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Time-resolved X-ray scattering study of actin polymerization from profilactin

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Abstract. The polymerization of actin in solutions of purified calf spleen actin or profilactin (1 – 10 $mg \cdot ml^{-1}$) was followed by synchrotron radiation X-ray solution scattering. At the concentration used, polymerization of actin from profilactin or actin occurs without any lag phase. It is shown by a combination of solution scattering, model calculations and electron microscopy that contrary to the conclusions from previous viscometry studies, filaments form without any lag phase in profilactin solution but aggregate in bundles or networks. This phenomenon is independent of the method used to induce polymerization: slow temperature increase, temperature jump in the presence of polymerizing salts or fast mixing with salt. This aggregation explains the lower final viscosity levels, as compared to actin solutions, observed during the polymerization of actin from profilactin.

Key words: Profilactin, actin polymerization, X-ray scattering

1. Introduction

Actin, one of the most abundant proteins in all eukaryotic cells, is the basic component of the microfilament system which plays an important role in many cellular processes. In muscle, actin filaments exist in specific arrangements serving the specialized functions of these cells. In other cells actin is found in different organizational states ranging from bundles of filaments to single filaments and unpolymerized monomers (see Cold Spring Harbour Symposia of Quantitative Biology 1982; Korn 1982; Lindberg et al. 1979 for reviews). The occurrence of a continuous turnover of actin between different states has been proposed (Markey et al. 1981; Lindberg et al. 1979; Tilney 1977) and proteins which can control the in vitro polymerization and depolymerization of actin filaments have been isolated (see Weeds 1982; Schliwa 1981 for reviews).

Profilin is a low molecular weight protein which can be isolated from a variety of tissues and cells as a 1:1 complex with actin (Blikstad et al. 1980; Reichstein and Korn 1979; Harris and Weeds 1978; Markey et al. 1978; Carlsson et al. 1977; Carlsson et al. 1976). The profilin-actin complex, profilactin, isolated from calf spleen was originally reported to be a polymerization resistant form of actin (Carlsson et al. 1976, 1977). It was later shown that in profilactin solutions the complex dissociates in the presence of 2–3 mM $MgCl_2$ and actin filaments form with sigmoid polymerization kinetics. The lag phase in this polymerization was found to be longer than with pure actin under comparable conditions. Furthermore, it appears that the lag phase after the addition of salt and the final level of polymerization monitored by viscometry vary widely between profilactin batches. Most preparations of spleen profilactin appear to be heterogenous with respect to polymerization behaviour. This may be due to the fact that proteolytic removal of the carboxy-terminal phenylalanine from actin probably occurs to varying extents during the preparation. This modification drastically reduces the inhibition of actin polymerization by profilin (Malm et al. 1980). Removal of the carboxy-terminal tyrosine from profilin also has an effect on the stability of profilactin (Malm et al. 1983). The inhibition of actin polymerization by profilin in recombination experiments suggests that the physiological function of profilin might be to keep actin in the unpolymerized form ready to serve as a precursor of actin filaments when needed (see Lindberg et al. 1981 for a review). It was found

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that addition of various nucleating agents overcomes the initial lag in polymerization of actin from reconstituted profilactin (Grumet and Lin 1980; Brenner and Korn 1980) as well as from purified calf spleen profilactin (Markey et al. 1982). Experiments were done using time resolved synchrotron X-ray solution scattering in order to obtain additional information about the kinetics of the early stages of actin polymerization from calf spleen profilactin. The advantage of solution X-ray scattering is that it is more sensitive than light scattering or viscometry to the formation of oligomers. Moreover, as the sample is not mechanically perturbed the effect of filament breakage, which tends to enhance the sigmoidal aspect of the polymerization curve, can be eliminated. To obtain precise results with small angle solution scattering it is, however, necessary to work at protein concentrations in the range $1-10\ mg \cdot ml^{-1}$ i.e. somewhat higher than viscometry or light scattering but more closely related to physiological conditions.

The combination of solution scattering results and model calculations described below indicates that both with purified actin and profilactin under polymerizing conditions actin filaments are formed without noticeable lag phase. In the case of profilactin structures other than filaments are also formed. Electron microscopy work carried out to complement the X-ray scattering studies shows that during actin polymerization from profilactin aggregates and bundles are formed.

2. Materials and methods

2.1. Biochemical methods

Profilactin was isolated from calf spleen as described by Carlsson et al. (1977). In some of the experiments, as indicated in the text, in the last step of purification profilactin was chromatographed on a Sephadex G-100 superfine (Pharmacia) column to achieve a better separation of contaminating factors present in the preparation (Malm et al. 1983). Profilactin was stored at 4 °C as a precipitate in 70% ammonium sulphate. Prior to the experiments the ammonium sulphate precipitate was dissolved at concentrations of $10-20\ mg \cdot ml^{-1}$ in G-buffer (2 m*M* potassium phosphate pH 7.6, 0.1 m*M* $CaCl_2$, 0.5 m*M* ATP, 0.5 m*M* dithiothreitol (DTT), 0.01 m*M* ethylenediaminetetraacetic acid (EDTA) and 1 m*M* NaN_3). Protein solutions were desalted on a Sephadex G-25 superfine (Pharmacia) column in the same buffer. No significant difference in the behaviour of the protein prepared from G-100 superfine chromatographed material was observed in our experiments. Profilactin solutions were kept at 4 °C and used within 5 days.

