Thermal Inactivation of *p*-Chloromercuribenzoate-dithiothreitol-treated Myosin ATPase

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Received September 18, 1975

Myosin ATPase is inactivated according to the first order kinetics by thermal treatment.¹⁻⁵⁾ Myosin from white skeletal muscle is composed of two heavy chains and three light chains, g_1 , g_2 and g_3 , in which g_1 and g_3 are prerequisite factor for ATPase activity.⁶⁻¹⁰⁾ It has been reported that the g_2 light chain has a Ca^{2+} -sensitivity and this sensitivity may play a role in regulating ATPase activity or in relaxation.^{10,11)} Perrie *et al.*¹²⁾ suggested that the phosphorylation of the g_2 light chain would increase the Ca^{2+} -binding ability of myosin. However, it remains uncertain what extent myosin is stabilized by the g_2 light chain against thermal denaturation. In order to elucidate the role of the g_2 light chain in stabilizing myosin, we have investigated the thermal inactivation of PCMB-DTT-treated myosin in which about a half of the g_2 light chain was released.¹³⁾

Myosin was prepared from rabbit back muscle by the method of Offer *et al.*¹⁴⁾ The PCMB-DTT-treated myosin was prepared according to the method of Hayashi *et al.*¹³⁾ As described by them,¹³⁾ about a half of the g_2 light chain was released from myosin molecules by this procedure. Two milliliters of the untreated or PCMB-DTT-treated myosins (1 mg/ml) in 0.5 M KCl, 0.01 M Tris-maleate (pH 7.0) was incubated at varying temperatures. The thermal treatment was terminated by adding the equal volume of an ice-cold 0.5 M KCl solution. ATPase activity of the myosin was determined in a medium (0.5 M KCl, 4 mM $CaCl_2$, 1 mM ATP and 0.02 M Tris-maleate, pH 7.0) at 25°C. The phosphorus liberated was determined by the method of Fiske and Subbarow.¹⁵⁾

ATPase activities of the untreated and PCMB-DTT-treated myosins after the incubation at varying temperatures are presented in Figs. 1a and 1b, respectively. The loss of ATPase activity of the PCMB-DTT-treated

myosin followed the first order kinetics. The apparent activation energies, E_a , of the untreated and PCMB-DTT-treated myosins calculated from the slopes of the Arrhenius plots of ATPase activity were 73 kcal/mole and 68 kcal/mole, respectively (Fig. 2). Further, by applying the Eyring theory of a absolute reaction rates¹⁶⁾ the apparent heat of activation, $\Delta H^\ddagger$, and the apparent entropy change, $\Delta S^\ddagger$, of the untreated myosin were calculated to be 73 kcal/mole and 162 cal/deg/mole, respectively. These values are consistent with those reported by Ouellet *et al.*¹⁷⁾ The values of $\Delta H^\ddagger$ and $\Delta S^\ddagger$ of the PCMB-DTT-treated myosin were calculated to be 68 kcal/mole and 147 cal/deg/mole, respectively.

FIG. 1. Logarithm of the Activity of Untreated and PCMB-DTT-Treated Myosins as a Function of Heating Time.

Prior to determining ATPase activity, myosin was incubated at 32.5°C (Δ), 35.0°C ($\circ$), 37.5°C ($\square$) and 40.0°C (∇), respectively.

a, untreated myosin; b, PCMB-DTT-treated myosin.

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Abbreviations: PCMB, *p*-chloromercuribenzoate; DTT, dithiothreitol; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid).

FIG. 2. Arrhenius Plot of the Effect of Temperature on the Rate of Denaturation of Myosin, as Measured by ATPase Activity.

a, untreated myosin; b, PCMB-DTT-treated myosin.

The values of $\Delta G^\ddagger$ of the untreated and PCMB-DTT-treated myosins were calculated as both 23 kcal/mole. A similarity in $\Delta H^\ddagger$, $\Delta S^\ddagger$ and $\Delta G^\ddagger$ between the untreated and PCMB-DTT-treated myosin indicates that the mechanism of the inactivation of the PCMB-DTT-treated myosin ATPase activity due to the thermal

treatment is quite similar to that of the untreated myosin. Therefore, it appears that the g_2 light chain released from myosin by the PCMB-DTT-treatment has little effect for protecting ATPase activity of myosin against the thermal treatment. It may be speculated that the DTNB light chain has also little effect for protecting the myosin ATPase site, since the DTNB and PCMB light chains have the same immunological reactivity.¹⁷⁾

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