CRYSTALLIZATION OF NATIVE STRIATED-MUSCLE ACTIN

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1. Introduction

Since the amino acid sequence of skeletal-muscle actin has already been established [1] the next step in the study of actin conformation should be an X-ray diffraction analysis, to elucidate its three-dimensional structure. So far, however, the numerous difficulties involving the crystallization of actin have not been overcome. The crystallization of proteins is most commonly obtained from ammonium sulfate but the addition of this salt increases the ionic strength of the medium and brings actin to its fibrous form. As the fibrous form of actin cannot crystallize, investigations have been carried out concerning means to prevent polymerization. For skeletal-muscle actin, various chemical modifications of one amino acid residue were performed [2–4]. The inhibition of polymerization has also been effected by the addition of another component [5] and in this way crystals of a three-component complex could be obtained with a non-muscle actin [6].

Adopting a different approach, we have attempted to crystallize non-modified muscle actin by using, as crystallization reagent, polyethylene glycol, which does not increase the ionic strength of the actin solution.

2. Experimental procedure

2.1. *Materials*

Rabbit skeletal-muscle F-actin was obtained by the KI-extraction procedure from myosin-removed myofibrils, as described by Maruyama et al. [7]. The desensitization of myofibrils and the depolymerization of F-actin to G-actin were carried out as already mentioned [8].

Polyethylene glycol 6000 was purchased from Touzart and Matignon (France) and not further treated.

2.2. *Methods*

Crystallization was performed in tubes containing different concentrations of PEG and 1 ml of G-actin in its depolymerization buffer, Tris 2 mM, $CaCl_2$ 0.2 mM, ATP 0.1 mM, pH 7.5, sodium azide 2 mM. For G-actin solution, the various concentrations employed were from 3–7 mg/ml and the PEG concentrations varied from 4–14% (w/v).

Before the addition of G-actin, PEG was dissolved in the depolymerization buffer of actin or in 10 mM sodium acetate buffer, pH 5.0–5.6.

The tubes were stored at 4–5°C, and the crystallization process was followed weekly under the microscope.

SDS–gel electrophoresis was performed using the method of Neville [9]. Acrylamide concentration of the separation gel was 11%, pH 9.50. The gels were stained with Coomassie Blue.

3. Results and discussion

3.1. *Conditions of crystallization*

The crystallization was carried out on KI-extracted actin because, as we have shown, the actin obtained by this method has a more stable conformation than that extracted from muscle acetone powder [8].

Crystals were obtained from all PEG solutions between 4–14%. They were found in PEG solutions at pH 7.5 and at pH 5.0–5.6. The biggest crystal

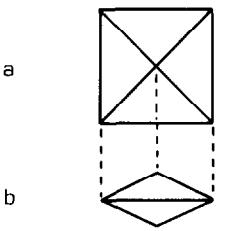

Fig.1. a, b. Shapes of rabbit skeletal-muscle actin crystals.

obtained so far was found in a solution containing 6 mg/ml of G-actin and 13% of PEG at pH 5.6.

Because of the very long crystallization time, it was necessary to add sodium azide to the solution in order to prevent bacterial growth. Indeed, actin is subject to bacterial breakdown, which gives a 37 000 dalton polypeptide, as also noted by Pollard [10].

3.2. *Size and shape of crystals*

The first crystals, of 0.02 mm, could be observed after three or four weeks and a growth up to 0.100 mm required three months.

At low magnification, most of the crystals appeared as bright squares with two diagonal bars (fig.1a) and a few as flat lozenges (fig.1b). These two shapes correspond to two different positions of the same crystal which is a flat square double pyramid, as can be seen from the photograph (fig.2).

3.3. *Identification of crystals as actin*

To check the identity of the crystals, they were picked out of the medium and washed four times in 20% polyethylene glycol. SDS–gel electrophoresis performed on these washed crystals gave a single band corresponding to actin. Both the washed crystals and the crystallization solution were free of any degradation product (fig.3).

4. Conclusion

This work demonstrates that striated-muscle actin can be crystallized without any chemical modification or the addition of any component. However, a major difficulty of the method is the slow growth of the crystals. Preliminary studies suggest that the crystallization process can be accelerated by vapor diffusion.

