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A THERMALLY POTENTIATED STATE FOR ACTOMYOSIN ATPase OF RABBIT SKELETAL MUSCLE

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SUMMARY

Heat-treatment of natural actomyosin at low ionic strength in the absence of substrate results in substantial augmentation of Mg-ATPase, and minor increase of Ca-ATPase and decrease of EDTA-ATPase. Changes in steady-state activity persist despite decrease of temperature. The effect appears to involve a thermally induced transition to a stable potentiated state for natural actomyosin. The phenomenon requires interaction between actin and myosin during heat-treatment; however, the presence of troponin and tropomyosin is needed for potentiation to be fully manifest. Thermal potentiation significantly modifies the Arrhenius behavior of actomyosin ATPase, and the augmented catalytic rate reflects a large increase of activation entropy.

INTRODUCTION

In general, myofibrillar contractile proteins undergo denaturation during prolonged incubation at high temperature, with irreversible loss of ATPase activity. However, in studies of myofibrillar proteins from rabbit skeletal muscle, we have found that heat treatment of natural actomyosin at low ionic strength in the absence of substrate is accompanied by substantial augmentation of ATPase activity, beyond what might be expected from conventional Arrhenius behavior of actomyosin ATPase. The effect appears to involve a stable change in the enzymatic state of the entire actomyosin complex. The present communication describes the general features of the thermal potentiation phenomenon, and its effect upon the Arrhenius dependence of actomyosin ATPase.

MATERIALS AND METHODS

Preparation of Natural Actomyosin. All procedures were at 5°C. Back

ABBREVIATIONS

DTT, dithiothreitol; EGTA, ethyleneglycol-bis-(β-aminoethyl ether) N,N'-tetraacetic acid; ATPase, ATP phosphohydrolase (EC 3.6.1.3)

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and leg muscles of freshly killed rabbits were minced and mixed with 3 vols/g of 0.4 M KCl, 0.1 M potassium phosphate, 1 mM DTT, 3 mM KN_3, pH 6.5. The mixture was stirred for 10-15 min and centrifuged at 5000 x g for 15 min. The ppt. was extracted for 15-20 h with 5 vols/g of 0.6 M KCl, 25 mM potassium phosphate, 3 mM KN_3, 1 mM DTT, pH 7.6. After centrifugation at 5000 x g for 15 min, the supernatant was diluted with 15 vols of water. The resulting ppt. was centrifuged at 10,000 x g for 20 min and dissolved in 1 volume of 1 M KCl. The solution of natural actomyosin was dialyzed against 0.5 M KCl, 5 mM $KHCO_3$, 3 mM KN_3, pH 7.0 and clarified by centrifugation at 15,000 x g for 20 min. On SDS-polyacrylamide gel electrophoresis, there are bands for myosin, actin, tropomyosin, troponin, and minor constituents of the myofibril, as reported (1).

Heat-treatment. Stock protein was diluted to 0.1-0.3 mg/ml in KCl-Tris solution at 5°C and heat-treated, as specified in Results. Aliquots were kept in an ice slurry at 0°C until assay of ATPase.

ATPase Assays. Reaction was initiated by addition of 1 ml substrate, thermally equilibrated at assay temperature, to 0.5 ml protein sample. Reaction was terminated with 1 ml 10% trichloroacetic acid, and inorganic phosphate in supernatant was determined using a Technicon Auto-Analyzer. Final reaction mixtures for Mg-ATPase contained 3.3 mM $MgCl_2$, 3.3 mM ATP, 16 μM $CaCl_2$, Tris, DTT and KCl as indicated, pH 7.8. For assays in the absence of Ca^{2+} ions, 3.3 mM EGTA was included. Ca-ATPase was assayed in 3.3 mM $CaCl_2$, 3.3 mM ATP, 20 mM Tris, 1 mM DTT, and either 50 mM KCl or 0.5 M KCl, pH 7.8. EDTA-ATPase was assayed in 3.3 mM EDTA, 3.3 mM ATP, 20 mM Tris, 1 mM DTT, 0.5 M KCl, pH 7.8. Ca^{2+}-sensitivity is defined as the ratio of Mg-EGTA-ATPase to Mg-ATPase. ATPase values are in μmol P_i/mg natural actomyosin · min.

