The influence of poly(ethylene glycol) 6000 on the properties of skeletal-muscle actin

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Poly(ethylene glycol) 6000 affected many of the properties of skeletal-muscle actin. It accelerated the rate and increased the extent of actin polymerization as measured by light-scattering and sedimentation studies respectively. Moreover, intrinsic-fluorescence measurements showed that addition of poly(ethylene glycol) 6000 decreased the rate of EDTA-induced denaturation of actin monomer and increased the temperature at which irreversible conformational changes occur in actin monomer. These effects occurred without any apparent direct binding interaction and are postulated to be a consequence of the effect of excluded volume on the thermodynamic activity of actin. A relationship based on spherical geometry was formulated which described the co-volume increment that occurs upon addition of a monomer to a long linear polymer in the presence of a space-filling macromolecule. The application of this relationship to the poly(ethylene glycol) 6000–actin system was not without assumption, but it permitted quantitative estimation of the co-volume increment which proved to be of the sign and magnitude required to explain the increased extent of actin polymerization found experimentally in the presence of various concentrations of poly(ethylene glycol) 6000. It is suggested that, *in vivo*, excluded volume may play a role in actin-filament formation and in the maintenance of the native G-actin structure.

Actin is found in most, if not all, eukaryotic animal cells and is thought to be important in many forms of cellular motility and in the maintenance of cellular morphology (Pollard, 1976; Clarke & Spudich, 1977; Korn, 1978; Taylor & Condeelis, 1979). The native form of actin monomer, or G-actin, contains a single tightly bound bivalent cation and ATP or ADP (Oosawa & Kasai, 1971). Removal of either the nucleotide or bivalent cation leads to a non-polymerizable form of actin (Oosawa & Kasai, 1971). *In vitro*, the polymerization of monomeric G-actin to filamentous F-actin, induced by millimolar concentrations of bivalent cations or physiological ionic strengths, has been described as a nucleation–condensation-type reaction (Kasai, 1969; Kasai *et al.*, 1962b; Oosawa & Kasai, 1962, 1971). In this process a small rate-limiting actin

nucleus is initially formed, which then elongates in a bi-directional manner to produce a long, ADP-containing, double-helical F-actin filament. The incorporation of actin monomer into polymer is usually associated with the hydrolysis of ATP, but these two events are not strictly coupled (Cooke, 1975; Pardee & Spudich, 1982). A steady state is finally achieved when the net addition equals the net dissociation of monomers at the two ends of the actin filament. The concentration of monomeric actin present at steady state is called the ‘critical actin monomer concentration’; above this value the concentration of F-actin increases markedly with the total concentration of actin.

The dynamic equilibrium between actin monomer and polymer *in vitro* is also found in some non-muscle cells (Carlsson *et al.*, 1979). However, *in vivo*, actin is excluded from a large volume of solution by high concentrations of other non-inter-active proteins. Volume exclusion increases the thermodynamic activity of a species in solution,

Abbreviations used: PEG-6000, poly(ethylene glycol) 6000; G-buffer, 2 mM-Tris/200 μ M-ATP/200 μ M-CaCl₂/1.5 mM-NaN₃ (pH 8.0)/0.5 mM-2-mercaptoethanol.

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which in turn may influence chemical equilibria involving that species (Minton, 1981). In order to arrive at an understanding of the various influences on actin polymerization *in vivo*, volume exclusion must be considered. In view of this we have investigated the effect of the space-filling polymer poly(ethylene glycol) 6000 (PEG-6000) on the properties of rabbit skeletal-muscle actin *in vitro* and the results are described in the present paper.

Experimental procedures

All reagents were of analytical grade with PEG-6000 being purchased from Fluka and ATP from Sigma. All solutions were made from deionized distilled water.

Actin purification

Rabbit skeletal-muscle actin was purified as previously described (Tellam & Frieden, 1982). The G-actin was either used immediately after Sephadex G-150 chromatography or stored by freeze-drying in the presence of sucrose (3 mg/mg of actin) at -20°C . Monomeric G-actin was prepared from freeze-dried material by extensive dialysis against G-buffer over a 2-day period at 4°C . This actin was subsequently centrifuged (165 000 g; 3 h; 4°C ; 50 Ti rotor) to remove any polymeric actin.

PEG-6000 solutions

A stock solution of PEG-6000 (200 g/litre) was prepared in G-buffer adjusted to pH 8.2. The final pH of the concentrated PEG-6000 solution was then 8.0. The number-average molecular weight of PEG-6000 was 8000 (Atha & Ingham, 1981).