Spleen actin was obtained from the calf spleen profilactin complex by dissolving the ammonium sulphate precipitate at $10-20\ mg \cdot ml^{-1}$ in a buffer with 5 m*M* potassium phosphate pH 7.6, 0.2 m*M* ATP, 0.01 m*M* ethyleneglycol-bis(β-aminoethylether)N,N′-tetraacetic (EGTA), 2 m*M* DTT and 1 m*M* NaN_3. After clarifying the solution by centrifugation at 30,000 *g* for 15 min, the complex was dissociated by addition of an equal volume of 2 *M* potassium phosphate pH 7.6, 6 m*M* $MgCl_2$, 4 m*M* ATP, 2 m*M* DTT and 1 m*M* NaN_3 and leaving the preparation at room temperature for 1.5 h. Filaments and paracrystals which form during this step were collected by centrifugation at 30,000 *g* at 15 °C for 30 min and the supernatant containing profilin was discarded. This pellet was resuspended in G-buffer with 2 m*M* $MgCl_2$ and F-actin was collected by centrifugation at 100,000 *g* for 4 h at 15 °C. The F-actin pellet was resuspended in G-buffer at a concentration of $3-4\ mg \cdot ml^{-1}$, homogenized and dialysed against G-buffer for 4 days. During this period the buffer was changed 4 times and each time the sample was homogenized. The dialysed material was centrifuged at 100,000 *g* for 3 h and the supernatant, concentrated to $8-10\ mg \cdot ml^{-1}$ by vacuum dialysis (Schleicher and Schull Ultrahulsen UH100), was chromatographed on a Sephadex G-100 superfine column in G-buffer. Fractions containing monomeric actin were pooled and concentrated by vacuum dialysis. Unless otherwise stated all procedures were carried out at 4 °C and the protein was used within 5 days of preparation.

Electrophoretic analysis of representative samples of proteins used in the experiments described below is illustrated in Fig. 1, right panel. The minor intermediate seen on the profilactin gel is thought to be a degradation product of actin as it can at times also be seen on gels of extensively purified actin preparations. The profilactin used in the following experiments was not assayed for the carboxy-terminal amino acids on actin or profilin. Protein concentrations were determined by absorption measurements at 280 nm, using absorbance values for 1 mg protein ml^{-1} (1 cm path length) of 1.1 for actin and profilactin.

In all experiments proteins were kept in G-buffer. Polymerization was performed in G-buffer containing 10 m*M* NaCl, 2 m*M* $MgCl_2$ and 0.5 m*M* EGTA, which is referred to in the following text as polymerizing buffer.

2.2. X-ray scattering experiments

Small angle X-ray scattering experiments were carried out at the EMBL Outstation on the DORIS

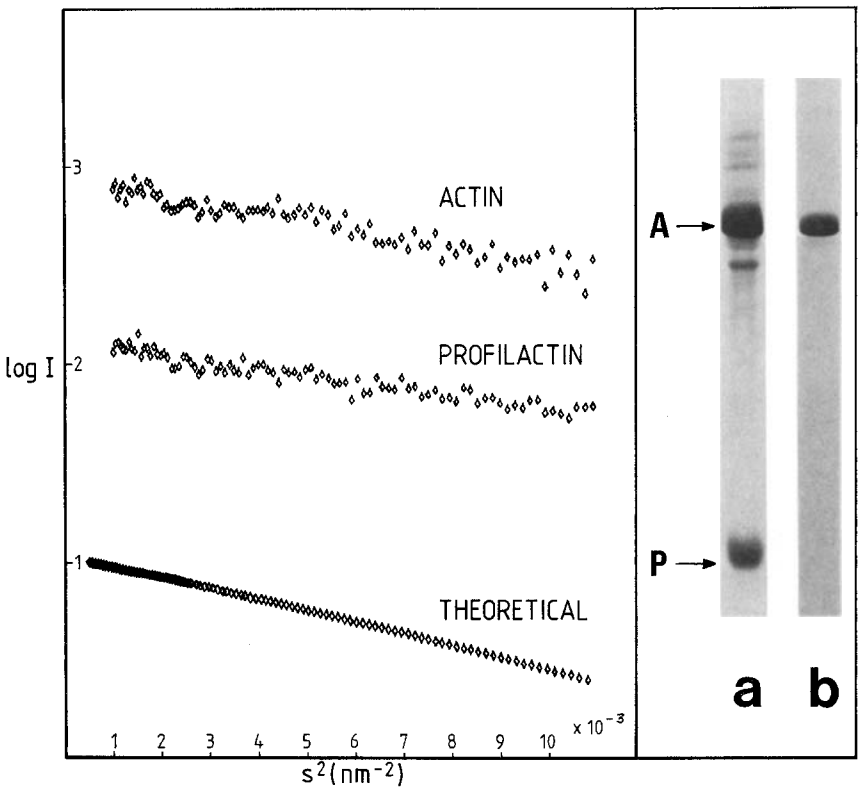

Fig. 1. *Left panel.* Guinier plots of the scattering patterns obtained from solutions of purified profilactin (10 mg · ml^{-1}) and actin (5 mg · ml^{-1}) and the calculated curve for a model of the actin monomer consisting of two contacting spheres of 2.0 nm and 1.5 nm radius. The curves have been offset for better visualisation. *Right panel.* The results of the electrophoretic analysis in the presence of sodium dodecyl sulphate of typical protein samples used in the scattering experiments **(a)** profilactin, about 20 µg, **(b)** actin, about 15 µg. The bands are identified as *A* (actin) and *P* (profilin). The band between *A* and *P* is probably due to a small amount of degraded actin