Protein concentration was determined by the biuret reaction (2) or the procedure of Lowry et al. (3). Other purification and analytic procedures are described elsewhere (4).

RESULTS AND DISCUSSION

Thermal Potentiation of Natural Actomyosin. Incubation of rabbit natural actomyosin at temperatures above 25°C at low ionic strength in the absence of substrate is accompanied by characteristic time dependent changes in ATPase activity with substantial augmentation of Mg-ATPase, as well as increase of Ca-ATPase and decrease of EDTA-ATPase to lesser degrees. There is also increase of Mg-EGTA-ATPase, resulting in progressive loss of Ca^{2+}-sensitivity. Ca^{2+}-sensitivity is lost at an appreciably slower rate than Mg-ATPase is augmented, and the two effects appear to represent independent results of heat-treatment.

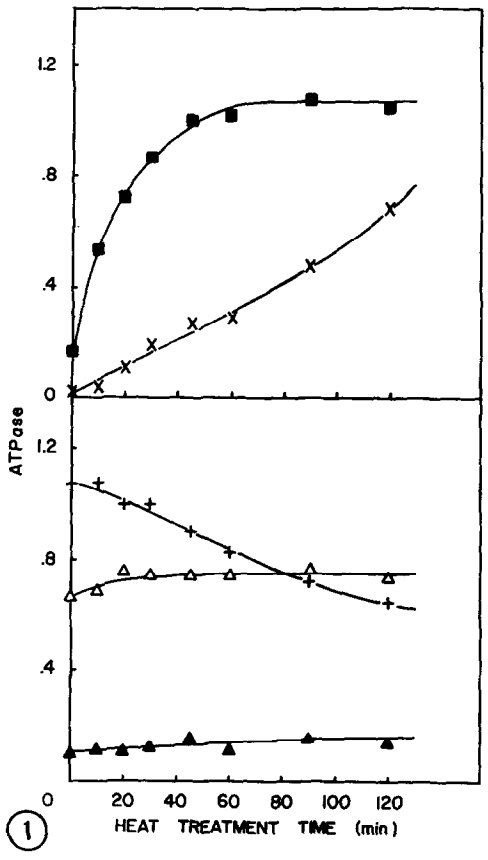
The overall changes are observed when ATPase activities are subsequently assayed at the same temperature at which heat-treatment is conducted. Furthermore, heat-altered activities are manifest during assays at temperatures below those at which heat-treatment was conducted. Altered levels of activity

remain unchanged for at least several hours after heat-treatment of natural actomyosin, and only minor decreases of activity are observed during prolonged storage at 5°C.

Figure 1 shows the characteristic changes which occur during incubation of natural actomyosin at 44°C. There is approx. 6-fold increase of Mg-ATPase over a period of 45 min, and slight additional increase during heat-treatment to 120 min. Mg-EGTA-ATPase shows progressive augmentation, but Ca^{2+}-sensitivity is largely preserved during the initial period when Mg-ATPase is undergoing rapid augmentation. Values of Ca-ATPase are slightly augmented during heat-treatment, as measured in 0.5 M KCl (38% increase) or 50 mM KCl (19% increase); and values of EDTA-ATPase decrease approx. 40% during 120 min at 44°C. Rates of hydrolysis are constant for assay periods as long as 20 min, so that the changes in specific activity reflect changes in steady-state behavior. Since the effects occur in the presence of DTT, the changes can not be attributed to oxidation of fast reacting sulfhydryl groups on myosin (5,6). In the absence of DTT, heat-treatment of natural actomyosin initially results in comparable augmentation of Mg-ATPase, but there is progressive loss of activity during prolonged heat-treatment.

