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EFFECT OF TEMPERATURE ON ACTIN ACTIVATION OF HEAVY MEROMYOSIN ATPase

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SUMMARY

The effect of temperature on the actin activation of heavy meromyosin ATPase has been analyzed by measuring the temperature dependence of double-reciprocal plots of ATPase activity against actin concentration. An Arrhenius plot of the maximum ATPase of fully complexed acto–heavy meromyosin, obtained from ordinate intercepts of such reciprocal plots, is linear from 6 to 30° and gives an activation energy of 28 kcal/mole. This is about twice the activation energy of heavy meromyosin ATPase in the absence of actin and is comparable to the highest reported values for actomyosin ATPase. Hence the high activation energy of actomyosin ATPase at low temperatures is probably a property of the actomyosin ATPase reaction itself and not a consequence of increased dissociation of the actomyosin. In fact, low temperature does not appear to favor the dissociation of acto–heavy meromyosin. On the contrary, at very low ionic strength, the apparent dissociation constant of acto-heavy meromyosin in the presence of ATP, obtained from the abscissa intercepts of reciprocal plots, decreases when the temperature is reduced.

INTRODUCTION

The interaction of actin and myosin, the two major contractile proteins of muscle, is usually studied near room temperature; however, a few investigations of the effect of varied temperature on the Mg^{2+}-activated ATPase of actomyosin or myofibrils at low ionic strength have been reported[1–5]. The Arrhenius plots of the actomyosin ATPase obtained in these studies have shown two particularly interesting features. First, at temperatures below about 16°, the actomyosin ATPase has a very high activation energy, in the neighborhood of 25–30 kcal/mole, which means a Q_{10} of about 5. This activation energy is about twice that of the Mg^{2+}-ATPase of myosin alone, and hence the actin activation of the myosin ATPase diminishes as the temperature is reduced, the activity of actomyosin approaching that of myosin near 0° (refs. 1 and 4). Second, several authors[1,2,4,5] observed a distinct change in the slope of the Arrhenius plot of actomyosin ATPase at about 16°, the slope at higher temperatures being considerably smaller. A change in Arrhenius activation energy at 16° was also seen in the ATPase of chemically modified myosin and in myosin ITPase, which led LEVY *et al.*[6] to suggest that it was caused by a temperature-induced con-

formational transition in the active site region of myosin. Other workers[2,4,5], however, ascribed the high Arrhenius slope of actomyosin, and the transition at 16°, to increasing dissociation of actomyosin with decreasing temperature.

The extent to which dissociation of the actomyosin influences the temperature dependence of its ATPase could not be ascertained directly from measurements of actomyosin ATPase at low ionic strengths because of the difficulty of distinguishing between changes in the ATPase activity of the actomyosin complex and changes in the extent of interaction of myosin with actin. However, if heavy meromyosin is used in place of myosin, these two variables can be investigated separately[7]. In this case, a double-reciprocal plot of ATPase rate against actin concentration is linear, and its intercepts on the ordinate and abscissa give independent measures, respectively, of the ATPase activity of fully complexed acto–heavy meromyosin (v_{max}) and of the effective dissociation constant (K_{app}) for the interaction of actin with the heavy meromyosin–ATP complex.

In a previous study of the acto–heavy meromyosin system[8] such measurements were made at two different temperatures, and the results indicated that lowering the temperature decreases v_{max} but does not promote dissociation of acto-heavy meromyosin. We have now studied the effect of temperature on the acto-heavy meromyosin ATPase system in more detail. We have found that an Arrhenius plot of v_{max} for the acto–heavy meromyosin ATPase is linear, in contrast to the biphasic plots reported for actomyosin systems, and gives an activation energy of 28 kcal/mole, *i.e.* a Q_{10} greater than 5. We find no evidence that low temperature promotes dissociation of acto–heavy meromyosin; on the contrary, at very low ionic strength, K_{app} decreases with decreasing temperature, although this temperature dependence of K_{app} vanishes when the ionic strength is increased.

METHODS

Actin and heavy meromyosin were prepared from rabbit skeletal muscle and their concentrations determined according to the customary procedures of this laboratory[7,9]. The final F-actin solution was prepared in 3 mM $MgCl_2$–10 mM imidazole-HCl buffer (pH 7), and the heavy meromyosin was finally dialyzed into 2 mM imidazole buffer, usually containing 50 mM KCl.

