ON THE OCCURRENCE OF A TEMPERATURE COEFFICIENT (Q_{10}) OF 18 AND A DISCONTINUOUS ARRHENIUS PLOT FOR HOMOGENEOUS RABBIT MUSCLE FRUCTOSEDIPHOSPHATASE

A. VAN TOL

Department of Biochemistry I, Faculty of Medicine, Erasmus University, Rotterdam (the Netherlands)

Received December 18,1974

SUMMARY. The allosteric inhibition of homogeneous rabbit muscle fructosediphosphatase (D-fructose-1,6-diphosphate 1-phosphohydrolase, EC 3.1.3.11) by AMP decreases with increasing temperature. Partly inhibitory amounts of AMP give rise to a 3-4 fold increase of the apparent energy of activation. In the presence of Mg^{++} ions and AMP the apparent energy of activation is more than 50 kcal/mole and the corresponding temperature coefficient (Q_{10}), between 30 and 40°C, is as high as 18. A discontinuous Arrhenius plot is observed in the presence of very small amounts of AMP. Some implications of these observations are discussed.

Fructosediphosphatase (D-fructose-1,6-diphosphate 1-phosphohydrolase, EC 3.1.3.11) catalyzes an irreversible step in the gluconeogenic pathway (for a review see ref. 1). The enzyme is inhibited by high concentrations of substrate and by the allosteric modifier AMP (2). In addition to gluconeogenic tissues like liver and kidney, skeletal muscle also contains a fructosediphosphatase isoenzyme. The muscle enzyme has a different primary structure and is much more sensitive to AMP inhibition than the liver or kidney enzyme (3).

It has been shown for fructosediphosphatase purified from several sources that the sensitivity for the inhibitor AMP decreases with increasing temperature (2,4-7). In order to investigate the temperature dependence of homogeneous muscle fructosediphosphatase, we constructed Arrhenius plots, both in the presence and in the absence of partly inhibitory amounts of AMP.

MATERIALS AND METHODS. Fructosediphosphatase was purified to homogeneity from rabbit muscle as described by Black et al. (3). The activity was measured by following the reduction of $NADP^+$ at 340 nm in a coupled assay. The standard incubation medium consisted of 50 mM triethanolamine (pH 7.3), 10 mM $MgCl_2$, 0.1 mM EDTA, 0.07 mM fructosediphosphate, 2 mM $(NH_4)_2SO_4$, 50 mM K_2SO_4, 0.1 mM $NADP^+$

(purified free of AMP by the method of Horecker and Kornberg (8), 1 µg of hexosephosphate isomerase and 1 µg of glucose-6-phosphate dehydrogenase (Boehringer, Grade I), an appropriate aliquot of homogeneous fructosediphosphatase and AMP as indicated. The reaction was started by the addition of substrate and the increase in extinction at 340 nm was followed with a recording spectrophotometer (Gilford, model 2400). Because of the great excess of the coupling enzymes over fructosediphosphatase, the rate of NADPH formation is a measure for the activity of fructosediphosphatase at every temperature (tested both in the presence and in the absence of AMP). Hill plots (cf. ref. 2) were constructed by way of the least squares method. The apparent energy of activation of purified rabbit muscle fructosediphosphatase, both without AMP and in the presence of partly inhibitory concentrations of AMP was calculated from Arrhenius plots. In this paper apparent energy of activation refers to the value of E obtained from the Arrhenius plot when log v is plotted against 1/T resulting in a straight line with slope -E/2.303 R. The enzyme must be saturated with substrate all the time, which is provided for by the substrate concentration used (70 µM). E includes the enthalpy change for the formation of the enzyme substrate complex (a constant value under the conditions used, because the substrate is present in excess) and, if applicable, the enzyme inhibitor complex (9,10). The temperature was measured in the cuvet with a thermocouple (YSI, model 42SC).

RESULTS AND DISCUSSION

Fig. 1 shows Hill plots of the AMP inhibition of rabbit muscle fructosediphosphatase at various temperatures. It is evident that the sensitivity for the inhibitor decreases sharply with increasing temperature. The I_{50}'s for AMP inhibition and the corresponding Hill coefficients are given in TABLE I. The Hill coefficient, which