The extent of thermal potentiation of Mg-ATPase is exquisitely dependent on the temperature during heat-treatment of natural actomyosin. Variation of temperature from 39°C to 48°C is accompanied by striking increases in initial rate and maximal extent of augmentation of Mg-ATPase (Figure 2). At 48°C, an initial increase of activity is followed by progressive loss, presumably due to heat denaturation.

Protein Interactions in Thermal Potentiation. When purified samples of myosin and thin-filament proteins (actin, tropomyosin, and troponin) are recombined in equivalent weight ratios and then heat-treated at low ionic strength in the absence of substrate, there is characteristic thermal potentiation, as in the case of natural actomyosin. In contrast, samples of myosin and thin-filament proteins which are heat-treated individually and then



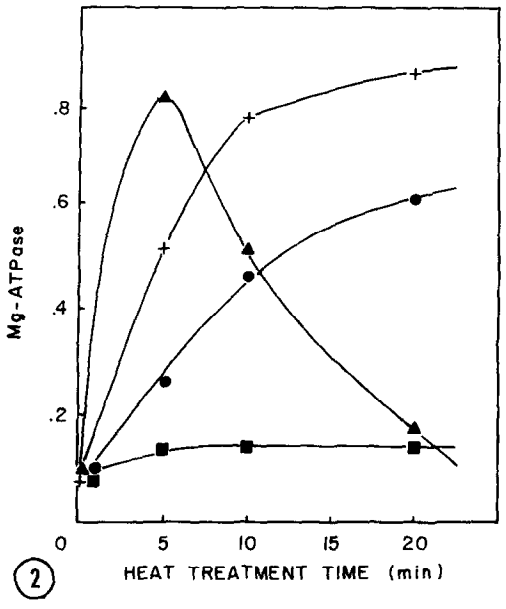


Figure 1: Thermal potentiation of natural actomyosin. Natural actomyosin in 0.5M KCl, 5 mM $KHCO_3$, 3 mM KN_3, pH 7.0, was diluted to a protein concentration of 0.14 mg/ml with final solvent composition 0.1 M KCl, 10 mM Tris, 1 mM DTT, pH 7.8. Aliquots (0.5 ml) were heat-treated at 44°C for specified intervals, and immediately placed in ice slurry until ATPase assays at 25°C. Data shown for Mg-ATPase (□) and Mg-EGTA-ATPase (X) in 50 mM KCl, 20 mM Tris, 1 mM DTT; Ca-ATPase in 50 mM KCl (△) and 0.5 M KCl (▲); and EDTA-ATPase (+) (see Methods).

Figure 2: Effect of prior heat-treatment on Mg-ATPase. Natural actomyosin was diluted to 0.13 mg/ml in 25 mM KCl, 50 mM Tris, pH 7.8 and heat-treated for specified times at 39°C (■), 42.5°C (●), 44.5°C (+) and 48°C (△). Mg-ATPase assayed in 25 mM KCl, 50 mM Tris, pH 7.8, at 25°C.

recombined show loss of specific activity. Thus, some kind of interaction between myosin and thin-filament proteins appears necessary for thermal potentiation to occur.

When purified samples of myosin and F-actin are recombined and then heat-treated, there is only slight augmentation of Mg-ATPase. However, upon

recombination of heat-treated actomyosin with troponin and tropomyosin, there is marked thermal potentiation. The extent of potentiation is approximately the same whether or not troponin and tropomyosin were also heat-treated prior to recombination. Thus, thermal potentiation appears to require direct interaction of actin and myosin during heat-treatment at low ionic strength, although the presence of troponin and tropomyosin is needed for potentiation to be fully manifest.

Thermal potentiation should be differentiated from the potentiation which results during formation of rigor complexes between actin and myosin active sites (7,8). Thermally potentiated activity is maintained during steady-state hydrolysis in 3.3 mM Mg-ATP, a condition under which rigor complexes are instantly dissolved. In addition, loss of Ca^{2+}-sensitivity is not coincident with thermal potentiation of Mg-ATPase, as in the case of rigor-complex potentiation.