The ATPase rates were measured at pH 7 by means of an automatic pH-stat[9] which was standardized separately at each temperature. The temperature of the reaction mixture was controlled by placing the sample beaker in a water jacket through which fluid from various thermostat baths could be circulated, and the actual temperature was recorded throughout the experiment by means of a small thermistor probe placed in the reaction mixture. In every case the temperature remained within $\pm$ 0.2° of the desired value.

The final composition of the reaction mixtures is specified in the figure captions. In all cases, 2 mM ATP and 2.4 mM $MgCl_2$ were present, together with 3–7 mM imidazole buffer (pH 7) and varying amounts of KCl. For each measurement, all components except the heavy meromyosin were initially mixed in a volume of 7.2 ml and brought to pH and temperature equilibrium, and the reaction was then started by addition of 0.3 ml of heavy meromyosin solution which had been brought to approximately the temperature of the sample. All the measurements for each Arrhe-

nius plot were done within 3 days, using the same preparations of actin and heavy meromyosin, and the temperature was varied at random rather than sequentially.

RESULTS

In order to analyze the effect of temperature on the acto–heavy meromyosin ATPase system, we measured the ATPase activity as a function of actin concentration at several temperatures. The data for each temperature were plotted in double-reciprocal form as $(v-v_{HMM})^{-1}$ against [free actin]$^{-1}$, according to the following equation which describes the kinetics of this system at saturating ATP concentration[7]:

$$\frac{1}{(v-v_{HMM})} = \frac{K_{app}}{(v_{max}-v_{HMM})} \cdot \frac{1}{[\text{free actin}]} + \frac{1}{(v_{max}-v_{HMM})} \qquad (1)$$

Here v is the measured rate of ATP hydrolysis per mg of heavy meromyosin; v_{HMM} is the rate of ATP hydrolysis per mg of heavy meromyosin measured in the absence of actin; v_{max} is the rate of ATP hydrolysis per mg of heavy meromyosin at infinite actin concentration; and K_{app} is the apparent dissociation constant of acto–heavy meromyosin in the presence of ATP. The concentration of free actin was obtained by subtracting the bound actin from the total added actin, and the bound actin was calculated from the measured ATPase rates assuming one-to-one stoichiometry of actin–heavy meromyosin binding[8]. Actually, this correction was significant only for certain points at the lower temperatures. Likewise, v_{HMM} was negligible in comparison to the measured v at all but the lowest actin concentrations and temperatures. Fig. 1 shows that double-reciprocal plots according to Eqn. 1 are linear at all temperatures. Extrapolation of each line to its ordinate intercept gives $(v_{max}-v_{HMM})^{-1}$, from which v_{max} for that temperature is easily obtained, and further extrapolation gives $-(K_{app})^{-1}$ as the abscissa intercept.

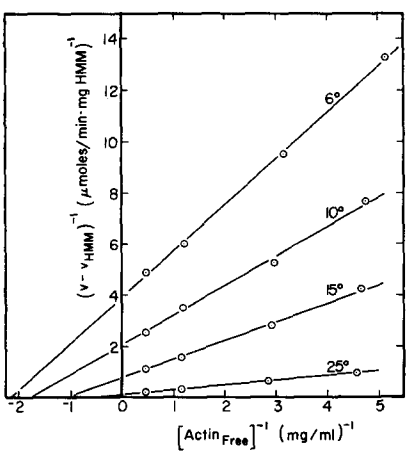

Fig. 1. Effect of temperature on double-reciprocal plots of acto–heavy meromyosin ATPase against actin concentration. All samples contained 2.0 mM ATP, 2.4 mM $MgCl_2$, 6.9 mM imidazole–HCl buffer (pH 7), and 12.7 mM KCl, for a total ionic strength of 0.027. An appropriate heavy meromyosin concentration, ranging from 0.045 to 0.56 mg/ml, was used at each temperature. Data plotted according to Eqn. 1. HMM, heavy meromyosin.