Protein determination

G-actin concentrations were measured spectrophotometrically at 290 nm by using the value $A_{1\text{cm}}^{1\%} = 6.3$ (Houk & Ue, 1974) and mol.wt. 42 000 (Elzinga *et al.*, 1973). It was found that there was little or no influence of PEG-6000 (100 g/litre) on the absorbance of actin corrected for light-scattering. Low concentrations of actin were measured by using the Bradford (1976) protein assay with G-actin or G-actin plus PEG-6000 as standards. PEG-6000 has a small effect on the colour development with G-actin in this assay, which never amounted to more than 5% of the total absorbance being measured and was compensated for by inclusion of the same concentration of PEG-6000 in the G-actin standards.

Actin polymerization as measured by light-scattering

Freshly centrifuged G-actin in G-buffer (or G-buffer plus PEG-6000) was thermostatically equilibrated to 25°C for 10 min in a Perkin-Elmer 3000 spectrofluorimeter with excitation and emission

monochromators set at 450 nm. At the end of this period any trapped air bubbles were dislodged by gentle inversion of the fluorescence cuvette. The actin polymerization was initiated by the introduction of a small portion of concentrated MgCl_2 (either 1.0 M or 0.1 M) into the actin solution, followed by four gentle inversions of the cuvette to ensure complete mixing. The resulting increase in the light-scattering intensity was monitored continuously until no further change occurred. The initial light-scattering intensity of the G-actin solution (in the presence or absence of PEG-6000) was subtracted from all measurements.

Although the light-scattering intensity at 450 nm of long (more than 50 nm) F-actin filaments is independent of filament length and proportional to the number of monomers in the filament, shorter (less than 50 nm) filaments have a light-scattering intensity that is dependent on filament length and mass (Wegner & Engel, 1975). Thus, during the early stages in the actin polymerization process when filaments are short, the light-scattering intensity per unit mass of filamentous actin will be different from that at the latter stages of the polymerization, where the filaments are very long (Kawamura & Maruyama, 1970). As a consequence, quantitative interpretation of the kinetics of actin polymerization by changes in light-scattering intensity is rendered difficult. Further, refractive-index changes of the solution in the presence of PEG-6000 may also complicate any analysis. However, the method has been shown to be a reasonable approximation in the monitoring of the actin-polymerization process (Wegner & Engel, 1975).

Determination of the critical actin monomer concentration

Duplicate G-actin solutions, in the presence of various concentrations of PEG-6000, were polymerized for 12 h at 25°C by the addition of small volumes of concentrated MgCl_2 . Samples (200 μl) were centrifuged in a Beckman Airfuge (178 000 g) for 60 min at 25°C . Portions (10–50 μl) of the supernatant were slowly and carefully removed so as not to disturb the F-actin pellets. The actin concentration in these samples was then measured by the Bradford (1976) protein assay and duplicate measurements were subsequently averaged.

The amount of non-polymerizable actin was estimated by polymerizing actin at six concentrations (2.36, 3.43, 5.20, 6.86, 8.98, 11.35 μM) with 5 mM- Mg^{2+} /0.1 M-KCl and then measuring the concentration of actin in the supernatant of these centrifuged samples at 25°C . Under these conditions the critical actin monomer concentration is less than 0.2 μM and is effectively independent of the total actin concentration (Detmers *et al.*, 1981). Thus the

dependence of the supernatant protein concentration in these samples on total actin concentration gives the fraction of non-polymerizable actin. Generally this amounted to 3–5% of the total actin concentration. The reported critical actin concentrations are the differences between the concentrations of protein in the supernatants of centrifuged samples and the non-polymerizable actin concentration.

Intrinsic fluorescence

Measurements of G-actin intrinsic fluorescence in 1 cm-path-length quartz fluorescence cuvettes were performed in a thermostatically controlled Perkin-Elmer 3000 spectrofluorimeter with excitation at 280 nm and emission at 325 nm. The kinetics of the EDTA-induced change of the intrinsic fluorescence of G-actin in G-buffer have been measured at 25°C. The initial fluorescence intensity was subtracted from all measurements and the results normalized to the change observed in the absence of PEG-6000.

