EXCHANGE OF F-ACTIN-BOUND NUCLEOTIDE IN THE PRESENCE AND ABSENCE OF MYOSIN*

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Received April 5, 1966

In one current proposal for the mechanism of muscle contraction, the relative motion of the myosin and actin filaments is regarded as resulting from a repeated cyclic conformational change in the actin (Oosawa, Asakura and Ooi, 1961). An experimental test for this hypothesis is suggested by the possibility that such a conformational change might lead to a transient increase in the exchangeability of the actin-bound nucleotide with free nucleotide in the medium. Martonosi, Gouvea, and Gergely (1960) observed that only a small amount of actin-bound nucleotide was released to the medium during superprecipitation of actomyosin; however, Szent-Györgyi has recently found exchange of up to 50% of the bound nucleotide (Szent-Györgyi, 1965; Szent-Györgyi and Prior, 1966). In our preliminary studies of nucleotide exchange, we also observed incorporation of labeled nucleotide into actomyosin during superprecipitation, but we found that considerable exchange took place in F-actin in the absence of myosin (Moos, 1965).

In view of the great significance which a change in bound-nucleotide exchangeability might have for considerations of the role of actin in muscle contraction, we have undertaken a detailed investigation of the incorporation of radioactive nucleotide into F-actin both with and without myosin. We have found that the bound nucleotide of F-actin is indeed exchangeable in the absence of myosin, showing a rapid incorporation of a limited amount of labeled

*Supported by USPHS Grants GM-10249 and 5T1-GM-718, and a grant from the Heart Association of Erie County, Inc.

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nucleotide immediately after the addition of H^3ATP, followed by further exchange at a slower steady rate. In superprecipitating actomyosin, we have found that the interaction of the actin with myosin does not affect the initial rapid burst of exchange, but that the steady rate of further exchange is considerably increased in the superprecipitated state.

Methods

Myosin and actin were prepared from rabbit skeletal muscle by the methods of Szent-Györgyi (1951) and Ulbrecht, Grubhofer, Jaisle, and Walter (1960), respectively. The exchange experiments were carried out by incubating F-actin with H^3ATP in the presence or absence of myosin. Creatine kinase (EC 2.7.3.2) and phosphocreatine were also added, and the turnover of ATP in the actomyosin suspension was followed by determination of the liberated creatine (Eggleton, Elsden, and Gough, 1943). For measurements of exchange in actomyosin, samples were quickly centrifuged after the desired reaction time, washed by repeated reprecipitation, and analyzed for radioactivity in the bound nucleotide. The actin samples were precipitated with myosin and then washed and analyzed in the same manner; however, either the free H^3ATP was first removed by treatment with Dowex-1 anion-exchange resin, or 1,2-diaminocyclohexanetetraacetic acid was added with the myosin to reduce the free magnesium ion concentration so that no additional exchange would occur on addition of the myosin to the actin. (Very little exchange occurs in actomyosin if the magnesium ion concentration is low (Szent-Györgyi, 1965; Moos, unpublished results). By "percent exchange", we mean the specific activity of the bound nucleotide expressed as a percentage of that of the free H^3ATP in the reaction mixture.

Results and Discussion

Contrary to the widespread assumption that F-actin-bound nucleotide is non-exchangeable, we consistently observe incorporation of radioactive

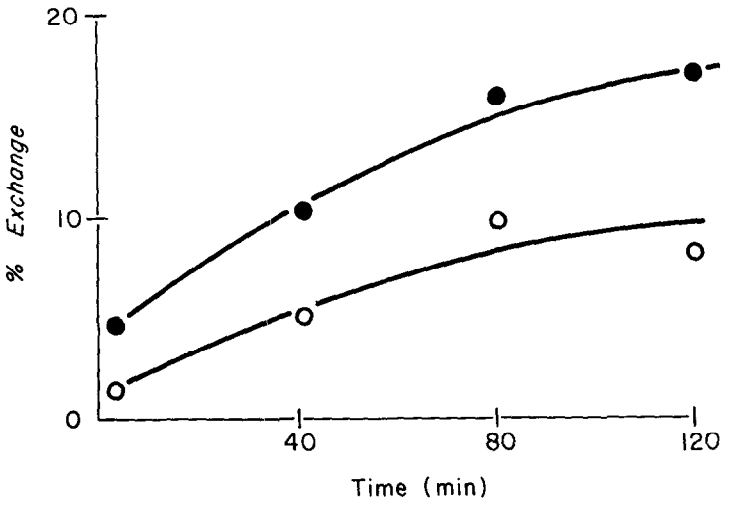

Figure 1. Bound nucleotide exchange in F-actin. Reaction mixture contained 0.2 mg/ml F-actin, 0.1 M KCl, 1 mM $MgCl_2$, 10 mM imidazole buffer, pH 7.0, and 50 μM H^3ATP; temperature, ca. 25°. Upper curve: at indicated times, 10 ml aliquots were removed and mixed with 3.5 ml of 0.1 M KCl solution containing 10 mg myosin and 50 μmoles 1,2-diaminocyclohexanetetraacetic acid at pH 7.0, and the suspension was centrifuged for exchange analysis. Lower curve: same, except 0.20 ml. 50 mM ATP added to each aliquot 1 min. before adding myosin mixture, bringing total ATP conc. to 1.0 mM.

