STUDIES ON THE POLYMERISATION OF ACTIN: A RAPID METHOD FOR THE SEPARATION OF THE MONOMERIC FROM THE POLYMERIC SPECIES

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1. Introduction

A main difficulty in the study of the kinetics of actin polymerisation is the time consuming procedure available for the separation of the monomeric from the polymeric species [1].

We have now found that G-actin and F-actin can be separated by filtration through 0.45 μm millipore filters. The procedure is very rapid and suitable to handle samples of very small volume. We expect this observation to provide a large impulse on the studies of the mechanism of actin polymerisation.

2. Experimental

Rabbit muscle ATP–G-actin was prepared as in [2]. The incubation mixtures contained G-actin from 0.15–0.6 mg/ml, 0.1 mM ATP, 0.1 mM $CaCl_2$, 0.5 mM mercaptoethanol, 0.2 mM NaN_3 and 2 mM Tris–HCl buffer (pH 7.9). Filtration was performed, by aspiration, through standard, white, plain, millipore filters, 0.45 μm pore size. Sample volume was usually either 1.5 ml (filter diam. 25 mm) or 0.2 ml (filter diam. 8 mm). Viscosity was measured with an Ostwald viscosimeter maintained at 20 ± 0.1 °C. The 90° light scattering intensity was measured with a fluorimeter (Perkin-Elmer MPF-3L) at 546 nm. The instrument was calibrated by measuring the scattering intensity of solutions of actin polymers of known weight concentration. Protein was determined by the Lowry method [3]. G-actin was employed as the standard.

3. Results and discussion

When either G-actin or F-actin solutions are filtered through 0.45 μm millipore, the polymeric species is retained while the monomeric species is found in the filtrate. The different behaviour does not depend on the nature of the medium (low and high ionic strength for G- and F-actin solutions, respectively). This was shown by comparing, in the course of the polymerisation, the amount of protein retained by the filter with the amount of polymer formed as monitored by the increase of either the relative viscosity or the intensity of the light scattering of the solution (fig.1). It is known that light scattering is a more sensitive tool, as

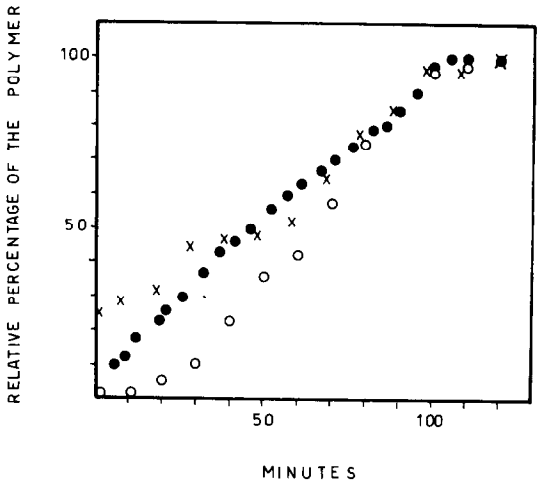

Fig.1. Actin polymerisation followed by millipore filtration. Polymerisation was started by the addition of 0.1 M KCl to a solution (pH 7.9) containing G-actin, 0.5 mg/ml; temp. 20°C. At time intervals samples were taken for filtration and for viscosity (η) and light scattering intensity (I) measurements:

$(I_t - I_o/I_{120'} - I_o) \times 100$ (•); $(\eta_t - \eta_o/\eta_{120'} - \eta_o) \times 100$ (○);

$$\frac{\text{(Total protein} - \text{Protein in the filtrate)}}{\text{Total protein}} \times 100 \text{ (x).}$$

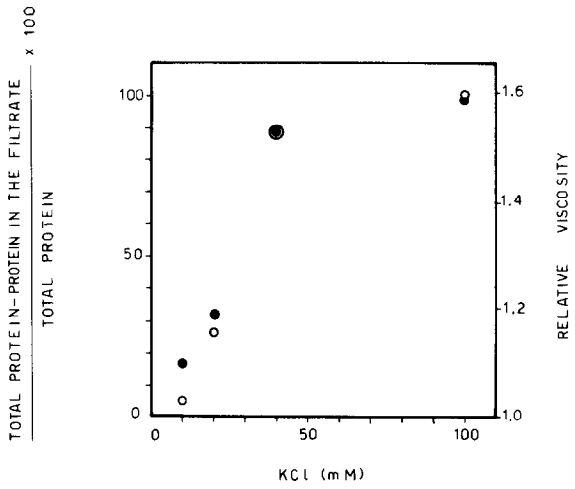

Fig.2. Actin polymerisation at different concentrations of KCl. Polymerisation was started by the addition of KCl, at the concentration indicated in the figure, to a solution (pH 7.9) containing G-actin (0.6 mg/ml); temp. 20°C. After 90 min incubation samples were taken for filtration and for viscosity measurements. Relative viscosity (○):

$$\frac{\text{(Total protein} - \text{Protein in the filtrate)}}{\text{Total protein}} \times 100\ (\bullet).$$

compared to viscosity, to detect incipient polymerysation. Filtration, however, appears to be even more sensitive. The most likely explanation of our data is that even small nuclei of actin are retained by the filter.

Similar results were obtained when the monomer-to-polymer ratio was altered by changing the concentration of the polymerising agent (fig.2).

Millipore filtration is also suitable to determine the critical concentration of actin. This is shown in fig.3 where the amount of protein present in the filtrate is constant for F-actin at 0.15–0.58 mg/ml.

The selectivity of the filter (not shown) is not altered by either changing the pH of the medium from 6.0–8.3 or by employing 1 mM $MgCl_2$ instead of 0.1 M KCl as the polymerising agent.

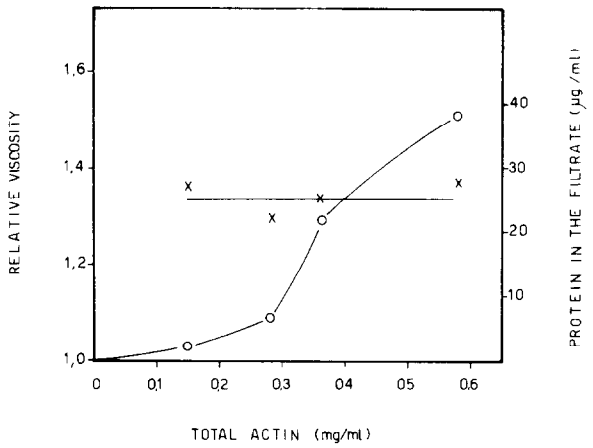

Fig.3. Determination of the critical concentration of actin by the millipore filtration method. Polymerisation was started by the addition of 0.1 M NH_4Cl to G-actin solutions (pH 7.9) at the concentrations indicated; temp. 20°C. The incubation was continued until no further increase of the viscosity was occurring. At that time samples were taken for filtration. Relative viscosity (○); protein in the filtrate (μg/ml) (x).

Acknowledgement

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References

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