Thermal denaturation of G-actin (in G-buffer) was monitored by changes in the intrinsic fluorescence with increasing temperatures. The temperature was directly measured by using a Yellow Springs Instrument Co. thermometer and thermocouple probe. At the high concentrations of PEG-6000 used in these experiments there was a significant contribution to the total fluorescence signal from background scatter, which was largely independent of temperature. The fluorescence intensity of solutions of G-actin in the presence of PEG-6000 were corrected for this scattering. The intrinsic fluorescence spectra of G-actin and G-actin with PEG-6000 (200 g/litre) corrected for light-scattering were not further corrected for variation of instrument sensitivity with wavelength.

Results

Actin polymerization

The change in the light-scattering intensities of actin solutions (17.3 μM) in the presence of various concentrations of PEG-6000 after making the solutions 1 mM in Mg^{2+} are shown in Fig. 1. The change in light-scattering intensity is a measure of the actin-polymerization process, as evidenced by the characteristic lag period in the kinetics of the process and the formation of highly viscous solutions (Oosawa & Kasai, 1971). The concentrations of PEG-6000 used in the present studies had no visible effect on the solubility of actin. In the absence of Mg^{2+} there was no time-dependent change of the light-scattering intensity of a solution of G-actin nor of a solution of G-actin plus PEG-6000 (80 g/litre) (results not shown). It is clear from Fig. 1 that, in the presence of 1 mM- Mg^{2+} , actin polymerization is progressively accelerated by in-

Fig. 1. Influence of PEG-6000 on the polymerization of actin

G-actin (17.3 μM) was polymerized at 25°C with 1 mM- Mg^{2+} in the presence of the following concentrations of PEG-6000 in G-buffer: 0 g/litre (●); 20 g/litre (▲); 40 g/litre (△); 80 g/litre (○). The change in light-scattering intensity was measured at 450 nm.

creasing concentrations of added PEG-6000; specifically, increasing the concentration of PEG-6000 decreased the lag period, increased the maximal rate, and increased slightly the final extent of polymerization. Additional information of the effect of PEG-6000 on the polymerization of actin may be gained by determining its influence on the critical actin monomer concentration. As Oosawa & Kasai (1971) have shown, this critical actin monomer concentration at steady state with regard to F-actin mass, is inversely related to the apparent net association equilibrium constant for the addition of monomer to the F-actin filament. Thus measurement of the critical actin concentration in the presence of PEG-6000 provides a means of monitoring the effect of PEG-6000 on the apparent equilibrium between monomer and polymer.

In view of the difficulties in examining the effect of PEG-6000 on the steady state between actin monomer and polymer via the final light-scattering extents of polymerization, we have directly measured, by ultracentrifugation, the critical actin monomer concentration as a function of PEG-6000 concentration. The results are shown in Fig. 2, for which an actin concentration (6.4 μM) and Mg^{2+} concentration (0.4 mM) were chosen in this experiment to allow a measurable critical actin concentration with minimal influence from non-polymerizable actin, as described in the Experimental procedures section. PEG-6000 decreased the critical actin concentration in an apparent exponential fashion, a PEG-6000 concentration of 35 g/litre being required to decrease the critical actin concentration by 50%. The inset of Fig. 2 shows a plot of the natural logarithm of the critical actin concentration ($\ln[G]$) as a function of PEG-6000 concen-

Fig. 2. Plot of the critical actin monomer concentration as a function of PEG-6000 concentration

G-actin ($6.4\mu\text{M}$) in G-buffer was polymerized for 12 h at 25°C with 0.4 mM-Mg^{2+} . The concentration of non-sedimentable actin ($178\,000\text{ g, 1 h}$) was measured and corrected for non-polymerizable actin. The inset shows a plot of the natural logarithm of the critical actin concentration as a function of the millimolar concentration of PEG-6000.

tration. Linear least-squares analysis of these data yields a slope of -160 M^{-1} .

The extent of the formation of F-actin (or alternatively, the critical actin concentration) has previously been shown to be strongly dependent on the concentration of Mg^{2+} in solution (Kasai *et al.*, 1962a). Fig. 3 confirms this observation and, further, shows that the decrease in critical actin concentration in the presence of PEG-6000 reported in Fig. 2 at 0.4 mM-Mg^{2+} occurs over a wide range of Mg^{2+} concentrations. The ratio of the critical actin concentration in the absence of PEG-6000 to that in its presence is approx. 1.75 ± 0.25 at Mg^{2+} concentrations less than 1.0 mM . However, at higher Mg^{2+} concentrations this ratio is variable, possibly reflecting the relatively small critical actin concentrations being measured under these conditions. Thus PEG-6000 appears to augment the effect of Mg^{2+} on the extent of polymerization of actin.