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nucleotide into F-actin during incubation with H^3ATP (Fig. 1, upper curve). This incorporation shows an initial rapid burst followed by a continuing slow increase. Sudden dilution of the H^3ATP by the addition of a 20-fold excess of unlabeled ATP removes nearly all the radioactivity incorporated in the initial burst; but after the exchange has continued for an hour or more, only a fraction of the incorporated radioactive nucleotide is subject to rapid back-exchange (Fig. 1, lower curve). Other experiments have shown that the remaining bound isotope is only gradually removed by continued incubation with unlabeled ATP, indicating that true exchange of isotope into the firmly bound nucleotide of the F-actin has occurred.

To investigate the influence of myosin on the actin-nucleotide exchange, we incubated identical samples of F-actin with H^3ATP either in the presence or absence of myosin, under conditions where actomyosin superprecipitates (Fig. 2). Here again we see exchange in F-actin alone, with a time course

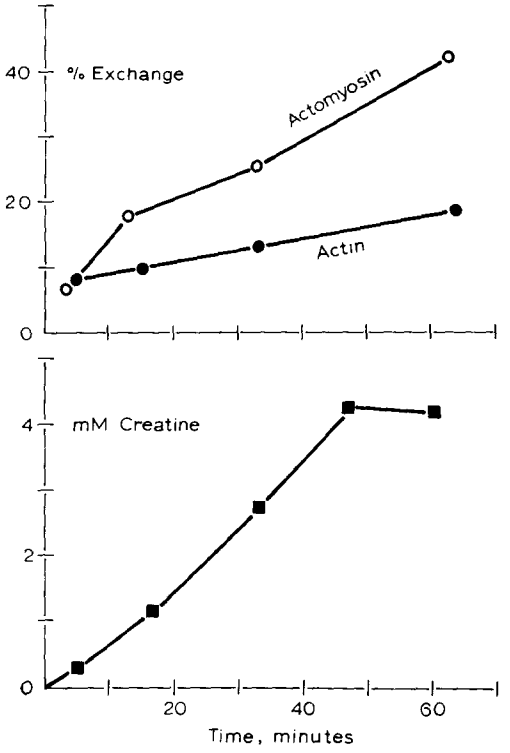

Figure 2. Bound nucleotide exchange in F-actin and actomyosin. Both mixtures contained 0.1 M KCl, 4 mM $MgCl_2$, 10 mM imidazole buffer, pH 7.0, 5 mM phosphocreatine, 50 ug/ml creatine kinase, 50 μM H^3ATP, and 0.1 mg/ml actin. Actomyosin mixture also contained 1 mg/ml myosin. Temperature, ca. 25°. Actin samples treated with Dowex-1 (Cl), then mixed with myosin (final conc. 1 mg/ml) and centrifuged for exchange analysis. Upper figure: incorporation of H^3-nucleotide. Lower figure: hydrolysis of phosphocreatine in actomyosin suspension.

similar to that shown in Fig. 1. In the actomyosin, on the other hand, the exchange of bound nucleotide was markedly greater, reaching nearly 50% at the end of the reaction. This increased exchange occurred entirely in the period following the initial rapid burst of exchange, whereas the magnitude of the initial burst was the same in actomyosin as in actin alone. This was true even though the actomyosin had completely superprecipitated before the time of the first exchange measurement.

In conclusion, we have shown that the bound nucleotide of F-actin is indeed exchangeable. On exposure to H^3ATP, there is an immediate burst of isotope incorporation, suggesting the presence of a certain number of rapidly-exchangeable "open" bound nucleotide sites in the actin. In the

course of time thereafter, additional exchange takes place at a slower steady rate, which may indicate a slow turnover of these "open" sites. The addition of myosin under conditions of superprecipitation does not increase the initial rapid burst of exchange but does greatly increase the steady rate of exchange thereafter. Hence, it appears that the formation of the actomyosin complex and the onset of superprecipitation do not cause the sudden appearance of any additional "open" actin-nucleotide sites; but the rate of turnover of these sites is greatly increased in superprecipitated actomyosin, suggesting that myosin does indeed affect the structural stability or conformation of the F-actin polymer.

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