A Study of Aggregation Phenomena Occurring in Actin Solutions during Depolymerization

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In cardiac muscle (Burch & Sohal, 1969), as in skeletal muscle, the thin filaments of actin and troponin are inserted centrally at the Z-line (Knappes

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& Carlsen, 1962), and interdigitate distally between the thick myosin filaments. It would therefore appear from the structural location of the thin filaments that the properties of the actin fibre may provide an essential link between the action potential and the

Fig. 1. Molecular-weight calibration of 7.5% polyacrylamide gel, 2.4% cross-linked with methylenebisacrylamide, and containing 0.78% end-chain NNN'-tetramethylethylenediamine relative to acrylamide

The buffer solution was 5mM-tris-38mM-glycine, pH8.1. In addition the upper-reservoir buffer (250ml) contained 0.25ml of 0.5% (w/v) Bromophenol Blue in 1% (v/v) acetic acid. The log(molecular weight) is plotted against the distance of migration, measured as the percentage of the distance run by the Bromophenol Blue tracker band (T). ○, Standard proteins of known molecular weight; ●, actin, the numbers indicating the calculated number of units in each oligomer.

mechanical response. As F-actin is composed of two strands, actin-actin interactions in the fibre comprise both horizontal pairing and vertical stacking, which gives rise to polymerization. The kinetics of F-actin formation (Asakura *et al.*, 1963) suggest intermediary forms of actin. Studies on Ca^{2+} exchange in F-actin and G-actin show that the structure of the unit undergoes conformational changes, which in the fibre tightly binds Ca^{2+} , although in the more open globular

actin Ca^{2+} is freely exchangeable (Kuehl & Gergely, 1969). It has been postulated that during excitation-contraction coupling the actin fibre locally and for a short period behaves as though it were G-actin (Szent-Gyorgyi & Prior, 1966). In the present paper some properties of actin aggregation phenomena in depolymerizing media, indicative of such conformational changes, are reported.

Actin was prepared from fresh dog hearts by using

Fig. 2. Electrophoresis of depolymerized actin solutions

A Joyce-Loebl automatic recording double-beam microdensitometer was used to measure the intensity of the stained protein bands in the polyacrylamide gel. (a) Comparison of G-actin purified by dialysis against 0.1 mM- Na_2CO_3 , pH 7.4 (lower trace), with the same preparation after addition of dilute salt, 10 mM-KCl-0.1 mM- MgSO_4 , for 10 min; 300 μg of protein was used. (b) Resuspension of cardiac F-actin in 0.2 mM-ATP-0.2 mM- CaCl_2 , pH 7.4, for 30 min; 200 μg of protein was used. (c) As for (b), except that the resuspension time was 18 h.

the technique of Barany *et al.* (1957) for the extraction and purification of actin. The method of Lowry *et al.* (1951) was employed for the determination of protein; dry freeze-dried bovine serum albumin was used as a reference standard. In order to study the process of depolymerization of the actin structure, the F-actin pellet was resuspended in depolymerizing media, pH 7.4, containing 0.2 mM-ATP, at a protein concentration in the range 5–8 mg/ml, for various times ranging from 10 min to 18 h. Pure G-actin was obtained by overnight dialysis of the F-actin solution at 4°C, against 0.1 mM- Na_2CO_3 pH 7.4. After centrifugation of the protein samples to remove insoluble material, up to 500 μg of protein was applied to polyacrylamide gels in volumes of 30–100 μl . The method of electrophoresis in a discontinuous buffer system was essentially based on the theory of Ornstein and Davis, as modified by Clarke (1964), where only the small-pore gel was used. The samples were run at constant current, of 3 mA/tube, and 200 V for 90 min.

In order to measure the molecular weight of the minimum reacting unit of the actin-actin interactions, a graph of $\log(\text{molecular weight})$ was plotted against the distance travelled relative to the Bromophenol Blue tracker band, as shown in Fig. 1. Fig. 1 demonstrates that, under these conditions, separation of the proteins is according to molecular size. The calibration plot was found to be reproducible for globular actin and the oligomeric forms of actin to an accuracy of $\pm 1.5\%$, although there was some deviation from linearity, perhaps as a result of charge density, above 500 000 and below 40 000 molecular weight. No artifacts were found that would have been caused by residual ammonium persulphate oxidation. The scanning absorbance at 650 nm gave a quantitative value for the peak intensity of each protein band.