Effect of PEG-6000 on G-actin structure

Removal of the single high-affinity exchangeable bivalent cation from G-actin monomer promotes a form of actin that readily and irreversibly loses the ability to polymerize (Oosawa & Kasai, 1971; Strzelecka-Golaszewska *et al.*, 1974). Accompanying this loss in polymerizability is an irreversible change in the intrinsic-fluorescence spectrum of the actin monomer (Lehrer & Kerwar, 1972). The wavelength of maximal fluorescence is shifted from 328 nm to 335 nm (excitation 280 nm) and the intensity is decreased by 25–30%. The kinetics of the change in the intrinsic fluorescence (excitation

Fig. 3. Dependence of the critical actin monomer concentration on the concentration of Mg^{2+}

A G-actin solution ($6.4\mu\text{M}$) in G-buffer was polymerized for 12 h at 25°C in the presence (●) and absence (O) of PEG-6000 (35 g/litre). The critical actin concentration was determined as described in the legend of Fig. 2 and in the Experimental procedures section.

Fig. 4. Effect of PEG-6000 on the EDTA-induced change of the intrinsic fluorescence of monomeric G-actin

The change in fluorescence (excitation 280 nm , emission 325 nm) of $0.52\mu\text{M}$ -G-actin in G-buffer (25°C) was measured after making the solution 1 mM in EDTA in the presence of the following concentrations (g/litre) of PEG-6000: 0 (●); 71 (Δ); 107 (□); 143 (■); 193 (○). A control experiment (▲) was performed in the presence of PEG-6000 at 107 g/litre and no EDTA. The results have been normalized to the fluorescence change observed in the absence of PEG-6000 and in the presence of 1 mM-EDTA .

280 nm , emission 325 nm) of G-actin ($0.52\mu\text{M}$) was monitored after the addition of EDTA (1 mM) and various concentrations of PEG-6000 (Fig. 4). Since the buffer (G-buffer) contained $200\mu\text{M-Ca}^{2+}$, the

effective EDTA concentration was 0.8 mM, representing a 1500-fold molar excess over the actin concentration. The concentration of actin in these experiments was below the critical actin concentration under these conditions, ensuring that the fluorescence properties being measured were those of actin monomer.

It is apparent from Fig. 4 that PEG-6000, in a concentration-dependent manner, decreases the rate of the EDTA-induced change of the intrinsic fluorescence of G-actin. The kinetics of this change in the absence of PEG-6000 describes an apparent single exponential decay process represented by a half-life of 2 min (●). The extents of the fluorescence changes in the presence of various concentrations of PEG-6000 are similar if measured at suitably long periods for the higher concentrations of PEG-6000. In the presence of PEG-6000 the decay process over an extended time scale cannot be described by a single exponential curve; rather it is biphasic, with a distinctly slower phase at longer times and higher PEG-6000 concentrations. G-actin in the absence of EDTA and PEG-6000 (result not shown) or in the absence of EDTA but in the presence of PEG-6000 (▲) was stable under these conditions.

The intrinsic-fluorescence spectrum of actin inactivated by addition of EDTA is very similar to that of thermally inactivated actin (Lehrer & Kerwar, 1972). We decided to investigate whether PEG-6000 would also have an influence on the temperature at which G-actin unfolds. The fluorescence intensity of a particular fluorophor in solution generally decreases with increasing temperatures (Gally & Edelman, 1964). However, for many proteins the dependence of the intrinsic fluorescence on temperature shows a discontinuity at a temperature where it is thought that the protein starts to unfold, possibly as a consequence of a change in the immediate environment of tyrosine and tryptophan residues (Steiner & Edelhoch, 1962; Gally & Edelman, 1964; Lehrer & Kerwar, 1972). Fig. 5 shows the dependence of the intrinsic fluorescence of G-actin ($1.18\mu\text{M}$ in G-buffer) on temperature in the presence and absence of PEG-6000 (100 g/litre). In agreement with Lehrer & Kerwar (1972), there was a discontinuity in the fluorescence temperature-dependence at approx. 45°C in the absence of PEG-6000. The scatter-corrected fluorescence data in the presence of PEG-6000 indicates that the discontinuity occurred at a slightly higher temperature, i.e. approx. 49°C . Thus PEG-6000 tends to stabilize G-actin against thermally induced conformational changes.

Two further experiments were necessary before discussion is possible of the major findings that PEG-6000 enhances the extent and rate of actin polymerization and tends to stabilize the native structure of G-actin against denaturing conditions.