The temperature dependence of v_{max} is shown in Fig. 2a, in the form of an Arrhenius plot. A striking feature of this plot is its linearity over the entire temperature range. There is no indication of a change in slope at 16° such as has been observed with actomyosin[1,2]. The slope in Fig. 2a gives an Arrhenius activation energy of 29 kcal/mole, which is comparable to the value reported for actomyosin ATPase below 16° and more than twice the value for actomyosin at higher temperatures[1,5]. An

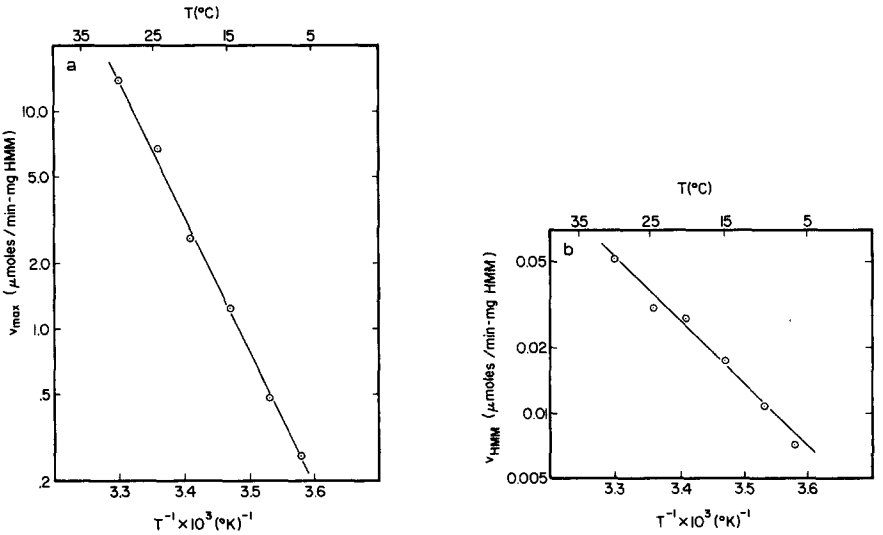

Fig. 2. Arrhenius plots for the ATPase activity of acto–heavy meromyosin (v_{max}) and heavy meromyosin alone (v_{HMM}). Conditions as in Fig. 1. (a) Arrhenius plot of v_{max}, determined from ordinate intercepts of reciprocal plots as in Fig. 1; (b) Arrhenius plot of v_{HMM}. The heavy meromyosin concentration used in (b) ranged from 1.7 to 3.6 mg/ml. HMM, heavy meromyosin.

TABLE I

ACTIVATION ENERGIES OF HEAVY MEROMYOSIN AND ACTO–HEAVY MEROMYOSIN ATPases AT VARIED IONIC STRENGTH

Values of the activation energy were determined from the slopes of Arrhenius plots like those of Fig. 2. The ATPase measurements were made with 2.0 mM ATP, 2.4 mM $MgCl_2$, 3–7 mM imidazole buffer (pH 7), and KCl concentrations varying between 2 and 32 mM. The heavy meromyosin concentration varied between 0.04 and 4 mg/ml as appropriate for each temperature and ionic strength.

Ionic strength	*Activation energy (kcal/mole)*	
	Acto–heavy meromyosin ATPase (v_{max})	*Heavy meromyosin ATPase (v_{HMM})*
0.017	27.4	—
0.017	25.9	—
0.027	28.9	13.5
0.036	27.2	13.5
0.036	27.9	13.5
0.045	27.2	—
0.045	30.9	13.5
Means	27.9 ± 1.5	13.5

Arrhenius plot of the Mg^{2+}–ATPase of heavy meromyosin alone (v_{HMM}) is also linear (Fig. 2b), but it gives an activation energy of only about 13 kcal/mole, in good agreement with the published values for myosin[1,3]. The activation energies for both v_{max} and v_{HMM} are independent of ionic strength, as shown in Table I; in fact the values of v_{max} themselves were independent of ionic strength, not only at 25° (ref. 8), but also at all other temperatures investigated.

The effect of temperature on the apparent dissociation constant of actin from the heavy meromyosin–ATP complex is shown in Fig. 3. In every case there is a decrease in K_{app} as the temperature is reduced, although this temperature dependence diminishes with increasing ionic strength. The increase in K_{app} with ionic strength[7,8] limited the range of ionic strength which could be investigated, because accurate determinations of K_{app} at higher ionic strengths would require actin concentrations in excess of experimentally feasible levels. However, the highest ionic strength investigated here is comparable to that used in the studies of actomyosin reported by BÁRÁNY[2] and BENDALL[5], and even in this case, we find no evidence of an increase in the dissociation of acto–heavy meromyosin with decreasing temperature.

