changes in heat capacity revealing the low- to physiological-temperature ADA nondenaturational conformational change as a function of scan rate.

process such as the conversion of an enzyme from its low-temperature conformation L to its physiological-temperature conformation P characterized by a high activation energy. The rate of conversion of L to P is $d[P]/dt = k_1[L]$ where k_1 is the first-order rate constant for the process. The appearance of P in the calorimetry measurement is dependent upon the scan rate $r_{scan} = dT/dt$. Dividing both sides of the rate equation by the scan rate gives $(d[P]/dt)(dt/dT) = k_1[L]/r_{scan}$ which leads to $(d[P]/dT)/[L] = k_1/r_{scan}$. At the midpoint of the transition T_t k_1 may be calculated from $k_1 = r_{scan}\{(d[P]/dT)/[L]\}$. Since all conversions observed in the present work were linear with respect to temperature dT may be well approximated by ΔT. If we take ΔT to be the temperature interval of the conversion and if we make the reasonable assumption that L is quantitatively converted to P the concentration units cancel and the above equation simplifies to $k_1 = r_{scan}/\Delta T$ at T_t. This equation allows us to calculate the temperature dependence of the rate constant.

T_t and ΔT were calculated by first fitting the pre-transition, transition, and post-transition each with a line. T_t was calculated as the temperature of the transition which was exactly half way between the pre- and post-transition trends. ΔT was calculated as the temperature difference between the transition and post-transition intersection and the pre-transition and transition intersection.

Results

Figure 2 shows the DSC scans of ADA as a function of scan rate. These scans are near the conformational transition temperature of 29°C and well below the heat unfolding temperature of 73°C. These scans differ from conventional DSC scans in that there is no apparent enthalpy component. This is due to the very slow scan rates employed here - the heat is released over a much longer time period than in conventional scans and goes undected by the calorimeter and is a well known phenomenon in the field. Each of these scans is reversible in the sense that if the solution used for the scan is recovered and kept in the refrigerator for over 72 hours and a scan repeated a nearly identical trace is obtained. Waiting for only two days does not reproduce the result. Scanning from high to low temperature also does not reveal a transition.

The trend is for both an increase in the transition temperature T_t and the temperature interval of the transition ΔT with increasing scan rate (Table 1). These scans reflect the increase in heat capacity that occurs when the folded enzyme transitions from the low-temperature conformer to the physiological-temperature conformer. This is supported by the trend of T_t with scan rate (Fig. 3) where at zero scan rate $T_t = 29$°C, the same temperature of the transition as indicated in the stability curve (Fig. 1).

With the scan characteristics from Table 1 we are able to calculate first order rate constants k_1 as a function of temperature using $k_1 = r_{scan}/\Delta T$. We are then able to construct an Arrhenius plot (Fig. 4) and characterize thermodynamically the transition state of the conversion. Analysis is at 29°C.

Table 1. Scan characteristics of the data from Fig. 1 describing the conversion of ADA from the low-temperature conformer to the physiological-temperature conformer

scan rate (°C/min)	T_t (°C)	ΔT (°C)	k_1 (s^{-1})
0.030	31.44	0.35	1.43×10^{-3}
0.040	32.22	0.35	1.90×10^{-3}
0.075	35.08	1.49	8.39×10^{-4}
0.125	37.25	3.19	6.53×10^{-4}
0.20	41.96	5.25	6.35×10^{-4}

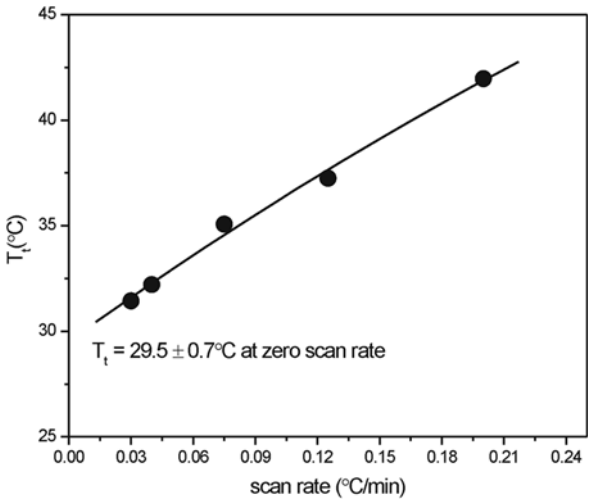

Fig. 3. Plot of T_t vs. scan rate. The trend is well modeled by a quadratic demonstrating how the extrapolated T_t at zero scan rate is 29°C, the same temperature of the conformational change as predicted by the stability curve (Fig. 1).

