Mechano-chemical energy transduction in biological systems

The effect of mechanical stimulation on the polymerization of actin: a kinetic study

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Mechanical stimulation (forced circulation in narrow tubing) accelerates as much as 10-fold the rate of polymerization of actin. The increase in the rate is proportional to the intensity of the stimulation for flow rates between 0 and 3 cm/s. This supports the hypothesis that a statistical factor (the orientation of the flowing particles) is influenced by the flow. Comparison of the kinetics of the polymerization of resting and of mechanically stimulated actin solutions shows that both the nucleation and the elongation steps are accelerated. It is thus concluded that flow orients not only the oligomeric structures but also the actin monomers. The elongation reaction, also in the flow-stimulated samples, occurs always by the addition of ATP-G-actin (or ATP-containing oligomers) and not by the fusion of ADP-containing oligomeric structures.

The polymerization of actin follows a quite complex mechanism, which is classically described by the following steps: nucleation, elongation, redistribution, association between F-actin particles (Arisaka *et al.*, 1975).

It is known that mechanical stimulation (rotation at a shear rate higher than 100 s^{-1} or repeated measurements of viscosity in a capillary viscometer) accelerates the G-F transformation of actin (Kasai *et al.*, 1960). More recently Boreido *et al.* (1981) have proposed that the acceleration by flow of polymerization of actin could be due to the conversion of circular nuclei of actin, not susceptible to elongation, into linear nuclei susceptible to elongation. They also stressed the significance of mechanical stimulation as a physiological regulatory factor of the polymerization of actin. Since we agree completely with the last proposition, we have re-investigated the phenomenon and have found that mechanical stimulation influences not only the elongation step but also the nucleation step by modifying a statistical factor (the orientation of the flowing particles).

Evidence has also been found that the elongation of the polymers takes place by the addition of ATP-G-actin (or ATP-containing oligomers) and not by the fusion of ADP-containing oligomers.

Materials and methods

G-actin from rabbit muscle (myokinase-free) was prepared as described by Spudich & Watt (1971).

Actin concentration was measured from the A_{290} , the absorbance of 1 mg of pure actin/ml (light-path 1 cm) being taken as 0.62 (Gordon *et al.*, 1976). Molar concentration of G-actin was calculated on the basis of a molecular weight of 42 000 (Collins & Elzinga, 1975). Inorganic orthophosphate was determined by the method of Tashima & Yoshimura (1975) after precipitation of the protein with 0.1 M-trichloroacetic acid. Viscosity was measured with an Ostwald viscometer (water flow time 60 s at 20°C).

Mechanical stimulation was obtained by forcing the actin solution successively up and down through the capillary of the viscometer under the pressure of 65 mmHg (8.7 kPa). The length of the capillary was 14 cm, and the diameter 0.44 mm. The flow rate was 0.66 ml/s. Alternatively the stimulation was performed by circulating, with the aid of a peristaltic pump, the actin solution in silicon-rubber tubing (1.1 mm diameter) at the rate of 8 cm/s. To do this the tubing was first filled with the actin solution by aspiration, the pump was stopped, the two ends of the tubing were connected and the pump was started again.

Results

Mechanical stimulation of actin accelerates both the nucleation and the elongation steps

The effect of mechanical stimulation on the polymerization of actin was initially studied in

20mM-KCl by comparing the increase in the viscosity of a resting solution with that of a solution which was previously forced (at a pressure of 65 mmHg) five times up and five times down through the capillary of the viscometer for 2 min. After this stimulation the specific viscosity of a 15 μ M (as the monomer) actin solution was increased by 0.06 to 0.1 viscosity unit, whereas in the resting solution the viscosity was practically unchanged (Fig. 1). This experiment also showed the following.

(1) Actin was not immediately susceptible to the mechanical stimulation, but required a preincubation of 10–15 min in 20mM-KCl solution. The lag, however, decreased with increasing concentration of actin (results not shown). This indicates that the species that accumulates is formed through a process of an order higher than first; it is thus a nucleation process (Kasai *et al.*, 1962).

(2) The increase in the specific viscosity was, under our conditions, a fairly good measure of the incorporation of the monomer into F-actin, and the elongation of the polymers in the mechanically

stimulated samples took place mainly through the incorporation of ATP-G-actin and not through a head-to-tail fusion of ADP-containing oligomeric structures. In fact the ratio between the concentration of the P_i released in the course of the polymerization and the increase in the specific viscosity of the solution was practically constant (37–41.4) both in the resting and in the mechanically stimulated samples and independently of the time of sampling. Furthermore, it was very close to the ratio found at complete polymerization: $15/0.39 = 38.4$.

We also performed the mechanical stimulation by circulating a solution of 15 μ M-ATP-G-actin in 20mM-KCl at the rate of 8 cm/s in tubing of 1.1 mm diameter. The polymerization was complete 6 min after the addition of KCl, a time shorter than the lag time in the resting conditions. (The different effects of the two systems of stimulation are partially explained by considering that in the tubing all the molecules of the solution are stimulated all the time, whereas in the viscometer the real time of stimulation is only a fraction of the total time and is given by:

$$\begin{aligned} & \text{Total time of stimulation} \times \frac{\text{volume of the capillary}}{\text{volume of the solution}} \\ &= 2 \text{ min} \times \frac{0.022 \text{ ml}}{0.6 \text{ ml}} = 0.073 \text{ min.} \end{aligned}$$

Fig. 1. Effect of mechanical stimulation on the polymerization of actin

The incubation mixtures contained 15 μ M-ATP-G-actin, 26 μ M-ATP, 0.2mM- CaCl_2 , 0.5mM-mercaptoethanol, 1mM- NaN_3 and 2mM-Tris/HCl buffer. pH was 7.9, temperature 22°C. Polymerization was started by the addition of 20mM-KCl. At the times indicated, samples of the solution were taken for determination of viscosity and of P_i content. These determinations were performed either immediately after collection (●, ▲) or after 2 min of mechanical stimulation in the capillary viscometer (see the Materials and methods section) (○, △). The intercepts between the continuous line and the broken lines indicate the times of collection of the samples that were submitted to the stimulation. ●, Specific viscosity of the resting mixture; ○, specific viscosity of the mixtures after 2 min of mechanical stimulation. P_i (μ M)/ η_{sp} , ratio of the P_i released to the increase in the specific viscosity in the resting samples (▲) and in the stimulated samples (△). ■, Specific viscosity of a sample after 6 min of stimulation at 8 cm/s in tubing of 1.1 mm diameter.