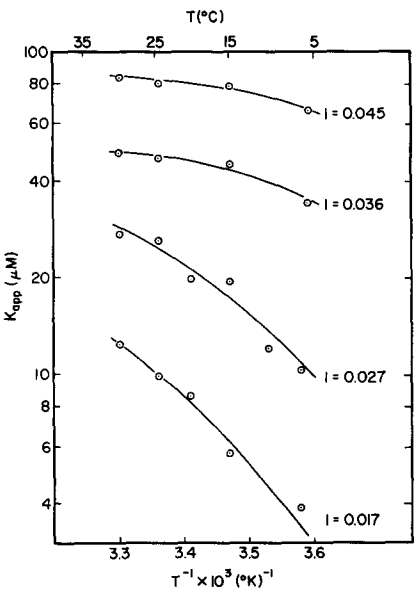

Fig. 3. Temperature dependence of K_{app}, the apparent dissociation constant for the interaction of actin with heavy meromyosin–ATP, at the various ionic strengths, I, indicated on the graph. K_{app} is calculated from the abscissa intercepts of reciprocal plots such as Fig. 1, assuming a molecular weight of 46000 for actin. Conditions identical to Table I.

DISCUSSION

Using double-reciprocal plots of ATPase rate against actin concentration, we have investigated separately the temperature dependence of the ATPase activity of the acto–heavy meromyosin complex and the temperature dependence of the interaction between actin and heavy meromyosin in the presence of ATP. Two principal findings have emerged: First, the Arrhenius plot for v_{max} the acto–heavy meromyosin ATPase is linear from 6° to 30°, with an activation energy about equal

to that of actomyosin ATPase below 16° (ref. 1) and roughly twice that of unmodified heavy meromyosin ATPase. Second, the temperature dependence of K_{app}, the apparent dissociation constant for the interaction of actin with heavy meromyosin–ATP, gave no indication that low temperature promotes dissociation of acto–heavy meromyosin in the presence of ATP; in fact, at very low ionic strength, K_{app} decreased with decreasing temperature. While the interpretation of K_{app} as a true thermodynamic equilibrium constant rests on the assumption of the particular kinetic mechanism which we have applied previously to the acto–heavy meromyosin ATPase system[7,10], it is clear from the present data, independent of any assumptions, that the actin concentration required to achieve half-maximal ATPase activation is not increased at lower temperatures. It may be desirable therefore to reexamine the view that lower temperature promotes the dissociation of actomyosin by ATP.

The major difference between our results with acto–heavy meromyosin and those for actomyosin reported by other workers[1–5] is that the Arrhenius plot of acto–heavy meromyosin ATPase does not show the change in slope at 16° which is usually observed in the case of actomyosin. Since the temperature coefficient of actomyosin ATPase at lower temperatures is equal to that of acto–heavy meromyosin, it is likely that this high temperature coefficient is a true property of the ATPase reaction at each site of actin–myosin interaction. Above 16°, where the properties of acto–heavy meromyosin and actomyosin diverge, we may regard the lower temperature coefficient of actomyosin ATPase as anomalous.

Increased dissociation of actomyosin with increasing temperature could give rise to a lower temperature coefficient for the ATPase, but, although we have seen such an effect in acto–heavy meromyosin in the present study, the effect was small, and furthermore it vanished when the ionic strength was raised to the level where most studies with actomyosin are done. It seems more likely that the difference in temperature dependence between acto–heavy meromyosin and actomyosin is related to the fact that myosin is aggregated at low ionic strength while heavy meromyosin molecules are free to interact independently with actin. The low actomyosin ATPase activity relative to the v_{max} of acto–heavy meromyosin[7] suggests that the interaction of myosin aggregates with actin filaments may involve some structural constraint which prevents the maximal activation of all the myosin molecules. Hence, an effect of temperature on the state of aggregation of the myosin, or perhaps the actin, might well influence the temperature dependence of the measured actomyosin ATPase. Of course this suggestion is highly speculative at this point, and further studies will be required to elucidate the reasons for the lower temperature coefficient of actomyosin ATPase above 16°.

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