A jump in an Arrhenius plot can be the consequence of a phase transition

The binding of ATP to myosin subfragment 1

J.A. Biosca, F. Travers and T.E. Barman

INSERM U 128, B.P. 5051, 34033 Montpellier Cedex, France

The temperature dependence of the kinetics of the binding of ATP to myosin subfragment-1 was studied by an ATP chase technique in a rapid-flow–quench apparatus:

$$M + ATP \overset{K_1}{\rightleftharpoons} MATP \overset{k_2}{\rightarrow} M^* ATP$$

A temperature range of 30°C to −15°C was obtained with ethylene glycol as antifreeze. The Arrhenius plot of k_2 is discontinuous with a jump at 12°C. Above the jump $\Delta H^{\neq} = 9.5$ kcal/mol, below $\Delta H^{\neq} = 28.5$ kcal/mol. Few such Arrhenius plots are recorded in the literature but they are predicted from theory. Thus, we explain our results as a phase change of the subfragment 1–ATP system at 12°C. This is in agreement with certain structural studies.

Arrhenius plots, discontinuous   *Phase transition*   *Myosin subfragment 1, ATP binding to*
Conformational change   *Enzyme kinetics*

1. INTRODUCTION

A study of the temperature dependence of an enzyme reaction can lead towards important mechanistic information [1,2]. Arrhenius and van 't Hoff plots are often linear but it is when they are non-linear that they are the more informative.

There are two main reasons for non-linear Arrhenius plots [1]. The more common of these is that there is a change in rate-limiting step. Enzyme reaction pathways consist of a number of steps, each described by rate constants with characteristic energies of activation. As the temperature is varied, a change in rate limiting step can occur. In these cases, Arrhenius plots consist of two straight lines joined by a curve with the higher activation energy dominating at low temperatures. Here the non-linearity is due to purely kinetic phenomena.

Another reason is that there is a temperature-induced conformational change in the enzyme or a phase change or both. The ΔH° values for such systems are usually high [3,4] and, if the two isomers have very different activation parameters for the common reaction that they catalyze, characteristic Arrhenius plots result. There is a priori no theoretical reason why such plots should be continuous with sharp 'breaks' [2,5] yet in actual practice this is often the case [1,3,6]. We have only found one discontinuous Arrhenius plot in the literature [4]: such plots are good evidence for a structural or phase change at a certain critical temperature, T_m [2,4].

During the course of our work on myosin subfragment 1, we have often observed that solutions of this protein show a sudden increase in turbidity at a certain critical temperature. This critical temperature is very sensitive to the experimental

conditions. It is possible that this phenomenon is related to studies that show the structure of myosin subfragment 1 is temperature dependent: NMR [7] and ultracentrifuge [8] studies and susceptibility to tryptic digestion [9]. This structure change does not appear to affect greatly the overall k_{cat}, an observation which can be explained by the insensitivity of the rate limiting step, k_4, to perturbations (unpublished).

We thought it was of interest to study the effect of this structural change on the kinetic parameters of myosin.

One is confronted with two problems in the temperature-dependent studies of enzyme reactions:

(1) The temperature range is limited to, at best, 40°C. A means of extending this range is the use of an antifreeze [10].

(2) It is often difficult to interpret the temperature dependence of composite kinetic constants such as a k_{cat} or K_m. To distinguish between two lines joined by a curve or cutting at a 'break' the data must be very good.

It is clear that the study of an early step on an enzyme pathway can lead to less ambiguous data.

The first two steps on the myosin pathway, namely the fixation of ATP, are rather sensitive to the experimental conditions [11]:

$$M + ATP \overset{K_1}{\rightleftharpoons} MATP \overset{k_2}{\longrightarrow} M*ATP$$

A way of specifically following this process is by the cold ATP chase method which leads directly to values for K_1 and k_2 [11]. Here, we exploit the binding of ATP to myosin as a kinetic probe to detect any temperature-induced phase change of the myosin. We extended the temperature range by the use of 40% ethylene glycol as the antifreeze.

2. MATERIALS AND METHODS

Myosin subfragment 1 was prepared from rabbit skeletal muscle [12]. [γ-^{32}P]ATP was obtained from Amersham International (Bucks).

ATP chase experiments were carried out by the rapid-flow–quench method [11]. The apparati used were thermostatically controlled to ± 0.2°C. In a typical experiment 1.1 ml enzyme solution (0.4–40 μM) was mixed with an equal volume of [γ-^{32}P]ATP (2–200 μM) and after ageing, quench-

ed in unlabelled ATP (2–200 mM). The mixture was incubated for 2 min at 25°C, quenched in 1.8% trichloroacetic acid and the ^{32}P$_i$ determined [13]. All the components except the acid quench were dissolved in KCl (5 or 150 mM) magnesium acetate (2 mM), dithiothreitol (0.1 mM), Tris–acetate buffer, pH 8.0 (50 mM) and 40% (v/v) ethylene glycol.

The data obtained were analyzed as in [11].