storage ring of the Deutsches Elektronen Synchrotron (DESY).

Measurements were taken on the double focusing mirror-monochromator camera X33 in HASYLAB (Koch and Bordas 1983) and recorded using a linear position sensitive detector with delay line readout, flushed with an 80 : 20 mixture of Ar/CO_2 (Gabriel 1977). Time-resolved scattering data were collected during temperature scan, temperature jump and mixing experiments using an enhanced version of the data acquisition system described earlier (Bordas et al. 1980). The data were collected in runs of 128 or 256 frames of 2 – 30 s duration.

In temperature scan and temperature jump experiments samples (200 µl) were kept in a temperature-controlled cell whose temperature could be changed rapidly by switching the circulation liquid from four thermostated baths (Renner et al. 1983).

For the mixing experiments a temperature-controlled cell in which the sample and an equal volume of the polymerizing buffer can be mixed within 50 ms was used (Renner et al. 1983).

The scattering data were reduced and interpreted using the PDP 11/45 computer of the EMBL Outstation and standard data manipulation programs (Koch and Bendall 1981). Data were normalized with respect to the intensity of the direct beam and detector response, and the background due to the buffer solution was subtracted. The correspondence between the detector channels and the reciprocal lattice vector was established using the diffraction pattern of rat tail tendon or beef corneal collagen (Meek et al. 1981) and the position of the direct beam.

2.3. Electron microscopy

In these experiments, solutions of profilactin (4 mg · ml^{-1}, G-100 superfine chromatographed) and actin (1.6 mg · ml^{-1}) were mixed with polymerizing buffer and put in a water bath at 37 °C for about one h. In the control experiments the proteins in G-buffer were kept at 37 °C. Aliquots of 10 µl were taken from the protein solutions just before they were placed in the water bath and at 10-min intervals thereafter. Samples were diluted to give a protein concentration of 0.5 to 1.0 mg · ml^{-1} and deposited on copper grids coated with formvar and carbon. After 2 min the grids were rinsed with experimental buffer followed by staining with a 1% aqueous solution of uranyl acetate. Excess stain was removed with a filter paper and the grids were air dried. The samples were then viewed in a Philips 400 electron microscope at 80 kV. Photographs were taken on Kodak electron microscope film 4463.

2.4. Viscometry

Viscometry measurements were performed in semi-micro Ostwald-type capillary viscometers (Cannon-

Manning size 100) with a flow time for buffer at 25 °C of about 65 s. Protein concentrations were 0.7 mg · ml^{-1} and polymerization was induced by adding the polymerizing buffer to the protein solution in the viscometer.

3. Results

3.1. Determination of radius of gyration of profilactin

The radius of gyration (R_g) of profilactin was calculated from the Guinier plots (Guinier and Fournet 1955) illustrated in Fig. 1, left panel, in the range $1.0\times10^{-3} < s^2 < 1.0\times10^{-2}$ nm^{-2} ($s = 2\sin\theta/\lambda$: 2θ is the scattering angle and λ the wavelength) for a concentration series in the range 1 – 10 mg · ml^{-1} and for different preparations. The region of the scattering pattern chosen can be used to determine R_g for monomeric forms. A value of 2.6 ± 0.2 nm for the radius of gyration of profilactin was consistently found.

The radius of the equivalent sphere which can be calculated from the molecular weight (MW = 58,000 Daltons) assuming a specific volume of 0.74 cm^3 · g^{-1} for profilactin is 2.57 nm, which would correspond to a radius of gyration (R_{calc}) of 2.0 nm. This indicates that profilactin is an anisotropic object with an anisotropy factor of $R_g/R_{calc} = 1.3$.

The value of R_g for monomeric actin under similar conditions was also found to be 2.6 ± 0.2 nm. The radius of the equivalent sphere which can be calculated from the molecular weight (MW = 42,000 Daltons) making the same assumptions as above is 2.3 nm. This leads to an expected radius of gyration of 1.8 nm and to an anisotropy factor of 1.44, a value in good agreement with the axial ratio obtained from the molecular dimensions of actin in the DNAse I-actin crystals: $6.7\times4.0\times3.7$ nm^3 (Suck et al. 1981).

The consistency of this data can be taken as an indication that we are dealing, within the limits of detectability (about 5%), with monodisperse solutions.

