SOME STABILITY EFFECTS OF ATP, Mg^{++}, AND DINITROPHENOL ON ACTOMYOSIN AND MYOSIN ADENOSINETRIPHOSPHATASE

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Decrease in adenosine triphosphatase activity of myosin when tested in the presence of 2,4 dinitrophenol, DNP and Mg^{++} at temperatures higher than 16° has been reported (Koshland, 1959). Analysis of Arrhenius plots of this phenomenon, together with the fact that the adenosine triphosphatase activity of actomyosin also yields nonlinear Arrhenius plots, has been interpreted recently as the result of reagent induced geometrical changes of myosin. Recently and independently we have reached similar conclusions in explaining our recent observations of decreased enzyme stability in the presence of substrates and cofactors (Grisolia and Joyce, 1960).

It was therefore of interest to test whether or not the anomalous Arrhenius plots of myosin and actomyosin are due to substrate induced enzyme instability. We have confirmed repeatedly the results of Koshland under a variety of conditions**, and at several levels of enzyme concentration to make conditions more stringent. However, as illustrated in Table I myosin sta-

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**We used 0.002 and 0.004 M DNP. While Koshland (1959) does not report concentration, about 0.01 M must have been used considering the high stimulation of adenosinetriphosphatase shown (Levy, Sharon and Koshland, 1959). On the other hand our myosin preparations were about twice as active as those of Koshland (1959).

bility to heat is not affected by DNP, moreover the enzymatic activity is protected by ATP or ATP plus DNP even at higher temperatures than used by Koshland (1959). On the other hand, as typified by Table II actomyosin is less stable (at high temperatures) in the presence of ATP and particularly in the presence of ATP and Mg^{++}. Since Mg^{++} per se has little effect, the potentiation of the ATP effect by Mg^{++} is of particular interest.

TABLE I

The Effect of ATP, DNP and Mg^{++} on Thermal Stability of Myosin

PREINCUBATION CONDITIONS		Adenosine Triphosphate Hydrolysis
Additions	Temperature	μmoles
None	0°	0.27
None	40°	0.15
4 μmoles ATP	40°	0.24
4 μmoles DNP	40°	0.13
4 μmoles each ATP and DNP	40°	0.31
4 μmoles each ATP and DNP plus 10 umoles $MgCl_2$	40°	0.28

Each tube containing 16 mg myosin prepared according to Perry (1955) and 50 μmoles Tris-Cl^- pH 7.3 plus the indicated additions in 1 ml was preincubated for 5 minutes as indicated. After cooling on ice, a 0.1 ml aliquot from each tube was made up to 1.0 ml containing the following components expressed in μmoles: ATP, 5; Tris-Cl^- pH 7.3, 50; $MgCl_2$, 10 and KCl, 75. Incubation was for 5 minutes at 15°.

TABLE II

The Effect of ATP, and Mg^{++} on the Thermal Stability of Actomyosin

PREINCUBATION CONDITIONS		Adenosine Triphosphate Hydrolysis
Additions	Temperature	μmoles
None	0°	0.904
None	52°	0.424
4 μmoles ATP	52°	0.114
4 μmoles $MgCl_2$	52°	0.404
4 μmoles each ATP and $MgCl_2$	52°	0.032

Each tube containing 4.5 mg actomyosin prepared according to Szent-Gyorygi (1953) and 100 μmoles Na^+ glycine buffer pH 8.5 plus the indicated additions, was incubated for 5 minutes, cooled in ice and 0.2 ml aliquots from each tube were made up to 1.0 ml containing the following components expressed in μmoles: ATP, 8; glycine buffer, pH 8.5, 100; $MgCl_2$, 10, and KCl, 24. Incubation was for 5 minutes at 35°.

Present belief is that the DNP stimulation of myosin adenosinetriphosphatase activity mimics actomyosin interaction or at least that there is a similar point of interaction, also that while ATP dissociates the actomyosin complex, Mg^{++} favors association (Bailey, 1954; Perry and Chappell, 1957; Koshland, 1959). Since under our conditions actomyosin is essentially stable at 50° for 5 minutes, while myosin is already unstable at 40°, it is clear that the instability of actomyosin when heated in the presence of both ATP and Mg^{++}, must be the result of structural changes, whether or not the inactivation is due to dissociation of the actomyosin complex at high temperature followed by myosin denaturation. Further, it is of considerable interest that even

if addition of DNP to myosin entails structural changes resembling actin interaction, the changes are not entirely comparable as indicated by the stability variations illustrated.

The experiments presented here extend the findings and are consistent except as indicated above with the interpretations of Koshland (1959), further they illustrate the power of the simple but highly specific technique (Caravaca and Grisolia, 1960) of modifications in enzyme stability by substrates and cofactors.

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