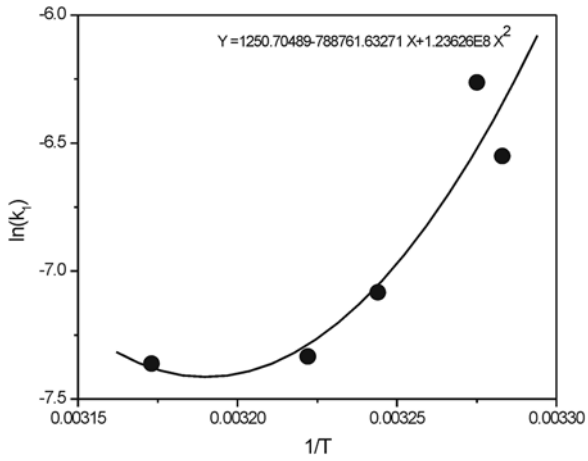

Fig. 4. Arrhenius plot describing the transition of ADA from the low-temperature conformer to the physiological-temperature conformer. The trend is modeled by the shown quadratic equation. R^2 for the fit is 0.88.

We calculate the activation free energy $\Delta G^{\neq}$ from standard transition state theory with $\Delta G^{\neq} = -RT[\ln(k_1 h/k_B T)]$ using an extrapolated value of $k_1 = 3.45 \times 10^{-3}$ s^{-1} at 29°C. $\Delta G^{\neq} = 88.1$ kJ/mole. The activation enthalpy $\Delta H^{\neq}$ is calculated from $\Delta H^{\neq} = E_a - RT$ where E_a is the activation energy obtained from the slope (derivative) of the quadratic fit at 1/(302.2K). $\Delta H^{\neq} = -247$ kJ/mole. Negative activation enthalpies associated with macromolecular conformational changes have been previously reported (Segawa *et al*., 1973; Urbanke *et al*., 1975; Strohmeyer *et al*., 2002). $\Delta S^{\neq}$ is calculated from $(\Delta H^{\neq} - \Delta G^{\neq})/T$ and is equal to −1.11 kJ/mole-K. $\Delta C_P^{\neq}$ is calculated from a $\Delta(\Delta H^{\neq})/\Delta T$ and is equal to 22 kJ/mole-K.

Discussion

It is generally assumed that the enzyme structure as determined by conventional applications of x-ray crystallography is the physiological structure. That is, it is assumed that enzymes do not undergo significant conformational changes between room temperature or below (where crystals for x-ray analysis are grown) and the organismal optimal thriving temperature. We have previously demonstrated that this is not the case with ADA (Anderson and Britt, 2002) and show here that the conversion from the low- to physiological-temperature conformer is under kinetic control. The general relevance of the low-temperature conformers of enzymes to their physiological-temperature conformers has been discussed (Britt, 2004).

$\Delta G^{\neq}$ for the conversion at 29°C is 88.1 kJ/mole. This value is approaching the typical activation free energies for enzyme unfolding. For example, the unfolding activation free energy for ADA at 38.3°C is 107 kJ/mole (Strohmeyer *et al*., 2002).

This system also provides a good example of why scan rate should be considered as a variable in DSC measurements (Sanchez-Ruiz *et al*., 1988; Lepock *et al*., 1992, Davoodi *et al*., 1998). Scanning at the conventional -- and arbitrary -- scan rate of 1°C/min reveals no sign of the transition observed here. Indeed, the trend of Fig. 2 indicates that the transition does not occur at this scan rate until unfolding begins. This raises the question of whether the transition may be observed at conventional scan rates realizing that it will be enveloped in the unfolding event. A value for k_1 of 41 s^{-1} at the thermal unfolding temperature of 73°C may be estimated from the trend shown in Fig. 4. If the conventional scan rate of 1°C/min is employed then a temperature interval for the transition ΔT of 4×10^{-4} °C can be inferred from $\Delta T = r_{scan}/k_1$. This is below the resolution of the calorimeter. Conventional scan rates could therefore not observe the transition reported here.