3.2. Temperature-induced polymerization

3.2.1. Temperature scan. A slow temperature scan from 4 °C to 37 °C was performed on a 9.5 mg · ml^{-1} profilactin solution in polymerizing buffer (G-buffer additionally containing 10 mM NaCl, 2 mM $MgCl_2$ and 0.5 mM EGTA, see *Materials and methods*, Sect. 2.1) and the scattering pattern was monitored with a time-resolution of 30 s for 2 h.

The Guinier plot of the scattering pattern of the initial solution yielded a radius of gyration of 2.6 nm. Figure 2a illustrates the changes in the integrated intensity of the most central part of the scattering pattern ($1.5\times10^{-2} < s < 2.9\times10^{-2}$ nm^{-1}) as well as the temperature record. The arrows marked A, B and C correspond to the different structural states of the protein in solution as indicated by a comparison of difference scattering patterns, discussed below, and by correlation plots in which the intensity at two scattering angles are plotted against each other (Bordas et al. 1983). Although they generally do not lead to unambiguous interpretation, difference plots can give indications about the various species developing at any time during a polymerization process.

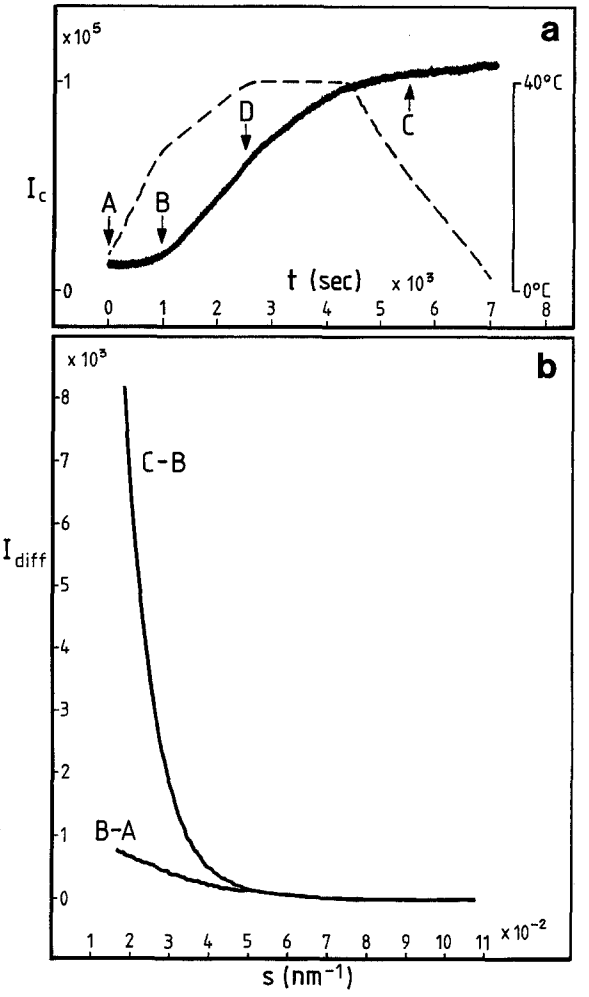

Fig. 2. **a** Time course of the central scatter and temperature (----) of a profilactin solution (9.5 mg · ml^{-1}) during a temperature scan experiment. *Arrows A, B* and *C* indicate different structural states of the protein in solution during actin polymerization. *Arrow D* indicates the change in the rate of increase of the scattering intensity when the temperature is kept constant after reaching 37 °C. **b** Plots showing the difference in the central scattering pattern of the profilactin solution at successive states *A*, *B* and *C* during the temperature scan

The plot of the difference (B–A) between the scattering pattern of the intermediate state (B) and

of the initial state (A) is shown in Fig. 2b together with the difference pattern (C−B) between the final (C) and the intermediate state (B).

Whereas the shape of the first difference pattern (B−A) is compatible with filament formation as judged by the results of the model calculations discussed below, the further increase of the central scatter cannot be explained by this process. Thus, at least two structural entities must coexist in solution and the relative amounts of these structures depend on the conditions, but the formation of the structures characteristic of state C is favoured by higher temperatures. It is, however, not possible from these experiments alone to determine whether one is dealing with a competitive or a consecutive process.

The heating rate influences the time course of the intensity as illustrated by the change of slope at point D in Fig. 2a. When the temperature was kept constant at 37 °C the central scatter continued to increase rapidly indicating that in spite of the relatively low heating rate, the system had not reached a steady-state. When the temperature was lowered back to 4 °C the central scattering continued to increase very slowly. In order to eliminate heating rate effects as much as possible and to obtain more details about the nature of the processes involved, temperature jump experiments were carried out.

3.2.2. Temperature jump. Temperature jump experiments between 4 °C and 37 °C were done with profilactin (5 − 10 mg · ml^{-1}) in polymerizing buffer. The changes in the scattering pattern were followed with 2 seconds time-resolution for 40 min. Guinier plots indicate that under these conditions F-actin filaments were already at least partially formed at 4 °C due to addition of polymerizing buffer immediately prior to the temperature jump.