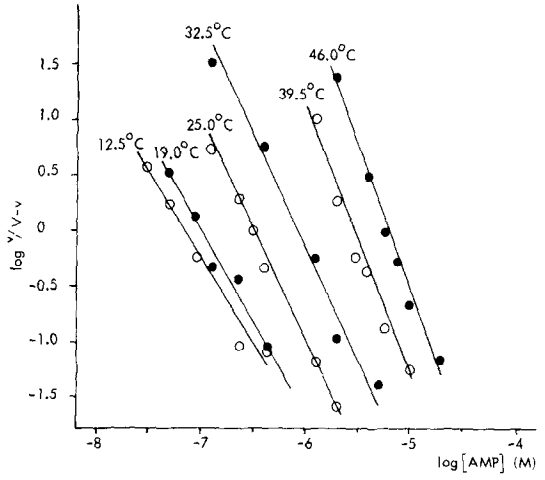

Fig. 1. Hill plots of the inhibition of rabbit muscle fructosediphosphatase by AMP at various temperatures. The standard incubation medium was used (see MATERIALS AND METHODS).

TABLE I

THE I_{50} FOR AMP INHIBITION OF RABBIT MUSCLE FRUCTOSEDIPHOSPHATASE AT DIFFERENT TEMPERATURES.

Temperature (°C)	12.5	19.0	25.0	32.5	39.5	46.0
I_{50} for AMP (μM)	0.07	0.10	0.31	0.82	2.8	6.2
Hill coefficient	1.5	1.6	2.0	2.0	2.4	2.6

The parameters were determined from the Hill plots, shown in Fig. 1. The activities were measured at the indicated temperatures under the conditions stated in MATERIALS AND METHODS. The I_{50} for AMP is the concentration of AMP giving 50% inhibition.

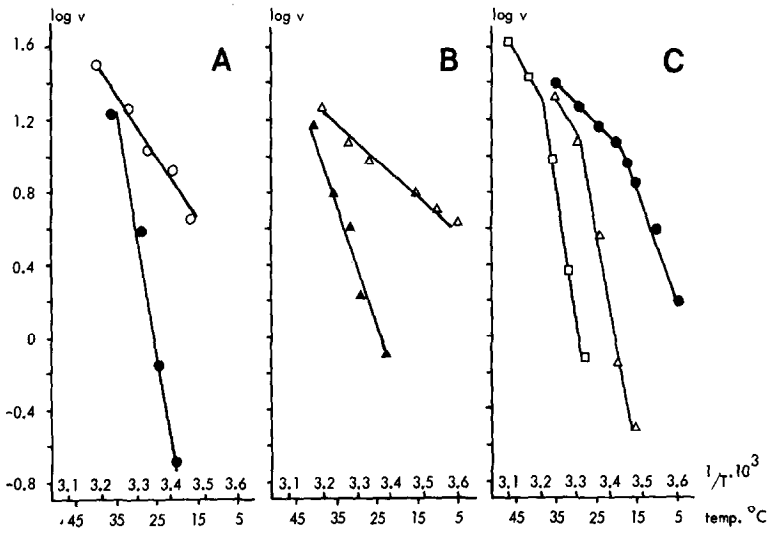

Fig. 2. Arrhenius plots of homogeneous rabbit muscle fructose-diphosphatase. The assay conditions were: A. 10 mM $MgCl_2$ without AMP (○-○) or in the presence of 0.92 μM AMP (●-●). B. 0.2 mM $MnCl_2$ (K_2SO_4 omitted) without AMP (△-△) or in the presence of 5.15 μM AMP (▲-▲). C. 10 mM $MgCl_2$ (K_2SO_4 omitted) and 2.12 μM AMP (□-□); 10 mM $MgCl_2$ and 0.12 μM AMP (△-△); 0.2 mM $MnCl_2$ and 0.12 μM AMP (●-●). For further details of the assay medium see MATERIALS AND METHODS.

is a measure for the interaction of the four subunits, interestingly increases with increasing temperature.

We constructed Arrhenius plots in order to measure the apparent energy of activation of purified rabbit muscle fructose-diphosphatase, both without AMP and in the presence of partly inhibitory concentrations of AMP. As the enzyme needs either Mg^{++} or Mn^{++} ions for activity (11), the Arrhenius plot was determined in the presence of either divalent cation (Fig. 2 A and 2 B,

