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The effect of organic cryosolvents on actin structure: studies by small angle X-ray scattering

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Abstract. Small-angle X-ray scattering was used to probe the structure of actin in the presence of cryosolvents: 1,2-propanediol, glycerol, or a mixture of both solvents. In media devoid of polymerizing salts, a radius of gyration of 23 Å is measured, as expected from the literature. In the presence of 1,2-propanediol alone, the scattering pattern begins to exhibit the characteristic slope of elongated objects with a non-negligible thickness, such as actin filaments polymerized in 40 mM KCl and 1 mM $MgCl_2$. However, only short fragments (radius of gyration 40 Å) are generated. We infer that in a medium of low ionic strength containing 15% 1,2-propanediol, actin assumes a structure closer to that of filamentous actin. 1,2-propanediol apparently induces nucleation of oligomers, as with polymerizing salts, but no propagation occurs. Glycerol and/or propanediol induce no alteration in the structure of individual salt-polymerized actin filaments. Aggregation occurs with propanediol, even in the presence of glycerol. Glycerol alone has no such effect. No shortening is detected within the scale covered, with either solvent, although 1,2-propanediol is known to shorten actin filaments. We suggest that in the absence of salts, 1,2-propanediol induces a conformational change in monomeric actin that is necessary for nucleation. This could correlate with a conformational change of actin protomers within microfilaments observed in the presence of 1,2-propanediol by other authors using different techniques.

Key words: Actin – X-ray scattering – Cryosolvents – 1,2-propanediol – Glycerol

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Abbreviations: SAXS, small-angle X-ray scattering; G-actin, globular monomeric actin; F-actin; filamentous polymerized actin

Introduction

The use of organic cryosolvents is necessary to protect cells against freezing damage in the process of cryopreservation. The colligative effect of these solvents has been extensively studied (Lovelock 1953; Mazur 1970; Farrant and Woolgar 1970; Leibo and Mazur 1978; Boutron and Kaufmann 1979; Meryman and Williams 1985), as has their influence upon cellular structures such as membranes (Baust 1973; Pringle and Chapman 1981; Quinn 1985; Crowe et al. 1990). Less information is available about their biological action on the cytoskeleton. Further progress in the field of cryobiology could be obtained through elucidation of the mechanisms underlying cryosolvent action on cytoskeleton proteins. Our main idea is that efficient cryoprotection might be achieved by obtaining a cytoplasmic gel that is able to reduce water flux and avoid crystallization by trapping it within small compartments. It could even induce vitrification of the cytoplasm upon cooling (Prulière et al. 1987; Prulière and Douzou 1989). Therefore, a range of studies involving both in vivo and in vitro investigations was undertaken to examine the action of 1,2-propanediol, an efficient cryoprotectant for early mammal embryos (Vincent et al. 1990; Renard et al. 1984; Glenister et al. 1990), on actin, which is a major cytoplasmic protein.

The dynamic polymerization equilibrium of globular G-actin [1] into filamentous F-actin [1], under the influence of solution parameters such as temperature, pH, ionic strength (Kasai 1969; Pollard and Craig 1982; Frieden 1983; Zimmerle and Frieden 1986; Wendel and Dancker 1986), and the regulation of its organization by a number of actin-binding proteins (Korn 1982; Stossel et al. 1985), control a number of cytoplasmic properties. In particular, interconnecting proteins may induce bundling of microfilaments or the formation of isotropic tridimensional networks likely to present the gel properties sought (Nguyen et al. 1988; Prulière and Douzou 1989). Several observations have been made on the modifications undergone by F-actin in the presence of 1,2-propanediol. Among them are shortening, bundling of microfilaments

(Vincent et al. 1987, 1990), and conformational change of actin protomers within the filaments which yields a conformation closer to that of monomeric actin (Nguyen 1991).