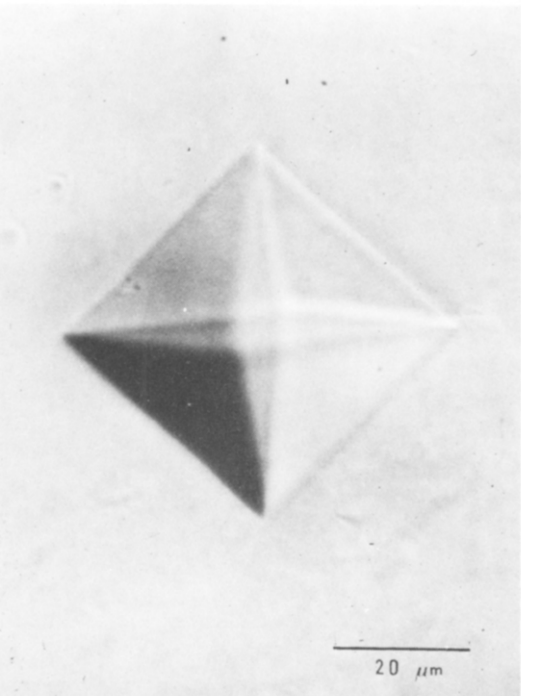

Fig.2. Photograph of actin crystal taken with a phase contrast Zeiss microscope. The lateral incidence of the light was obtained by a slight displacement of the condenser.

Acknowledgements

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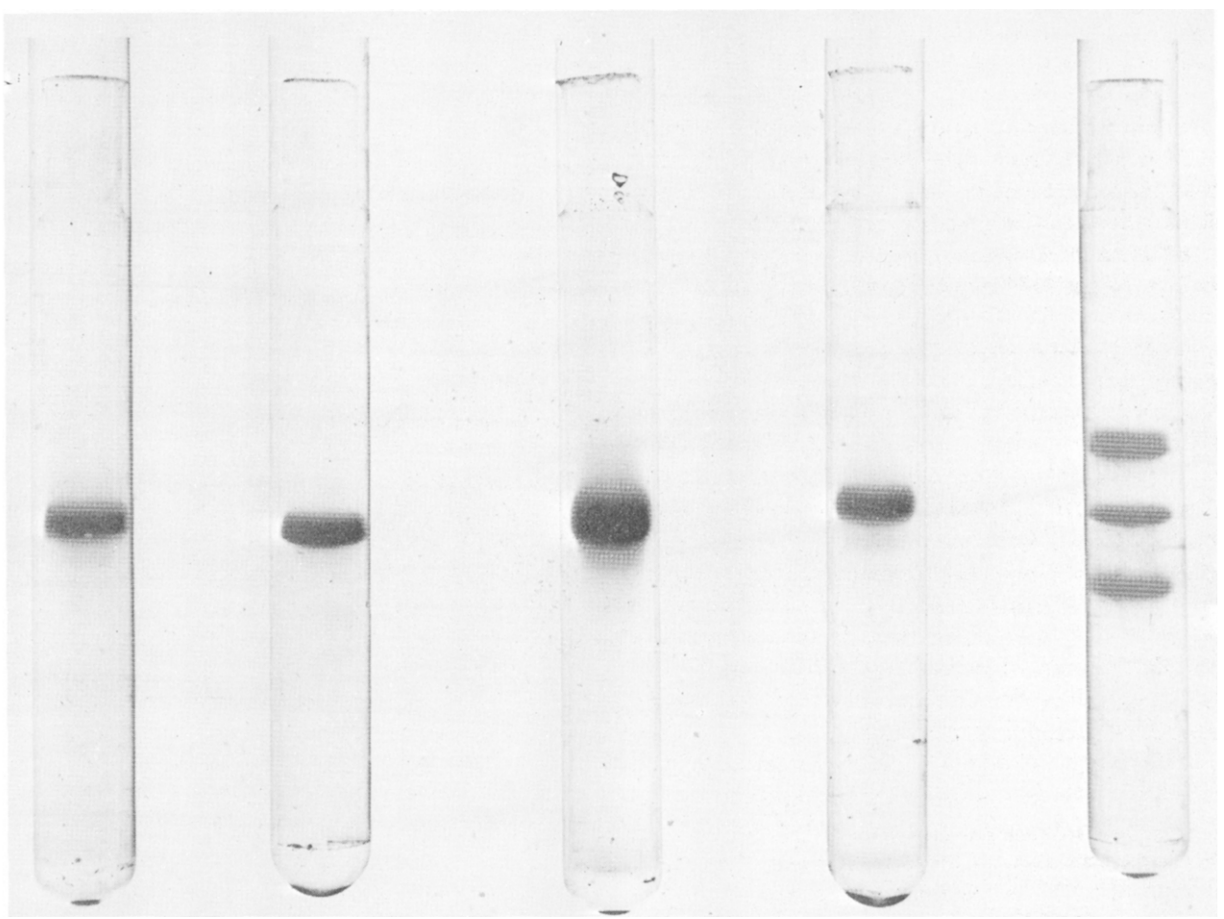

Fig.3. SDS–polyacrylamide gel electrophoresis. (1) KI-extracted actin. (2) Washed actin crystals. (3) Washed crystals plus freshly prepared actin marker. (4) Actin left in the supernatant after crystallization. (5) Markers of tubulin (56 000 and 54 000 daltons) actin (42 000 daltons) and glyceraldehyde phosphate dehydrogenase (36 000 daltons).