The Rate-Limiting Step of the Protamine-Induced Adenosine Triphosphatase Activity of Adenosine Triphosphate–G-Actin

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The release of P₁ from the P₁-G-actin-ADP complex is the rate-limiting step in the ATPase activity that is shown by ATP-G-actin in the presence of protamine.

We have previously reported that, by interacting with protamine, ATP-G-actin is converted into ADP–G-actin (Magri et al., 1978a, b), and, by lowering the free Mg²⁺ concentration to 0.1 μM, G-actin is induced by protamine to work like an ATPase through the cyclic conversion of ATP–G-actin into ADP–G-actin (Magri et al., 1978b). We show here that the rate-limiting step of the overall reaction is the release of P₁ from the P₁-G-actin-ADP complex.

Materials and Methods

Rabbit muscle ATP–G-actin (myokinase-free) was prepared by the method of Spudich & Watt (1971). Actin concentration was measured from the absorbance at 290 nm, the absorbance of 1 mg of pure actin/ml (light-path 1 cm) being taken to be 0.62 (Gordon et al., 1976). Molar concentration of G-actin was calculated on the basis of a molecular weight of 42000 (Collins & Elzinga, 1975). Protamine sulphate from salmon (mol. wt. 9640) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Free Mg²⁺ and Ca²⁺ concentrations were calculated from a log(equilibrium formation constant) for the 1:1 chelate of 8.69 and 10.6 respectively, which refer to the most strongly basic form of EDTA: pK₄ and pK₅ of EDTA are 10.26 and 6.16 respectively (Schwarzenbach & Ackerman, 1947, 1948). The contributions of ATP and of G-actin were ignored because of the lower association constant (10⁶M⁻¹) of the ATP¹⁻–Mg²⁺ complex (O’Sullivan & Perrin, 1961) and the low concentration of G-actin.

P₁ production was determined by the method of Tashima & Yoshimura (1975) after precipitation of the protein with 0.1 M-trichloroacetic acid.

Conductivity measurements were performed with an apparatus consisting of a Wayne Kerr AF Signal S121 a.c. generator operated at 20 kHz, energizing a Wayne Kerr B221A universal bridge equipped with a conductivity cell (cell constant 1.5 cm). The departure from the electrical equilibrium was measured with a P.A.R. lock-in amplifier–detector equipped with a recorder. The lock-in sensitivity was 20 μV and the recorder sensitivity was 2.5 V/10 cm. In all the experiments the capacitive component of the impedance was found to be constant; changes in conductivity were thus related exclusively to changes in the resistance of the conductivity cell. To perform the measurements the bridge was first balanced in the absence of protamine in the reaction cell. Protamine was then added and the progressive departure of the bridge from balance, due to the increase in conductivity, was recorded. At the end of the experiment the bridge was balanced again to provide the final value for the conductivity. Owing to the very small imbalance of the bridge the readings in the imbalanced condition were linearly related to the change in conductivity.

The equivalence between the changes of conductivity and the hydrolysis of ATP was determined as follows. A solution containing 0.2 mm-ATP, 0.5 mm-EDTA and 50 μM-MgCl₂, adjusted to pH 7.0, was prepared (solution A). A sample (5 ml) of this solution was treated with myosin S-I and actin (40 μg/ml each) to hydrolyse ATP. At the end of the hydrolysis the solution was heated for 3 min at 100°C and then cooled, and the protein was removed by centrifugation (solution B). Conductivity of solution A (5 ml) was varied by the subsequent addition of 0.05 ml portions of solution B. The increase in conductivity was linearly related to the change of composition of the solution in the range 200–180 μM-ATP/20 μM-P₁. It was found that in this range of concentrations, at pH 7.0 and 23°C, the hydrolysis of 1 nmol of ATP/ml was accompanied by a conductivity increase of 0.01 mS.

Results and Discussion

The ATPase activity of G-actin, induced by protamine at low Mg²⁺ concentration, can be studied either by measuring, colorimetrically, the P₁ released after precipitation of the protein with trichloroacetic acid, or by following the increase of the conductivity of the solution due to the hydrolysis of ATP. There
Fig. 1. \textit{ATPase activity of ATP-G-actin induced by protamine}

The incubation mixtures (5 ml) contained 0.5 mm-EDTA, 20 μM-CaCl\textsubscript{2}, 50 μM-MgCl\textsubscript{2}, 200 μM-ATP, 5.7 μM-ATP-G-actin and 2 μM-protamine. The temperature was 23°C and the pH was 7.0. The reaction was started by the addition of protamine. Control experiments without protamine were also performed. The release of P\textsubscript{i} (○) and the increase of conductivity (●) were followed as described in the Materials and Methods section. Δ[P\textsubscript{i}] represents the difference between P\textsubscript{i} released in the complete system and in the control samples without protamine.

![Graph showing ATPase activity](image)

**Scheme 1. Interaction of ATP-G-actin with protamine**

Experimental details are given in the text. ●, Protamine; ○, actin.

is an initial rapid release of P\textsubscript{i} before the steady-state release is established (Fig. 1). As we have shown previously (Magri et al., 1978b), the rate of the rapid phase increases with the concentration of protamine, and its amplitude equals the initial concentration of ATP-G-actin. The rate of the hydrolysis of ATP, in the steady state, is 0.1–0.2 mol/min per mol of actin. In contrast, the conductometric assay shows only the steady-state rate of reaction (Fig. 1).

The P\textsubscript{i} data indicate that the hydrolytic transformation of ATP-G-actin into P\textsubscript{i}-G-actin-ADP is not the rate-determining step in the steady-state reaction. The absence of the initial rapid phase in the conductivity data therefore indicates that it is the release of P\textsubscript{i} from P\textsubscript{i}-G-actin-ADP that is rate-limiting in the steady state. It also follows that P\textsubscript{i} is released from P\textsubscript{i}-G-actin-ADP when it is treated with acid in the assay for the P\textsubscript{i}.

It was shown previously (Magri et al., 1978a,b) that protamine induces the formation of small nuclei of 3–4 molecules of ATP-G-actin, which are rapidly converted into ADP-G-actin (k = 0.045 s\textsuperscript{-1}).

When the actin present in the medium exceeds the ratio 3–4 mol/mol of protamine, the rate of the dephosphorylation decreases (k = 0.02 s\textsuperscript{-1}), but is still higher than the steady-state rate. It is thus clear that the exchange between the molecules of actin bound to protamine and those free in the medium is faster than the release of the hydrolytic products. Accordingly, the release of actin from protamine (Scheme 1, step 3) precedes the release of P\textsubscript{i} and ADP from actin (step 4). The formation of ATP-G-actin from actin and ATP (step 5) completes the cycle of the protamine-induced ATPase activity of G-actin.

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**References**


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