respectively). Without AMP the energy of activation is about 8 kcal/mole in the presence of Mn^{++} ions and 12 kcal/mole in the presence of Mg^{++} ions. The addition of a small amount of AMP gives a big (3-4 fold) increase in the apparent energy of activation (see TABLE II). From figures 2 A and 2 B it is evident that the Arrhenius plot will be discontinuous in the presence of AMP, if the measurements are extended to about 40 or 45°C, respectively. The break in the Arrhenius plot may also occur at temperatures ranging from 20-38°C, as is demonstrated in Fig. 2 C. It is apparent that the temperature at which the break in the Arrhenius plot occurs, is completely dependent on the interaction of the enzyme with AMP. An increase in the AMP concentration causes a shift of the break to higher temperatures (cf. Fig. 2 A and Fig. 2 C (Δ-Δ)). Ligands which increase the sensitivity for AMP inhibition, like K^+ or NH_4^+ ions (3), also cause a shift to higher temperatures (not shown). As the enzyme is more sensitive to AMP inhibition if measured with Mg^{++} ions compared to Mn^{++} ions (11), the break shifts to lower temperatures if Mg^{++} is replaced by Mn^{++} at constant AMP concentration (Fig. 2 C).

TABLE II summarizes the apparent energies of activation which can be calculated from the experiments shown in Fig. 2. The energy of activation is dependent on the nature of the divalent cation and on the presence of the inhibitor AMP. From the data shown in Fig. 2 C two apparent energies of activation can be calculated; one approaching the value found in the absence of AMP and one close to the value measured in the presence of AMP. The divalent cations Mg^{++} and Mn^{++} not only give different energies of activation but also different affinities for the substrate (12) and the inhibitor (11). It has been shown recently that the divalent cations affect the enzyme conformation (12). K^+ ions, however, which increase the activity of muscle fructosediphosphatase by 50-75% (3), do not affect the energy of activation and may influence the enzymatic activity by interaction with the substrate (cf. ref. 13).

Discontinuous Arrhenius plots have been described for membrane-bound as well as soluble enzymes (9,10). In the latter case (temperature-induced) conformational changes are believed to cause the discontinuity. The high apparent energy activation (in the presence of AMP) described in this paper can be explained by

TABLE II

APPARENT ENERGIES OF ACTIVATION OF RABBIT MUSCLE FRUCTOSEDIPHOSPHATASE

Additions	AMP (μM) --	0.12	0.92	2.12	5.15
Mg^{++}	14.9*	-	-	56.1**	-
Mg^{++} + K_2SO_4	13.4,14.0*	51.2**	52.5	-	-
Mn^{++}	7.7	-	-	-	25.4
Mn^{++} + K_2SO_4	8.0*	23.2**	-	-	-

* These values were calculated from the experiments shown in Fig. 2 C using the data obtained at high temperatures in the presence of AMP.

** Id. using the data obtained at low temperatures in the presence of AMP.

The apparent activation energy was calculated from the experiments shown in Fig. 2 and is given in kcal/mole. The measurements were done with either 10 mM $MgCl_2$ or 0.2 mM $MnCl_2$ in the presence of 50 mM K_2SO_4 and/or variable amounts of AMP, as indicated. For further details of the assay medium see MATERIALS AND METHODS.

TABLE III

TEMPERATURE COEFFICIENT (Q_{10}) OF RABBIT MUSCLE FRUCTOSEDIPHOSPHATASE IN THE PRESENCE AND ABSENCE OF AMP

Conditions	without AMP 20-30°C	without AMP 30-40°C	with AMP 20-30°C	with AMP 30-40°C
Mg^{++}	2.2	2.1	21	18
Mn^{++}	1.6	1.5	4.2	3.8

The Q_{10}'s were calculated from the experiments shown in Fig. 2 for the temperature range 20-30°C or 30-40°C. The various conditions used in the assay were 10 mM $MgCl_2$ or 0.2 mM $MnCl_2$, in the presence or absence of AMP, as indicated. For further details of the assay conditions see MATERIALS AND METHODS.

the decrease of AMP inhibition with increasing temperature (Fig. 1). Therefore the breakpoints in the Arrhenius plots coincide with temperatures at which the AMP becomes inhibitory (cf. Figs. 1 and 2). The effect of temperature on the AMP inhibition may be caused by a temperature-induced conformational change of the protein. The possibility of a ligand-induced discontinuity in the

Arrhenius plot, as demonstrated in this paper for a homogeneous enzyme, should also be considered in the case of more crude systems like membrane-bound enzymes.

TABLE III shows the temperature coefficients (Q_{10}) calculated from experiments similar to those of Fig. 2 (cf. ref. 10). It appears that the temperature dependence of fructosediphosphatase is normal in the absence of inhibitor. In the presence of AMP, however, the Q_{10} increases to about 4 or even 20 in the presence of Mn^{++} or Mg^{++} ions, respectively.

