ACTIN POLYMERIZATION BY DIRECT TRANSPHOSPHORYLATION[1]

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The "mechanochemical coupling" in muscle contraction may be defined in its general form as the relationship of the contractile elements to the chemical factors of the surrounding sarcoplasm. The work of Carlson and Siger (1960) has resulted in a specification of the underlying mechanism involved, where the key element is "compartmentalized" actin. The question involved is whether or not the bound nucleotide of the actin can be phosphorylated by an external enzyme system.

Strohman (1959) has shown that the bound nucleotide of polymerized (F)[2] actin is inert to the action of external enzymes. In dialysis experiments, he has demonstrated that the ADP of depolymerized actin can be phosphorylated by CP and CPK, but the interpretation has been questioned by Martonosi et al. (1961) on technical grounds. The work presented herein extends the original interpretation of Strohman and presents evidence that the bound nucleotide of actin can be directly phosphorylated by the CP and CPK system.

The CPK used was prepared by the method of Noda, Kuby, and Lardy (1955) to the penultimate step and lyophilized. For use, this material was dissolved in 1 mM $MgCl_2$ to the desired concen-

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2. Abbreviations: F = polymerized actin, G = non-polymerized actin, ATP = adenosine triphosphate, ADP = adenosine diphosphate, CP = creatine phosphate, CPK = creatine phosphokinase.

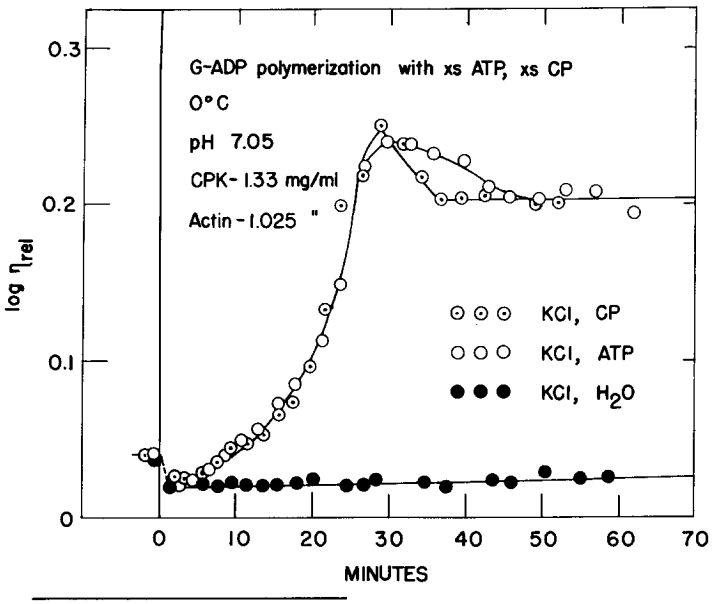
tration and treated with Dowex-1 (Hayashi and Tsuboi, 1960) to remove contaminating nucleotides. Purified F-ADP actin was prepared by a modification of the method of Ulbrecht et al. (1960) and converted to G-ADP essentially according to the quick method of Grubhofer and Weber (1961). As with the CPK, the G-ADP actin was cleaned of free nucleotide before use.

G-ADP actin can be induced to polymerize simply upon the addition of salt (0.1 M KCl, 0.001 M $MgCl_2$) according to Hayashi and Rosenbluth (1960) and Grubhofer and Weber (1961). This "salt-induced" polymerization occurs readily at room temperature; it will be shown that the process apparently has a high temperature coefficient, since at 0°C this type of polymerization is completely suppressed. Polymerization of G-ADP can also be induced with ATP (100 μM) added with the salt ("ATP-salt-induced"); this second induction method causes G-ADP to polymerize to undiminished extent at both 29°C and 0°C. This type of polymerization is due to the conversion of the G-ADP to G-ATP actin by replacement of the bound ADP by the added ATP, since Hayashi and Rosenbluth (1962) have shown a quantitative dephosphorylation accompanying the polymerization. A third method of induction is termed "CP-salt-induced" where CP is included in the inducing agent instead of ATP, and CPK is present with the actin..

The behavior of G-ADP actin with all three methods of induction used at 0°C is depicted in Figure 1.

The conditions of the experiment follow. Three Ostwald viscosimeters with similar efflux times are prepared in a 0°C bath, each containing the same amounts of G-ADP actin, CPK, $MgCl_2$, and tris-maleate buffer, final pH at 0°C being 7.05. The final[3] concentrations are 1.025 mg/ml of G-ADP, 1.33 mg/ml of

3. "Final" concentrations are calculated after all agents added.



CPK, 1 mM $MgCl_2$, and 0.01 M buffer. The two proteins G-ADP and CPK are present in essentially equimolar concentrations. To each viscosimeter is now added an inducing agent: to the first, CP-KCl, to the second, ATP-KCl, and to the third, H_2O-KCl. The final concentration of the KCl in the viscosimeters is 0.1 M. The changes in viscosity are then followed.

The first two viscosimeters show polymerizations of the actin which are indistinguishable in rate and extent, whereas the third shows no polymerization. The third viscosimeter is an example of "salt-induced" polymerization of G-ADP suppressed at $0^{\circ}C$; from the behavior at $0^{\circ}C$, it is apparent that the G-ADP actin in the first two viscosimeters has been converted to G-ATP actin, by CP in the first case, and by ATP in the second.

It is possible that the phosphorylation of the actin-associated ADP by CP-CPK takes place by way of free nucleotide lost from the actin. That is, the bound ADP of the actin dissociates to a free ADP. This free ADP is then phosphorylated by CP-CPK to ATP, which then displaces more bound ADP to the free condition.

This in turn is converted to ATP, which displaces more bound ADP, etc., and the mechanism is not different from that stated for "ATP-salt-induced" polymerization.

Hayashi and Rosenbluth (1960) have stated that the ADP loss at 0°C and in the presence of 1 mM Mg is quite slow. Under the conditions of the experiment, the half-life of the bound ADP is about 7 hours, and it can be shown that the maximum amount of free ADP present when the polymerizing agent is added is about 3% of the bound nucleotide. With such an amount of unbound nucleotide, it is highly unlikely that the cycling of this nucleotide can account for the polymerization exhibited in the first viscosimeter. Especially is this true when comparison is made with the second viscosimeter, wherein ATP replacement of the bound ADP is accomplished with 100 μM of free ATP, an amount more than 200 times that present at any time in the first viscosimeter.

It may be concluded therefore that the bound nucleotide of actin can be phosphorylated by the CP-CPK system. Physiologically, this finding provides a basis for the mechanism suggested by Carlson and Siger (1960), but with this significant modification: that the actin, to be susceptible to transphosphorylation by external enzymes, must be in the G-ADP form.

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