Fig. 5. Temperature-dependence of the intrinsic fluorescence of G-actin

The fluorescence intensity (F) of G-actin ($1.18\mu\text{M}$) in G-buffer at 325 nm (excitation 280 nm) was measured in the absence (○) and presence (●) of PEG-6000 (100 g/litre) as a function of temperature. The broken vertical line allows comparison of results in the absence and presence of PEG-6000. The results in the presence of PEG-6000 have been corrected for scatter and arbitrarily displaced by 2 units along the vertical axis for purposes of comparison.

First, since the actin polymerization kinetics, critical actin concentration and native G-actin stability are all strongly dependent on the concentrations of bivalent cations in solution (principally Ca^{2+} and Mg^{2+}), a possibility exists that significant concentrations of contaminating metals may arise from the high concentrations of PEG-6000 used in these studies. Contaminating bivalent cations might then account for the influence of PEG-6000 on the properties of actin. We have therefore directly measured the Ca^{2+} and Mg^{2+} content of a 200 g/litre solution of PEG-6000 dissolved in distilled deionized water. Atomic-absorption spectroscopy of this solution indicated only trace levels of Ca^{2+} and no detectable Mg^{2+} content. Secondly, the effects of PEG-6000 on actin polymerization and G-actin stability may be a consequence of a conformational change of G-actin induced by PEG-6000 binding. A change in the conformation of a protein is often manifested by an alteration in its intrinsic-fluorescence spectrum. Since actin contains many dispersed tyrosine and tryptophan residues (Elzinga *et al.* 1973), the intrinsic-fluorescence spectrum of actin should be a good monitor of any PEG-6000-induced conformational change. Fig. 6 shows intrinsic-fluorescence spectra of G-actin in the presence and absence of PEG-6000, the former being corrected for light-scattering. It is evident that the spectra of the two solutions are nearly identical, indicating the

Fig. 6. Influence of PEG-6000 on the intrinsic fluorescence of G-actin

The relative fluorescence spectrum (excitation 280 nm) of G-actin ($1\mu\text{M}$) in G-buffer at 25°C was measured in the absence (—) and presence (---) of PEG-6000 (100 g/litre). The spectra were corrected for the scatter of PEG-6000, but remain uncorrected for the variation of instrument sensitivity with wavelength.

lack of any major conformational change of G-actin caused by binding of PEG-6000. This conclusion is in conformity with other studies that have demonstrated a lack of binding between PEG-4000 and albumin (Busby & Ingham, 1980) and ribonuclease (Atha & Ingham, 1981).

Discussion

The first finding which merits detailed examination is that an increase of concentration of PEG-6000 effectively increases the extent of association of actin (Figs. 1, 2 and 3). Particular attention is drawn to the finding, in Fig. 2, that a linear relationship exists between the concentration of PEG-6000 and $\ln[G]$ with associated slope of -160M^{-1} . The self-association of actin may be viewed as a combination of two types of indefinite self-association, the first leading to a nucleus of critical size and the second describing extended chain formation by the successive addition of monomeric units. Provided sufficiently large total concentrations of actin are being considered, the equilibrium concentration of unbound monomeric actin in the absence of added space-filling polymer remains approximately constant at its critical value, $[G]$, and the indefinite self-association involved in chain growth may be described in terms of a single apparent equilibrium constant:

$$K^{\text{app.}} = \frac{[F_{n+1}]y_{n+1}}{[F_n]y_n[G]y_G} \quad (1)$$

where $[F_n]$ and $[F_{n+1}]$ are concentrations of F-actin polymers consisting of n and $n+1$ monomers respectively. The symbol y is used to denote the thermodynamic activity coefficient of the designated species. It should be stressed that $K^{\text{app.}}$ is an apparent constant for two reasons. First, the assumption has been made that successive addition of monomer to either end of the filament does not alter the reactivity of the end group of the growing polymer chain. Secondly, the apparent constant incorporates that governing the hydrolysis of ATP to ADP which accompanies each successive monomer addition. The latter reaction is a potentially complicating feature, since two forms of actin monomer may exist in the equilibrium mixture, the original G-actin with ATP bound and that with ADP bound as an equilibrium reactant; it was for this reason that excess ATP was included in all reaction mixtures to ensure a predominance of ATP-actin-monomer to which the symbol G pertains. For sufficiently large values of n it has been shown (Timasheff, 1981) that $[F_{n+1}]/[F_n] \sim 1$ and thus eqn. (1) may be rewritten as:

$$K^{\text{app.}} \sim [G]^{-1}y_{n+1}y_n^{-1}y_G^{-1} \quad (2)$$

Comment is now required on the activity coefficient ratio appearing in eqn. (2). In the absence of any added inert compound this ratio approximates to unity for moderately dilute solutions of actin (Adams & Fujita, 1963; Wills *et al.*, 1980). In the presence of an inert compound, consideration of composition-dependent activity coefficients reveals that the term containing the concentration of the inert compound dominates (Nichol *et al.*, 1981). Accordingly, with consideration given only to the second virial coefficient we may write:

$$\frac{y_{n+1}}{y_n y_G} = \exp[(a_{n+1,\text{PEG}} - a_{n,\text{PEG}} - a_{G,\text{PEG}})m_{\text{PEG}}] \quad (3)$$

where the symbol a denotes a non-ideality coefficient (M^{-1}) and m_{PEG} is the molar concentration of PEG-6000. Since PEG-6000 bears no charge, it follows from eqns. A-2 and A-9 of Wills *et al.* (1980) that:

$$a_{i,j} = U_{i,j} - M_j \bar{v}_j$$

where $U_{i,j}$ denotes a co-volume and $M_j \bar{v}_j$ the molar volume of j (molecular weight multiplied by partial specific volume). Combination of this relation with eqns. (2) and (3) yields:

$$K^{\text{app.}} = [G]^{-1} \exp[(U_{n+1} - U_n - U_G + M_{\text{PEG}} \bar{v}_{\text{PEG}})m_{\text{PEG}}] \quad (4)$$

where for reasons of brevity the subscript for co-volumes denotes the actin species, it being

understood that the second member of the pair is PEG-6000 in each case.

It follows from eqn. (4) that a plot of $\ln [G]$ versus m_{PEG} yields a straight line with slope $U_{n+1} - U_n - U_G + M_{\text{PEG}} \bar{v}_{\text{PEG}}$, a linearity indeed evident in Fig. 2. It remains, therefore, for us to comment on the magnitude and sign of the observed slope in relation to the corresponding coefficient of m_{PEG} appearing in eqn. (4). It is shown in the Appendix that the co-volume increment ($U_{n+1} - U_n - U_G$) may be estimated on the basis of spherical geometry to be -105 M^{-1} , whereupon the addition of the molar volume of PEG-6000 of 7 M^{-1} yields an anticipated slope of -98 M^{-1} . This value compares favourably in both sign and approximate magnitude with the experimental value of -160 M^{-1} obtained from Fig. 2. Certainly there is not exact agreement in the magnitude of these quantities, which is not surprising in view of the spherical approximations, discussed fully in the Appendix, as a model for PEG-6000 or for the formation of F-actin filaments. In the latter connection the model envisaged for chain growth as a linear array of spheres may well underestimate the co-volume increment in the actual growth of the F-actin filament, since these filaments are known to be of a double-helical nature. However, the F-actin filament can equally be described by its 'genetic' helix as a one-start single-stranded left-hand helix which approximates the proposed linear model more closely (Korn, 1982). The important point emerges that a rational basis in terms of excluded-volume effects has been provided for the increase of extent of association of actin by the addition of an inert space-filling polymer. This interpretation is in accord with other similar findings (Minton, 1981; Nichol *et al.*, 1981) that addition of such inert compounds may tend to favour structures that have minimal excluded volumes (Minton, 1981); specifically it does appear that actin association is favoured, since polymers of it have less overall excluded volume than the sum of their individual components (Minton, 1981). The results of Fig. 3 also find explanation on the basis of eqn. (4) in that $[G]_{\text{without PEG}}/[G]_{\text{with PEG}}$ is predicted to be 1.53 on the basis of the excluded volume term of -98 M^{-1} at the fixed PEG-6000 concentration of 4.4 mM : with Mg^{2+} concentrations less than 1.0 mM that ratio was found to average 1.75 ± 0.25 .