The Q_{10} of 18, measured in the presence of Mg^{++} ions between 30 and 40°C, may have implications *in vivo*. Fructosediphosphatase needs free (uncomplexed) Mg^{++} or Mn^{++} ions for catalytic activity (11). Recently it has been shown that, at least in liver, a substantial amount of free Mg^{++} ions is present (14). The intracellular concentration of Mn^{++} ions is low (15) and most of it will be in a complexed form. Therefore Mg^{++} ions probably activate fructosediphosphatase *in vivo*. The reported intracellular contents of fructosediphosphate (16-17) and AMP (17,18) are higher than the measured K_m for the substrate (12) and I_{50} for the inhibitor, respectively (TABLE I). It seems likely therefore that the conditions necessary for measuring a Q_{10} of 18 are fulfilled *in vivo*. This will cause a striking dependence of the activity of fructosediphosphatase on the temperature. An elevation of about 3°C over normal body temperature will give a 5-fold increase in the rate of fructosediphosphate hydrolysis. Fructosediphosphatase is the rate limiting step in the "substrate cycle" between fructosediphosphate and fructose-6-phosphate which operates in muscle (19-22). It follows that a 3°C rise in temperature will give a 5-fold increase in the rate of hydrolysis of ATP by this "substrate cycle". This mechanism may contribute to the observed increased substrate cycling and ATP hydrolysis during malignant hyperthermia (22).

ACKNOWLEDGEMENTS

The author acknowledges with gratitude the assistence of Miss A.C. Hanson and Mr. T. van Gent in typing the manuscript and performing the experiments, respectively.

REFERENCES

1. Pontremoli, S. and Horecker, B.L. (1971) in The Enzymes (Boyer, P.D., Ed.) Vol. IV, pp. 611-646, Academic Press, New York.

2. Taketa, K. and Pogell, B.M. (1965) J. Biol. Chem. 240, 651-662.
3. Black, W.J., Van Tol, A., Fernando, J. and Horecker, B.L. (1972) Arch. Biochem. Biophys. 151, 576-590.
4. Colombo, G., Hubert, E. and Marcus, F. (1972) Biochemistry 11, 1798-1803.
5. Cooperman, B.S. and Buc, H. (1972) Eur. J. Biochem. 27, 503-512.
6. Gonzalez, A.M., Gonzalez, F., Golf, S. and Marcus, F. (1972) J. Biol. Chem. 247, 6067-6070.
7. Villanueva, J. and Marcus, F. (1974) J. Biol. Chem. 249, 745-749.
8. Horecker, B.L. and Kornberg, A. (1957) in Methods in Enzymology (Colowick, S.P. and Kaplan, N.O., Eds.) Vol. III, pp. 879-882, Academic Press, New York.
9. Dawes, E.A. (1964) in Comprehensive Biochemistry (Florkin, M. and Stotz, E.H., Eds.) Vol. 12, pp. 89-125, Elsevier Publishing Company, Amsterdam, London, New York.
10. Dixon, M. and Webb, E.C. (1964) Enzymes, 2nd edition, pp. 145-166, Longmans, Green and Company Ltd., London.
11. Van Tol, A., Black, W.J. and Horecker, B.L. (1972) Arch. Biochem. Biophys. 151, 591-596.
12. Van Tol, A. (1974) Arch. Biochem. Biophys. 162, 238-247.
13. Mc. Clure, W.R., Lardy, H.A. and Cleland, W.W. (1971) J. Biol. Chem. 246, 3584-3590.
14. Veloso, D., Guynn, R.W., Oskarsson, M. and Veech, R.L. (1973) J. Biol. Chem. 248, 4811-4819.
15. Wimhurst, J.M. and Manchester, K.L. (1970) FEBS Lett. 10, 33-37.
16. Woods, H.F., Eggleston, L.V. and Krebs, H.A. (1970) Biochem. J. 119, 501-510.
17. Faupel, R.P., Seitz, H.J., Tarnowski, W., Thiemann, V. and Weiss, Ch. (1972) Arch. Biochem. Biophys. 148, 509-522.
18. Williamson, J.R. (1966) J. Biol. Chem. 241, 5026-5036.
19. Newsholme, E.A. and Crabtree, B. (1970) FEBS Lett. 7, 195-198.
20. Clark, M.G., Bloxham, D.P., Holland, P.C. and Lardy, H.A. (1973) Biochem. J. 134, 589-597.
21. Hue, L. and Hers, H.G. (1974) Biochem. Biophys. Res. Commun. 58, 532-539.
22. Bloxham, D.P., Holland, P.C., Taylor, C.A. and Lardy, H.A. (1973) Nature 245, 99-101.