Resuspensions of F-actin in 0.1 M-KCl–1 mM- MgSO_4 gave electrophoretic runs in which protein was incorporated only at the surface of the gel. A preparation of globular (G) actin is shown in Fig. 2(a) (lower trace). This consists of a single fast-moving peak and a small dimer peak. G-actin migrated at 69.5% of the tracker-band distance from the origin (T), which from the calibration graph was equivalent to a molecular weight of $47\,000 \pm 900$ s.d. (25); this agrees well with published values for the molecular weight of G-actin (Rees & Young, 1967; Johnson & Perry, 1968; Kuehl & Gergely, 1969).

On the addition of salt to a concentration of 10 mM-KCl–0.1 mM- MgSO_4 and allowing the mixture to stand for 10 min, this component partially polymerized, and gave a series of peaks (Fig. 2a, upper trace). The intensity of the G-actin peak has been diminished and the oligomers 2–7 have appeared. It was found that each peak was an integral number times 47 000 molecular weight; the dimer migrated at

50.5%T, which corresponded to a molecular weight of $94\,000 \pm 1800$ s.d. (25), and the trimer migrated at 40.3%T, calculated to correspond to a molecular weight of $141\,000 \pm 2700$ s.d. (25), as shown in the calibration graph.

No evidence of a smaller unit was found, even in solutions of 12 M-urea or 5 M-guanidinium chloride, that would correspond to a single chain of molecular weight 28 000 (Adelstein *et al.*, 1963; Mihashi & Ooi, 1965). An electrophoretic run producing only one G-actin peak is at variance with the results of Krans *et al.* (1965), who suggested that actin produces inactive artifacts that are attributable to the electrophoretic process. If this were indeed happening, then the resolution between the actin oligomers would be lost, and it would not be possible to obtain pure G-actin electrophoretically.

Resuspensions of actin in 0.2 mM-ATP–0.2 mM- CaCl_2 for longer periods show evidence of equilibria between higher oligomers of actin. In Fig. 2(b), where the actin pellet was resuspended for 30 min, the G-actin peak has almost disappeared and the most intense peak is that of the trimer.

Fig. 2(c), in which resuspension was for 18 h, shows high-molecular-weight actins, and the presence of a minimum peak-intensity position corresponding to the tetramer.

A comparison of these results shows that an equilibrium between intermediary forms of actin can occur, and that this can be displaced to favour different conformational states of actin-actin interactions.

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- Adelstein, R. S., Godfrey, J. E. & Kielley, W. W. (1963) *Biochem. Biophys. Res. Commun.* **12**, 34–38.
 Asakura, S., Taniguchi, M. & Oosawa, F. (1963) *J. Mol. Biol.* **7**, 55–69.
 Barany, M., Barany, K. & Guba, F. (1957) *Nature (London)* **179**, 818–819.
 Burch, G. E. & Sohal, R. S. (1969) *Amer. Heart J.* **78**, 358–368.
 Clarke, J. T. (1964) *Ann. N.Y. Acad. Sci.* **121**, 428–436.
 Johnson, P. & Perry, S. V. (1968) *Biochem. J.* **110**, 207–216.
 Knappeis, G. G. & Carlsen, F. (1962) *J. Cell Biol.* **13**, 323–335.
 Krans, H. M. J., Van Eijk, H. G. & Westenbrink, H. G. K. (1965) *Biochim. Biophys. Acta* **100**, 193–201.
 Kuehl, W. M. & Gergely, J. (1969) *J. Biol. Chem.* **244**, 4720–4729.
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
 Mihashi, K. & Ooi, T. (1965) *Biochemistry* **4**, 805–813.
 Rees, M. K. & Young, M. (1967) *J. Biol. Chem.* **242**, 4449–4458.
 Szent-Gyorgyi, A. G. & Prior, G. (1966) *J. Mol. Biol.* **15**, 515–538.