Immediately after the *T*-jump to 37 °C one observes a continuous increase of the central scatter which saturates after about 20 min as illustrated in Fig. 3a. Since in these experiments the initial state already contains some filaments, only the transition from state B to C is obvious. Plots of the difference between the scattering patterns of state B and the initial state A and between the patterns in the final state C and in state B are shown in Fig. 3b. The difference pattern (C−B) is very similar to the one observed in the *T*-scan experiments. Lowering the temperature back to 4 °C had little effect on the scattering pattern.

When the same experiment was repeated in G-buffer the changes in the scattering pattern upon increasing or decreasing the temperature were not significant. The radius of gyration remained constant at 2.6 ± 0.2 nm. This can be taken as a proof that

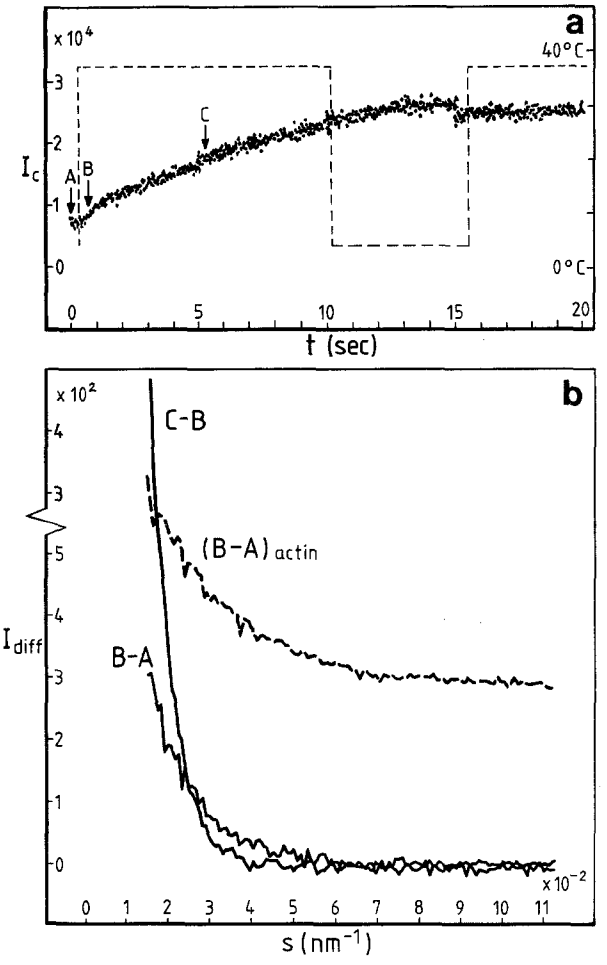

Fig. 3. a Time dependence of the central scatter of a profilactin solution (5 mg · ml^{-1}) and (---) the temperature during a temperature jump experiment. *Arrows A, B* and *C* indicate different structural states of the protein in solution. **b** Patterns showing the difference in the central scattering pattern of a profilactin solution at successive states *A, B* and *C* during a temperature jump experiment. The pattern $(B-A)_{actin}$ corresponding to the difference between the states *B* and *A* during a similar experiment on a purified actin solution (4 mg · ml^{-1}) has been offset for better visualization

the phenomena observed in the previous experiments do not result from radiation damage during these long exposures.

Spleen actin (4 mg · ml^{-1}) under polymerizing conditions at 4 °C gives a scattering pattern characteristic of the formation of filaments.

The radius of gyration of the cross section of the actin filaments in solution can be determined from the slope of plots of log $(s \cdot I(s))$ vs. s^2 (Porod 1982) in the range $1.0 \times 10^{-3} < s^2 < 1.0 \times 10^{-2}$ nm^{-2}. The value of this parameter is 2.5 ± 0.2 nm corresponding to an equivalent solid cylinder diameter of 7.2 ± 0.5 nm for the F-actin filament. The plot of the difference $((B-A)_{actin})$ between the scattering pattern of the final state − state B in this case − and that of the initial state A is shown in Fig. 3b. It is

similar to the difference between the patterns of states B and A in the temperature scan and temperature jump experiments with profilactin.

Model calculations indicate that the process observed in polymerization of purified actin and polymerization of actin from profilactin between states A and B is compatible with filament elongation.

Under non-polymerizing conditions a small increase in the central scatter was observed immediately after the *T*-jump possibly indicating the formation of a small amount of oligomers. However, the effect was small and the value of the radius of gyration remained constant.

During the temperature jump experiment the starting solution contained a small amount of filaments due to the addition of salt immediately prior to the experiment. This effect was eliminated by inducing polymerization at 37 °C with salt.

3.3. Salt-induced polymerization

Profilactin in G-buffer (5 mg · ml^{-1}, G-100 superfine chromatographed) was mixed in about 50 milliseconds with an equal volume of polymerizing buffer 10 s after the beginning of the experiment at 37 °C. The changes in the scattering pattern were followed with a time resolution of 2 s for 20 min. The time course of the changes in the integrated intensity in the most central part of the scattering pattern as well as the transitions between states A, B and C are shown in Fig. 4 a.

The Guinier plots for the first minute of this experiment yield a radius of gyration of 2.6 nm indicating that the solutions were monodisperse at the start of the experiments. A progressive increase in the central scatter was observed upon mixing with salt. After 3 min the central scatter had increased by a factor of about 6 and after 16 min the integrated intensity had reached about 20 times its initial value.