The concept that PEG-6000 acting as a space-filling macromolecule may perturb an equilibrium reaction in the direction of that reactant that occupies minimal excluded volume is also relevant to the results presented in Figs. 4 and 5, both of which indicate that PEG-6000 tends to stabilize the native conformation of G-actin against denaturing conditions. In relation to Fig. 4 it is noted that the presence of excess EDTA ensures the effective removal of Ca^{2+} from G-actin, converting it to a

form which undergoes a rate-limiting irreversible transformation to a non-polymerizable actin (Kuehl & Gergeley, 1969). This set of reactions has been postulated by Strzelecka-Golaszewska *et al.* (1974) to be of the type:

The rate-limiting process leading to G^{**} -actin, which has a different intrinsic fluorescence spectrum than undenatured forms, may proceed in a sequence of steps as evidenced by the biphasic intrinsic fluorescence changes observed in the presence of PEG-6000 in Fig. 4. Nevertheless, the overall rate of this process will be strongly dependent on the concentration of the intermediate species, G^* -actin, which may well be reduced in concentration on the addition of PEG-6000 by a shift of the first equilibrium reaction in favour of a more compact native G-actin. A similar mechanism involving compact and unfolded states of G-actin can be postulated to account for the small PEG-6000-induced protection of G-actin against thermal denaturation (Fig. 5).

The details of the geometrical considerations leading to an explanation of the observed effects of PEG-6000 on both the extent of association and the stability of G-actin are not yet precisely defined, because it is entirely possible that preferential hydration of actin by PEG-6000 may operate conjointly with excluded volume effects. However, it is fair to note that the present study has provided both experimental and theoretical indications that the properties of G-actin, and especially the formation of the F-actin filaments, may be markedly influenced by the presence of space-filling macromolecules. In this connection it is noted that the cytoplasm of cells contains high concentrations of space-filling macromolecules that may well favour the formation of F-actin filaments as a result of co-volume effects. Consequently, the 'critical actin monomer concentration' *in vivo* may be significantly less than that determined *in vitro* under similar ionic conditions but in the absence of space-filling macromolecules.

References

- Adams, E. T., Jr. & Fujita, H. (1963) in *Ultracentrifugal Analysis in Theory and Experiment* (Williams, J. W., ed.), p. 119, Academic Press, New York
- Atha, D. H. & Ingham, K. C. (1981) *J. Biol. Chem.* **256**, 12108–12117
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Busby, T. F. & Ingham, K. C. (1980) *J. Biochem. Biophys. Methods* **2**, 191–206
- Carlsson, L., Markey, F., Blikstad, I., Persson, T. & Lindberg, U. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6376–6380

- Clarke, M. & Spudich, J. A. (1977) *Annu. Rev. Biochem.* **46**, 797–822
- Cooke, R. (1975) *Biochemistry* **14**, 3250–3256
- Detmers, P., Weber, A., Elzinga, M. & Stephens, R. E. (1981) *J. Biol. Chem.* **256**, 99–105
- Elzinga, M., Collins, J. H., Kuehl, W. M. & Adelstein, R. S. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2687–2691
- Gally, J. A. & Edelman, G. M. (1964) *Biopolym. Symp.* **1**, 355
- Houk, T. W., Jr. & Ue, K. (1974) *Anal. Biochem.* **62**, 66–74
- Kasai, M. (1969) *Biochim. Biophys. Acta* **180**, 399–409
- Kasai, M., Asakura, S. & Oosawa, F. (1962a) *Biochim. Biophys. Acta* **57**, 13–21
- Kasai, M., Asakura, S. & Oosawa, F. (1962b) *Biochim. Biophys. Acta* **57**, 22–31
- Kawamura, J. M. & Maruyama, J. (1970) *J. Biochem. (Tokyo)* **67**, 437–457
- Korn, E. D. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 588–599
- Korn, E. D. (1982) *Physiol. Rev.* **62**, 672–737
- Kuehl, W. M. & Gergeley, J. (1969) *J. Biol. Chem.* **244**, 4720–4729
- Lehrer, S. S. & Kerwar, G. (1972) *Biochemistry* **11**, 1211–1217