Arrhenius Dependence of Mg-ATPase. Figure 3 shows the effect of thermal potentiation on the Arrhenius behavior of Mg-ATPase. Natural actomyosin which had not been subjected to prior heat-treatment exhibits a non-linear Arrhenius plot, with an apparent transition about 22°C, and there is progressively increasing thermal potentiation as the incubating temperature is increased. Thus, at temperatures greater than 25°C, measurements of specific activity may include temperature-dependent components from conventional Arrhenius activation and thermally-induced potentiation. The apparent Arrhenius curve may vary with the conditions employed during thermal equilibration of natural actomyosin prior to assay. This consideration may account for differences between the present data and previous data on the Arrhenius dependence of rabbit actomyosin Mg-ATPase (9).

Calculations of activation energies from the data in Figure 3 indicate that at 10°C, values for $\Delta H^{\ddagger}$ are 28.7 kcal/mol and for $\Delta S^{\ddagger}$ are 43 e.u. At 40°C, unheated natural actomyosin exhibits values of 7.3 kcal/mol for $\Delta H^{\ddagger}$ and - 29 e.u. for $\Delta S^{\ddagger}$, whereas potentiated natural actomyosin exhibits values

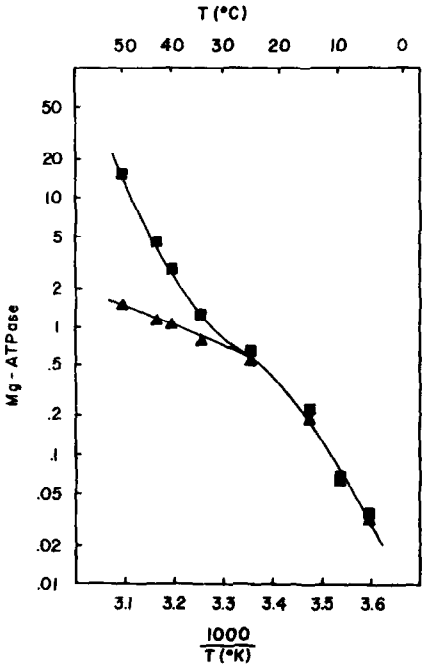


Figure 3: Effect of thermal potentiation on Arrhenius dependence of Mg-ATPase. Samples of natural actomyosin in 60 mM KCl, 10 mM Tris, 1 mM DTT, pH 7.8, were incubated at temperatures of from 5°C to 50°C, and aliquots were periodically removed for assay of Mg-ATPase in 38 mM KCl, 20 mM Tris, 1 mM DTT at the *same* temperature as during prior heat-treatment. Data are shown for unheated natural actomyosin, (▲); and for *maximally* potentiated natural actomyosin after heat treatment (■). Periods of thermal incubation vary from several min at 50°C, to as long as 25 h at 25°C, or below. Activation energies were calculated assuming that natural actomyosin contains 50% (w/w) myosin, having 470,000 molecular weight.

of 25.3 kcal/mol for $\Delta H^{\ddagger}$ and 30 e.u. for $\Delta S^{\ddagger}$. Hence, the augmented catalytic rate of thermally potentiated natural actomyosin reflects a large increase in activation entropy, which overcomes the change in $\Delta H^{\ddagger}$, which actually tends to decrease the catalytic rate.

Previously reported values of actomyosin ATPase are comparable in general with those here obtained for unheated natural actomyosin; however, considerably higher levels of ATP hydrolysis are thought to occur *in vivo* (10). The present evidence indicates that natural actomyosin possesses the potential for considerably greater activity than is usually measured, and that thermally potentiated natural actomyosin exhibits activity which approaches *in vivo* levels. In particular, natural actomyosin which has been heat-treated at

37°C exhibits Mg-ATPase of 4 to 5 μmol P_i/mg myosin · min upon subsequent assay at 37°C.

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