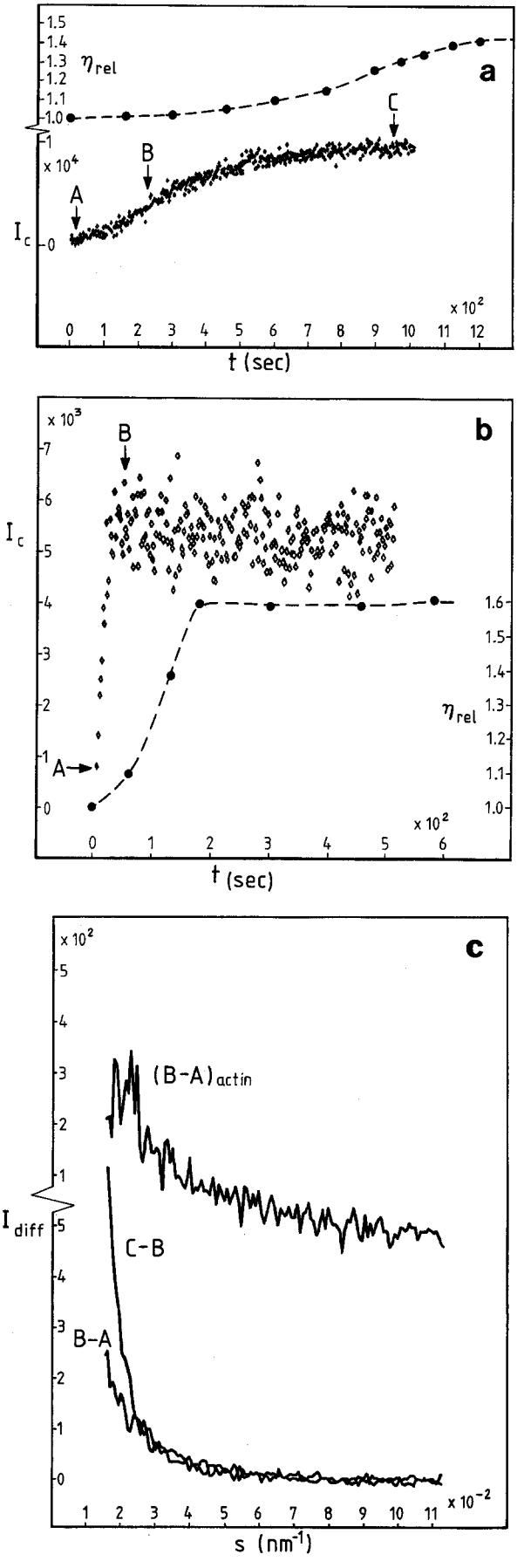

Fig. 4. **a** Time course of the central scatter of a profilactin solution (2.5 mg · ml^{-1}) in the mixing experiment. *Arrows A, B* and *C* indicate different structural states of the protein in solution. (–●–●–) Time course of the relative viscosity increase of a profilactin solution (0.7 mg · ml^{-1}) mixed with polymerizing buffer at time zero. **b** Time course of the central scatter of an actin solution (2 mg · ml^{-1}) in the mixing experiment. *Arrows A* and *B* indicate different structural states of protein in solution. (–●–●–) Time course of the relative viscosity increase of an actin solution (0.7 mg · ml^{-1}) mixed with polymerizing buffer at time zero. Note that the final relative viscosity is higher than in **a**. **c** Plots showing the difference in the central scattering pattern of a profilactin solution at successive states *A*, *B* and *C* after mixing with polymerizing buffer. The difference pattern $(B-A)_{actin}$ for an actin solution during a similar experiment has been offset for better visualization

Plots corresponding to the difference between the patterns of state B and state A and between state C and B are shown in Fig. 4c. These are directly comparable with those of the temperature scan and temperature jump experiments indicating that the same processes take place in all cases. When similar measurements were made on purified spleen actin (4 mg · ml^{-1}) the central scatter increased by a factor of 4–6 within 10 seconds but remained constant afterwards as illustrated in Fig. 4b. Determination of the radius of gyration of the cross section yield an equivalent solid cylinder diameter of 7.2 nm for the filaments.

Plots of the difference between the final and the initial scattering patterns illustrated in Fig. 4c agree with those of the model calculations corresponding to elongation described in the next section.

It is seen from Fig. 4a and b that although the polymerization of actin from profilactin takes place at a slower rate, no lag phase is detectable with either profilactin or purified spleen actin at the concentrations used in the X-ray experiments.

The value of the slope of the $\log(s \cdot I(s))$ vs. s^2 (Porod 1982) plots in the final state of the profilactin solution is about 20% larger than that for purified actin in the same range of s values. This gives strong evidence for the presence of structures other than filaments. However if a phenomenon like bundling occurs no physical meaning can be ascribed to the value of this slope since for a bundle i.e. a structure with a large cross section, the radius of gyration of the cross-section would have to be determined at much smaller values of the scattering vector.