The shortening of the lag time showed that a sufficiently intense mechanical stimulation could accelerate not only the rate of the elongation of the polymers but also the rate of the conversion of G-actin into the nuclei on which the polymers were built. This point was proved when we succeeded in distinguishing the formation of the nuclei from the elongation step by finding conditions under which actin does not polymerize and is converted only into the nuclei. This was obtained by lowering the concentration of KCl to 7.5mM. Under these conditions, in the ATP-G-actin resting solution no P_i release or increase in viscosity or in light scattering was observed. Nevertheless nuclei were formed, since, when KCl was increased to 20mM and the actin solution was stimulated by flow for 2 min in 1.1mm-diameter tubing at the rate of 8 cm/s, polymerization started, with a rate depending on the preincubation time in 7.5mM-KCl (Fig. 2).

When the preincubation in 7.5mM-KCl was performed in flow (8 cm/s, tubing diameter 1.1 mm), the process occurring in this phase was accelerated 10-fold compared with the same process occurring in rest.

Fig. 2. Effect of preincubation in 7.5 mM-KCl on the mechanically stimulated polymerization of ATP-G-actin in 20 mM-KCl

(A) Preincubation in rest. The incubation mixtures contained $38\mu\text{M}$ -ATP-G-actin, 7.5 mM-KCl, $65\mu\text{M}$ -ATP, $65\mu\text{M}$ -CaCl₂, 0.5 mM-mercaptoethanol and 2 mM-Tris/HCl buffer. pH was 7.8, temperature 22°C. At the times indicated, samples were taken, actin was diluted to $15\mu\text{M}$ with identical buffer, KCl concentration was immediately raised to 20 mM, the sample was stimulated by 2 min flow at 8 cm/s in 1.1 mm-diameter tubing and viscosity was measured immediately thereafter. In separate experiments it was shown that the increase in viscosity was approximately proportional to the time of stimulation up to 2 min. ●, Specific viscosity. (B) Preincubation in flow. Experimental conditions were the same as in (A), except that the preincubation in 7.5 mM-KCl, instead of being in rest, was performed in flow, in 1.1 mm-diameter tubing, at the rate of 8 cm/s. ○, Specific viscosity.

The initial rate of the polymerization is directly proportional to the rate of the flow

To elucidate the mechanism of action of the mechanical stimulation, we investigated the relationship between the rate of polymerization and the rate of flow. For this, $17\mu\text{M}$ -ATP-G-actin in 20 mM-KCl was stimulated by flowing the solution for 1, 4 or 6 min at different rates in tubing of 1.1 mm diameter. As shown in Fig. 3, the increase in the specific viscosity of the solution was proportional to the flow rate up to about 70% of the final viscosity value.

This means that the principal effect of the flow is to orient the actin particles. This is because, at the low velocity gradients used in these experiments, friction is the only significant orienting force, and its intensity is proportional to the flow rate.

Discussion

The mechanical stimulation accelerates the elongation of the actin polymers as well as the formation

Fig. 3. Direct proportionality between the rate of flow and the rate of polymerization

The incubation mixtures (0.9 ml) contained $17\mu\text{M}$ -ATP-G-actin, $40\mu\text{M}$ -ATP, $40\mu\text{M}$ -CaCl₂, 0.5 mM-mercaptoethanol, 1 mM-Na₂S₂O₄ and 2 mM-Tris/HCl buffer. pH was 7.9, temperature 22°C. Polymerization was started by the addition of 20 mM-KCl. Immediately after this addition, the incubation mixtures were made to circulate for 1 (○), 4 (●) or 6 (▲) min in tubing of 1.1 mm diameter at the flow rates indicated, and the viscosity of the solution was determined immediately thereafter.

of the nuclei, in agreement with the demonstration by Boreido *et al.* (1981) that flow decreases the critical concentration for actin polymerization. The rate of the overall process of polymerization is proportional to the intensity of the stimulation. This confirms the view that a statistical factor (the orientation of the flowing particles) is influenced in the process. It is also clear that, in order to accelerate the formation of the nuclei, flow must be able to orient also the actin monomers. There is a large discrepancy in the literature about the axial ratio of these molecules. Axial ratios as large as 10:1 were reported for the monomer in solution (Straub, 1942; Tsao, 1953). The axial ratio, however, is likely to be substantially smaller. A value of 5.5 to 3.3 was found in electron-microscopic observations (Dos Remedios & Dickens, 1978). Our experiments, in any case, indicate that the actin monomers are oriented by moderate flow (0–8 cm/s). We show also that the elongation reaction takes place mainly by the addition of ATP-G-actin (or ATP-containing linear oligomers) and not by the fusion of ADP-containing oligomers. The formation of linear polymers, which presumably would still contain ATP, was postulated (Oosawa & Kasai,

1962), but, as far as we know, no experimental proof of their existence has yet been obtained.

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