The temperature dependence of the light absorption of solutions of subfragment 1 was carried out in a thermostatically controlled Cary 219 spectrophotometer. The wavelength used was 350 nm.

3. RESULTS

3.1. The effect of temperature on the turbidity of solutions of S-1

We studied the turbidity of solutions of myosin subfragment 1 (0.1–5 mg/ml) in 40% ethylene glycol, 5 mM KCl, 2 mM magnesium acetate and 50 mM Tris–acetate buffer, pH 8 over – 20°C to 40°C. At – 20°C, the turbidity was slight; as the temperature was increased, there was little change up to 12–14°C where there was a sharp increase in absorption at 350 nm. Several minutes were re-
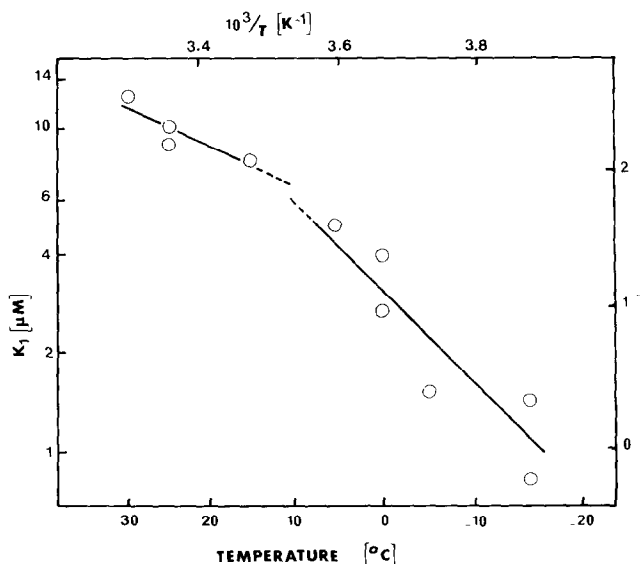

Fig.1. A van't Hoff plot of the variation of K_1 with the temperature. The conditions were 5 mM KCl (pH 8) in 40% ethylene glycol. For full experimental details, see the text.
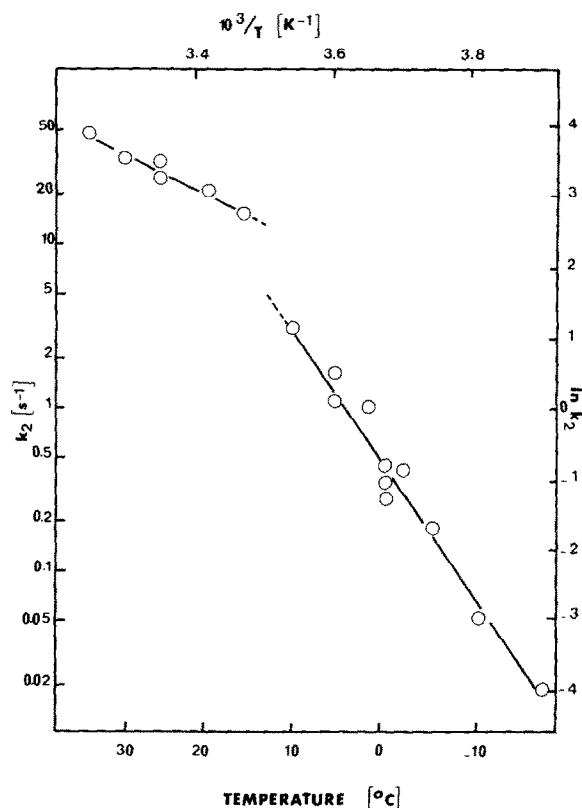
Fig.2. An Arrhenius plot of the variation of k_2 with the temperature. The conditions were 5 mM KCl (pH 8) in 40% ethylene glycol. For full experimental details, see the text.

quired for the optical density to stabilize; at 1 mg/ml of subfragment 1, an absorbance of 0.5 was reached. No further increase in turbidity occurred on further increasing the temperature. This experiment suggests that there is a temperature-dependent phase transition at a $T_m = 12-14°C$.

The transition exhibited the following characteristics: it was reversible, reproducible and independent of [protein] over 0.1–5 mg/ml subfragment 1. The T_m was very sensitive to the experimental conditions thus when the KCl was raised to 0.15 M, $T_m = 28-30°C$. In pure water and at 5 mM KCl, the $T_m = 34-37°C$.

3.2. The effect of temperature on K_1 and k_2

A van't Hoff plot of K_1 is shown in fig.1. K_1 is not very sensitive to a change in temperature (12 μM at 30°C and ~1 μM at −15°C). Nevertheless, there could be a jump at 10–15°C.

An Arrhenius plot of k_2 is shown in fig.2. k_2 is very sensitive to the temperature, particularly at <10°C: at 30°C $k_2 = 50$ s^{-1} and at −15°C 0.02 s^{-1} which represents a 2500-fold difference. There is a sharp discontinuity in the 10–15°C region (representing a 2.5-fold jump in k_2) at 12.5°C.