Figure 4a and b also illustrate the results of viscosity measurements performed on the same samples but at somewhat lower concentrations (0.7 mg · ml^{-1}). Since a lower protein concentration has to be used and the technique is not sensitive to structures that form during the early stages of the polymerization process, the kinetics as detected by viscometry appear to be slower than that followed by X-ray scattering.

As reported earlier (Markey et al. 1982) and also seen from Fig. 4a and b the final level of viscosity reached with profilactin solutions upon actin polymerization is lower than that reached with actin solutions. The continued increase in the central scatter in the X-ray experiments and the larger value of the slope of the log $(s \cdot I(s))$ vs. s^2 plots are compatible with this observation since less anisotropic aggregates than filaments should result in a lower viscosity and a larger central scatter. Model calculations and electron microscopy work described in the following section suggest bundling of filaments as an explanation of these observations.

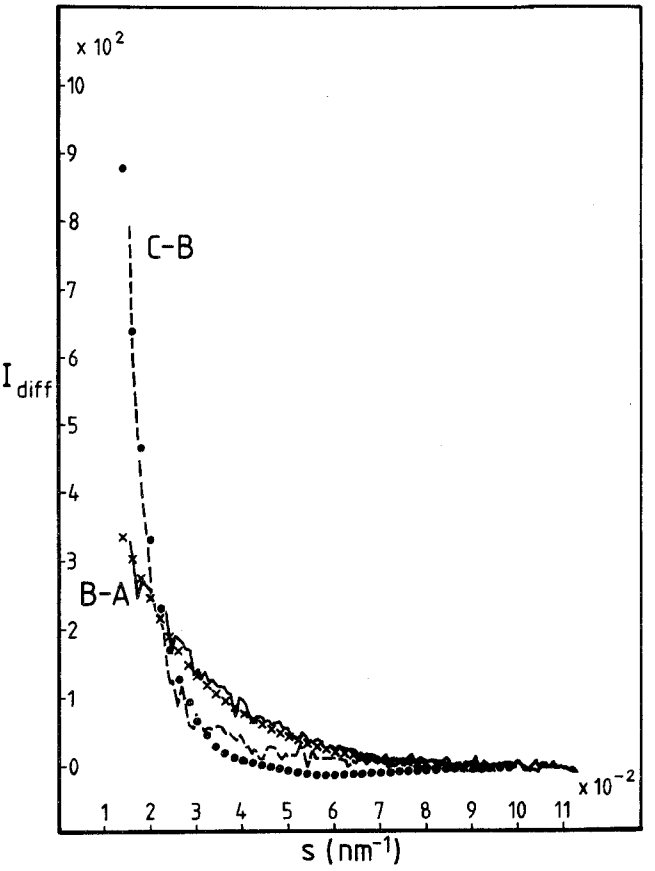

Fig. 5. Calculated and experimental difference intensity plots showing the difference patterns between states *A*, *B* and *C* during actin polymerization from profilactin. *B*−*A*: (——) experimental, (×××) calculated. *C*−*A*: (– – –) experimental, (●●●) calculated. The difference *B*−*A* corresponds to filament formation whereas *C*−*B* corresponds to lateral aggregation of the filaments

4. Model calculations

The theoretical scattering curves of models of monomeric actin, profilactin, actin filaments and bundles were computed using assemblies of spheres of various diameters to represent the structures, and the intensities were calculated using Debye's formula (Debye 1915).

The anisotropic shape based on the known dimensions of actin (Suck et al. 1981) used as the model of the monomeric form of actin, profilactin and F-actin subunits consisted of two contacting spheres of radii 2.0 nm and 1.5 nm. These theoretical curves yield Guinier plots in good agreement with the experimental data as can be seen in Fig. 1, left panel.

The calculated difference between the scattering from a solution of randomly oriented filaments constructed with the subunit axis perpendicular to the long axis of the filament and an initial pattern corresponding to monomers is illustrated in Fig. 5. This

