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

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The release of P_i from the P_i -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), and, by lowering the free Mg^{2+} concentration to $0.1\mu M$, 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 P_i from the P_i -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 290nm, the absorbance of 1 mg of pure actin/ml (light-path 1cm) 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 Mg^{2+} and Ca^{2+} 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: pK_4 and pK_3 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 ($10^5 M^{-1}$) of the ATP^{4-} - Mg^{2+} complex (O'Sullivan & Perrin, 1961) and the low concentration of G-actin.

P_i 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 20kHz, energizing a Wayne Kerr B221A universal bridge equipped with a conductivity cell (cell constant 1.5cm). 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 $20\mu V$ and the recorder sensitivity was 2.5V/10cm. 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 0.2mM-ATP, 0.5mM-EDTA and $50\mu M$ - $MgCl_2$, adjusted to pH7.0, was prepared (solution A). A sample (5ml) of this solution was treated with myosin S-1 and actin ($40\mu g/ml$ each) to hydrolyse ATP. At the end of the hydrolysis the solution was heated for 3 min at $100^\circ C$ and then cooled, and the protein was removed by centrifugation (solution B). Conductivity of solution A (5ml) was varied by the subsequent addition of 0.05ml 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 - P_i . It was found that in this range of concentrations, at pH7.0 and $23^\circ C$, 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 Mg^{2+} concentration, can be studied either by measuring, colorimetrically, the P_i 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. ATPase activity of ATP-G-actin induced by protamine

The incubation mixtures (5ml) contained 0.5mM-EDTA, 20μM-CaCl₂, 50μM-MgCl₂, 200μM-ATP, 5.7μM-ATP-G-actin and 2μM-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_i (○) and the increase of conductivity (●) were followed as described in the Materials and Methods section. Δ[P_i] represents the difference between P_i released in the complete system and in the control samples without protamine.

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_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 0.1–0.2mol/min per mol of

actin. In contrast, the conductometric assay shows only the steady-state rate of reaction (Fig. 1).

The P_i data indicate that the hydrolytic transformation of ATP-G-actin into P_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_i from P_i-G-actin-ADP that is rate-limiting in the steady state. It also follows that P_i is released from P_i-G-actin-ADP when it is treated with acid in the assay for the P_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 ($k = 0.045\text{s}^{-1}$).

When the actin present in the medium exceeds the ratio 3–4mol/mol of protamine, the rate of the dephosphorylation decreases ($k = 0.02\text{s}^{-1}$), 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_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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