The thermodynamic parameters for the binding of ATP to S-1 are summarized in table 1.

4. DISCUSSION

The results of our turbidity experiments show that there is a temperature-induced phase transi-

Table 1

Thermodynamic parameters for the binding of ATP to S-1 in 50 mM Tris–acetate (pH 8), 5 mM KCl and 40% ethylene glycol

	K_1	$\Delta H°$ (kcal/mol)	$\Delta G°$ (kcal/mol)	$\Delta S°$ (cal.mol^{-1}.degree^{-1})
0°C	3.5 μM	−11	−6.9	−7
25°C	10 μM	−5	−6.9	3
	k_2	$\Delta H^{\neq}$ (kcal/mol)	$\Delta G^{\neq}$ (kcal/mol)	$\Delta S°$ (cal.mol^{-1}.degree^{-1})
0°C	0.3 s^{-1}	28.5	16.4	44
25°C	30 s^{-1}	9.5	15.4	−21

The parameters were calculated from fig.1 and 2

tion of S-1. The transition occurs at a critical temperature T_m; the large increase in turbidity observed above T_m suggests a protein aggregation phenomenon. Our observations could be related to the published structure–temperature studies of S-1 [7–9]; but it would certainly be premature to relate the phase change of S-1 to any physiological function of myosin. In any event, it was not our intention in the present work to study the structural details of this phenomenon.

We consider that the discontinuity in the Arrhenius plot for k_2 is convincing – it represents a jump by a factor of 2.5 which is well outside our experimental errors. Further, the jump is at a temperature very close to the T_m obtained from the diffusion studies. The Arrhenius plot was linear on either side of the jump and this allowed us to calculate the activation parameters of the two forms.

If the calculation of activation parameters is an easy matter their interpretation is not. The S-1 ATP system involves the interaction of a macromolecule with a highly charged substrate. Further, solvation and counter ion effects make a major contribution to the observed thermodynamic quantities [14]. Attempts have been made to separate effects such as local viscosities and the specific effect of the solvent [15].

There is a striking difference between the values of $\Delta S^{\neq}$ for k_2 for the two forms of S-1: $+44$ cal.mol^{-1}.degree^{-1} below the jump and -21 cal.ml^{-1}.degree^{-1}, above. This drastic change in $\Delta S^{\neq}$ is most probably due to a change in the environment of the subfragment 1 rather than to a conformational change of itself. This would be in accord with our aggregation studies and with the observation that k_2 is very sensitive to the experimental conditions such as [KCl], pH and solvent composition [11].

There was also a difference between the values for ΔS° for K_1, for the two forms of S-1, but as this constant varied much less with the temperature than did k_2, there is less certainty in its interpretation. Nevertheless, there appears to be a break, if not a jump, at the same temperature as for k_2.

Here, we wished to show experimentally that if a phase transition of an enzyme occurs at a critical temperature T_m, then a jump in the Arrhenius plot of an individual rate constant at the same temperature could result. As we indicate above, this situation has been predicted by several authors and the S-1–ATP system provides a good example of this.

ACKNOWLEDGEMENTS

REFERENCES

[1] Dixon, M. and Webb, E.C. (1964) Enzymes, 2nd edn, pp.145–165, Longmans Green, London.
[2] Londesborough, J. (1980) Eur. J. Biochem. 105, 211–215.
[3] Massey, V., Curti, B. and Ganther, H. (1966) J. Biol. Chem. 241, 2347–2357.
[4] Kumamoto, J., Raison, J.K. and Lyons, J.M. (1971) J. Theor. Biol. 31, 47–51.
[5] Krug, R.R., Hunter, W.G. and Grieger, R.A. (1976) J. Phys. Chem. 80, 2325–2341.
[6] Lehrer, G.M. and Barker, R. (1976) Biochemistry 9, 1533–1539.
[7] Shriver, J.W. and Sykes, B.D. (1982) Biochemistry 21, 3022–3028.
[8] Morel, J.E. and Garrigos, M. (1982) Biochemistry 21, 2679–2686.
[9] Moez, G., Lu, R.C. and Gergely, J. (1982) Biophys. J. 37, 38 (a).
[10] Douzou, P. (1977) Cryobiochemistry, Academic Press, New York.
[11] Barman, T.E., Hillaire, D. and Travers, F. (1983) Biochem. J., in press.
[12] Weeds, A.G. and Taylor, R.S. (1975) Nature 257, 54–56.
[13] Reimann, E.M. and Umfleet, R.A. (1978) Biochim. Biophys. Acta 523, 516–521.
[14] Jencks, W.P. (1969) Catalysis in Chemistry and Enzymology, McGraw-Hill, New York.
[15] Beece, D., Einsenstein, L., Frauenfelder, H., Good, D., Marden, M.C., Reinisch, L., Reynolds, A.H., Sorensen, L.B. and Yve, K.T. (1980) Biochemistry 19, 5147–5157.