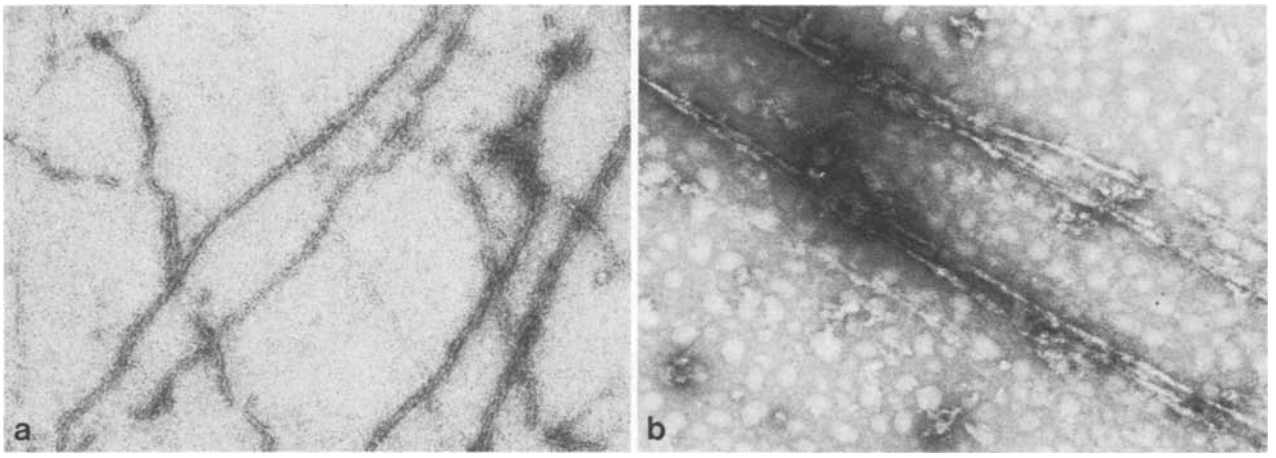

Fig. 6. Negatively stained preparations of actin **(a)** and profilactin **(b)**. Grids were prepared after the proteins (approximately 1 mg/ml) had been under polymerizing conditions at 37 °C for 1 h. The electron micrographs were taken at a magnification of 29,000. Note the formation of aggregates with parallel filaments in the case of profilactin

difference plot corresponds to the elongation process and agrees well with the experimental difference between states B and A.

Comparison of the results for purified actin with those of profilactin shows that in the case of actin polymerization from profilactin the elongation process is accompanied by another structural organization. To determine the nature of the second process theoretical scattering curves expected from bundles of filaments were calculated. The plot of the difference between the scattering pattern expected from a solution containing bundles of ten filaments and that from a solution with randomly distributed filaments is also shown in Fig. 5. This is in good agreement with the experimental difference plots between states C and B for profilactin solutions. This agreement should however only be interpreted qualitatively. Ideally a distribution of a number of filaments in the aggregates should be considered but the information in the experimental data does not justify a more detailed model.

The calculations and the experimental results do not allow us to rule out the possibility that the formation of other aggregates (e.g. profilin aggregates) may cause the observed increase in the central scatter. In order to elucidate this point, electron microscopy studies were carried out and the final states of actin polymerization from purified actin and profilactin at 37 °C were compared. After the proteins had been under polymerizing conditions the grids prepared with profilactin contained not only filaments and bundles but also other aggregates: either unpolymerized profilactin or profilin. These aggregates may cause the filaments to bundle as seen on the electron micrograph in Fig. 6 b.

5. Discussion

Polymerization of G-actin to F-actin at low protein concentration has been studied in a number of ways including viscometry, light scattering, flow birefringence, fluorescence labelling and electron microscopy (see Korn 1982 for review).

These methods indicate that the association of actin monomers to form filaments is a highly cooperative condensation polymerization reaction characteristic of the self-assembly process which takes place in two phases. The slow initial phase (nucleation) has a rate proportional to the third or fourth power of the actin concentration. Less is known about the nucleation phase than about the rapid second (elongation) phase mainly because nucleation involves only a very small fraction of the total actin. Thus, what is measured is not the formation of nuclei but that of filaments under conditions where nucleation is believed to be the rate limiting step. The results obtained by these methods are consistent with the theoretical models of actin polymerization at low concentration levels proposed by Oosawa and Kasai (1962) and by Wegner (1976).

However the actin concentrations expected to be found in cells are, at least locally, above 10 mg · ml^{-1} (Lindberg et al. 1981; R. Karlsson private communication), and the polymerization process takes place in the presence of several regulatory proteins which may influence the types of structures formed (separate filaments, bundles etc.). Caution is therefore needed in interpreting the kinetics of e.g. viscometry measurements, in relation to actual cellular processes. This prompted us to undertake the present study using X-ray solution scattering methods.

The static solution scattering measurements indicate that both profilactin and actin have anisotropic shapes in G-buffer, in agreement with the findings of Lanni et al. (1981). At the concentrations used, in a high salt buffer polymerization of actin from profilactin or of purified spleen actin sets in immediately. During the initial phase the time course of the scattering pattern is in both cases characteristic of the formation of filaments. In the case of profilactin, however, the difference scattering patterns indicate that besides filaments other structures are also formed. Model calculations and electron micrographs of samples of polymerized actin and profilactin obtained under conditions close to those of the X-ray experiments indicate that aggregates and bundles of filaments may be formed when actin is polymerized from profilactin.

This phenomenon also explains that the relative viscosities observed during the polymerization of actin from calf spleen profilactin in the presence of various nucleating agents are always lower than in similar experiments on actin (Markey et al. 1982). The present results suggest that the aggregation phenomenon is a property of profilactin solutions and that the changes of viscosity observed during polymerization are at least partly due to the formation of F-actin bundles and networks, independently of the presence of nucleating agents.

The results illustrate that a detailed picture of the observed phenomena can only be obtained by an appropriate combination of physical techniques, taking the specific limitations of each method into account. Synchrotron radiation solution scattering techniques which enable the study of the kinetics of assembly processes at high protein concentration levels with minimum perturbation of the system allow to achieve a situation somewhat closer to physiological conditions than other current techniques. Further extension of the studies presented here to systems where the polymerization kinetics of actin are investigated in the presence of nucleating agents, end-blocking proteins and bundling proteins or even in vivo would thus provide useful information about the processes taking place in cells.

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