- Minton, A. P. (1981) *Biopolymers* **20**, 2093–2120
- Nichol, L. W., Ogston, A. G. & Wills, P. R. (1981) *FEBS Lett.* **126**, 18–20
- Oosawa, F. & Kasai, M. (1962) *J. Mol. Biol.* **4**, 10–21
- Oosawa, F. & Kasai, M. (1971) in *Subunits in Biological Systems* (Timasheff, S. & Fasman, G. D., eds.), Part a, pp. 261–322, Marcel Dekker, New York
- Pardee, J. D. & Spudich, J. A. (1982) *J. Cell. Biol.* **93**, 648–654
- Pollard, T. D. (1976) *J. Supramol. Struct.* **5**, 317–334
- Steiner, R. F. & Edelhoch, H. (1962) *Nature (London)* **193**, 375–376
- Strzelecka-Golaszewska, H., Nagy, B. & Gergeley, J. (1974) *Arch. Biochem. Biophys.* **161**, 559–569
- Taylor, D. L. & Condeelis, J. S. (1979) *Int. Rev. Cytol.* **56**, 57–144
- Tellam, R. & Frieden, C. (1982) *Biochemistry* **21**, 3207–3214
- Timasheff, S. (1981) in *Protein-Protein Interactions* (Frieden, C. & Nichol, L. W., eds.), pp. 315–335, John Wiley and Sons, New York
- Wegner, A. & Engel, J. (1975) *Biophys. Chem.* **3**, 215–225
- Wills, P. R., Nichol, L. W. & Siezen, R. J. (1980) *Biophys. Chem.* **11**, 71–82

APPENDIX

Calculation of the co-volume increment

The co-volume increment that occurs upon addition of a monomer to a long linear polymer may be expected to be constant and similar to that occurring upon dimerization. Appendix Fig. 1 represents this proposal diagrammatically for spheres, but it may be applied to any regular, orientationally specific, linear self-association. If V is the volume of a shaded area in Fig. 1, then:

$$U_1 = -\frac{4}{3}\pi N(r+a)^3 \quad (1a)$$

$$U_2 = 2U_1 - 2NV \quad (1b)$$

$$U_3 = 3U_1 - 4NV \quad (1c)$$

and

$$U_n = nU_1 - 2(n-1)NV \quad (1d)$$

where $U_i (i=1,2\dots n)$ is the co-volume (M^{-1}) of a spherical inert macromolecule of radius r and the polymer i formed from a spherical monomer ($i=1$) of radius a and N is Avogadro's number. Thus the co-volume increment is:

$$U_{n+1} - U_n - U_1 = -2NV = -2N\pi r^2(3a+2r)/3 \quad (2)$$

The formulation of eqn. (2) above in simple form permits ready calculation of the difference in co-volumes appearing in eqn. (4) of the main paper, provided values for r and a may be assigned. In this

Fig. 1. Schematic representation of the co-volume increment accompanying association of spherical monomers into a linear polymer

The radius of monomer is a , and r is the radius of the inert spherical macromolecule creating the non-ideality of the solution through volume exclusion. The shaded areas represent the loss of co-volume that occurs upon association of monomer to monomer or to polymer.

connection it is noted that specific assumptions must be made in relation to the actin-PEG-6000 system, notably that a Stokes radius estimated from measurements on the individual species is appropriate in the calculation of effective co-volumes; the implicit assumptions are that no marked volume or shape changes occur in the successive growth of the F-actin filament, that spherical geometry is an appropriate approximation and that preferential hydration of actin by PEG-6000 is negligible. The molecular dimensions of actin monomer within an F-actin filament have been estimated as $5.6\text{ nm} \times 4.0\text{ nm} \times 3.3\text{ nm}$ (Aebi *et al.*, 1980, 1981), referring to lengths in three-dimensional space of a slightly asymmetric entity. On the basis of an arithmetic average of these lengths, a value of 2.15 nm for a may be inferred. It is reassuring to note that standard calculation on the basis of M_r (42 000) and v (0.732 ml/g) reported respectively by Elzinga *et al.* (1973) and Mihashi (1964) leads to a similar value (2.3 nm for G-actin monomer). The radius, r , of an equivalent rigid sphere of PEG-6000 viewed as monodisperse was taken as 2.65 nm , a

value determined by Atha & Ingham (1981) from intrinsic-viscosity measurements. It is not possible to be more precise in relation to the values r or a , but as the lower value for the latter of 2.15 nm was used in all calculations, it is likely that the calculated co-volume difference of -105 M^{-1} may be an underestimate and this is in accord with the main paper, where comparisons are made with experimental results.

References

- Aebi, U., Smith, P. R., Isenberg, G. & Pollard, T. D. (1980) *Nature (London)* **288**, 296–298
Aebi, U., Fowler, W. E., Isenberg, G., Pollard, T. D. & Smith, P. R. (1981) *J. Cell Biol.* **91**, 340–351
Atha, D. H. & Ingham, K. C. (1981) *J. Biol. Chem.* **256**, 12108–12117
Elzinga, M., Collins, J. H., Kuehl, W. M. & Adelstein, R. S. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2687–2691
Mihashi, K. (1964) *Arch. Biochem. Biophys.* **107**, 441–448