Our previous analysis of the ADA stability curve suggested that the low-temperature conformation is a more compact structure than the physiological-temperature conformation and that the physiological-temperature conformation has considerably more exposed nonpolar amino acid side surface area (Anderson and Britt, 2002). This may be demonstrated by a comparison of the equilibrium thermodynamics of unfolding of either conformation as shown in Fig. 1. For the conversion from the low-temperature conformer to the physiological-temperature conformer $\Delta H^0_{(LP)} = -625$ kJ/mole and $\Delta S^0_{(LP)} = -2.31$ kJ/mole-K. Since the heat capacities shown in Fig. 1 refer to the unfolding event, the heat capacity change accompanying the conformational change $\Delta C_{P(LP)}$ is actually 50 kJ/mole. The signs and magnitudes of these thermodynamic parameters in enzyme conformational changes are characteristic of increased exposure of nonpolar amino acid side chain components to the aqueous environment and imply a conformational opening up of the enzyme (Chen and Schellman, 1989; Chen *et al*., 1989).

Our current results suggest that the transition state for the conformational change possesses an unfolding quality and

appears to be on the direct path to the physiological-temperature conformation in that the parameters of $\Delta H^{\neq} = -247$ kJ/mole, $\Delta S^{\neq} = -1.11$ kJ/mole-K, and $\Delta C_P^{\neq} = 22$ kJ/mole-K are 40%, 48%, and 44%, respectively, on the way to the equilibrium thermodynamic change values calculated above.

Conclusions

Though the conversion of ADA from the low- to physiological-temperature conformation is associated with a high heat capacity change the transition is revealed in DSC only at slow scan rates indicating the transition is under kinetic control. $\Delta G^{\neq}$ is determined to be 88.1 kJ/mole. Determination of the other transition state thermodynamic parameters suggest that the transition state lies on the direct path to the final state and possesses an unfolding quality. To our knowledge, this is the first demonstration of the use of slow scan rate DSC to describe the transition state thermodynamics of a process.

Acknowledgments This work was supported by a departmental grant from the Robert A. Welch Foundation and a Petroleum Research Fund grant from the American Chemical Society. We would also like to thank two anonymous reviewers for their helpful comments.

References

Anderson, E. and Britt, B. M. (2002) The stability curve of bovine adenosine deaminase is bimodal. *J. Biomolecul. Struct. Dynamics* **20**, 375-380.

Britt, B. M. (2004) Understanding enzyme structure and function in terms of the shifting specificity model. *J. Biochem. Mol. Biol.* **37**, 394-401.

Chen, B. and Schellman, J. A. (1989) Low-temperature unfolding of a mutant of phage T4 lysozyme. 1. Equilibrium studies. *Biochemistry* **28**, 685-691.

Chen, B., Baase, W. A. and Schellman, J. A. (1989) Low-temperature unfolding of a mutant of phage T4 lysozyme. 1. Kinetic investigations. *Biochemistry* **28**, 691-699.

Davoodi, J., Wakarchuk, W. W., Surewicz, W. K. and Carey, P. R. (1998) Scan-rate dependence in protein calorimetry: the reversible transitions of *Bacillus circulans* xylanase and a disulfide-bridge mutant. *Prot. Sci.* **7**, 1538-1544.

Gill, S. C. and von Hippel, P. H. (1989) Calculation of protein extinction coefficients from amino acid sequence data. *Anal. Biochem.* **182**, 319-326.

Lepock, J. R., Ritchie, K. P., Kolios, M. C., Rodahl, M. A., Heinz, K. A. and Kruuv J. (1992) Influence of transition rates and scan rates on kinetic simulations of differential scanning calorimetry profiles of reversible and irreversible protein denaturation. *Biochemistry* **31**, 12706-12712.

Sanchez-Ruiz, J. M., Lopez-Lacomba, J. L., Cortijo, M. and Mateo, P. L. (1998) Differential scanning calorimetry of the irreversible thermal denaturation of thermolysin. *Biochemistry* **27**, 1648-1652.

Segawa, S., Husimi, Y. and Wada, A. (1973) Kinetics of the thermal unfolding of lysozyme. *Biopolymers* **12**, 2521-2537.

Strohmeyer, E. A., Beckley, J. R. and Britt, B. M. (2002) Direct measurement of local and global contributions in the binding of coformycin to bovine adenosine deaminase. *J. Enzyme Inhibit.* **17**, 77-86.

Urbanke, C., Roemer, R. and Maass, G. (1975) Tertiary structure of tRNAPhe (yeast). Kinetics and electrostatic repulsion. *Eur. J. Biochem.* **55**, 439-444.