The techniques employed in the previous studies (high-speed sedimentation, electron microscopy, viscometry, fluorimetry) were not well suited to the detection of the influence of 1,2-propanediol on monomeric actin. We therefore initiated this study by using small-angle X-ray scattering (SAXS[1]) on actin solutions (1 or 2 mg/ml). This technique represents a non-perturbing method which is sensitive to oligomer formation. The combination of results obtained on F-actin and on G-actin provides more coherent information on how 1,2-propanediol acts on the various actin species involved in the polymerization equilibrium.

Materials and methods

Proteins

Rabbit skeletal muscle actin was prepared from acetone powder as described by Spudich and Watt (1971), and filtered as described by MacLean-Fletcher and Pollard (1980). It was dialyzed against 2 mM Tris-HCl, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM β-mercaptoethanol, 1 mM NaN_3, pH 8.0 at 4 °C before use. Concentrations were assessed as described by Bradford (1976), using the Bio-Rad reagent and bovine serum albumin as a standard. For comparison with other experiments performed in the presence of α-actinin (not reported here), for which interaction with actin is maximum at low temperature (Jockusch and Isenberg 1981), measurements were conducted at 4 °C. Samples were prepared so as to reach final concentrations of 2 mg/ml for actin in the absence of polymerizing salts (monomeric globular actin, G-actin), and 1 mg/ml for polymerized actin (filamentous actin, F-actin). In both cases, 1,2-propanediol (later referred to as propanediol) and glycerol (Janssen Chimica) were used at concentrations of 15% (v/v) (2 M) and 10% (v/v), respectively. After gentle mixing the sample was loaded into a cell with thin mica windows. The cell was tightly closed and thermostated at 4 °C. Scattering from solutions of G-actin was measured immediately after sample preparation. For experiments on F-actin, solutions of actin were allowed to polymerize for 2 hours at 4 °C in a medium containing 40 mM KCl, 1 mM $MgCl_2$ (final concentrations), and solvent depending on the system.

Small-angle X-ray scattering

Scattering data were collected on beamline D24 on the storage ring DCI at LURE (Orsay, France), at a wavelength $\lambda = 1.608$ Å, in the range from $s = 0.0019$ Å^{-1} to $s = 0.044$ Å^{-1}, $s = (2/\lambda) \sin\theta$, where 2θ is the scattering angle. A gas-filled linear position-sensitive detector was used (Depautex et al. 1987; Boulin et al. 1986). The data were collected in runs of 6 frames of 15 min each, scaled for absorption and normalized for the integrated intensity of the direct beam. The scattering of the solvent was subtracted.

A radius of gyration R_G is obtained from the slope of the Guinier plot, $\log I$ versus s^2, within the lower angles region up to the s limit such that $s \cdot R_G \sim 0.2$ (Guinier and Fournet 1955):

$$\log I \sim -(4/3)\,\pi^2 R_G^2 s^2 \log e. \quad (1)$$

For anisotropic molecules such as rod-like particles, a radius of gyration of the cross-section, R_C, is determined from the slope of $\log(sI)$ versus s^2:

$$\log(sI) \sim -2\pi^2 R_G^2 s^2 \log e. \quad (2)$$

If we assume a homogeneous circular cross-section for the rod, the related radius is equal to $R_C\sqrt{2}$.

In a mixture containing only two types of particles with very different radii of gyration, two different slopes can be extracted, yielding the two radii of gyration (Guinier and Fournet 1955, p. 151). This type of scattering is obtained, for instance, when the system is a mixture of single G-actin units and very small aggregates, consisting of two or three single units.

In a log-log plot of I versus s, a slope slightly steeper than -1 indicates elongated particles. The steeper the slope, the more dense the scattering particles.

Results

Non-polymerizing ionic conditions

SAXS patterns from G-actin in solutions with or without propanediol are plotted on a log-log scale in Fig. 1, and compared with that obtained from F-actin without any cryosolvent. At low angles, the intensity of the propanediol-containing sample of G-actin is higher than that of G-actin alone. The two curves intersect for s around 0.011 Å^{-1}, as also occurs in the case of G-actin and F-actin. A linear section appears in the trace of G-actin with propanediol in the range of 0.0045 Å$^{-1} < s < 0.009$ Å^{-1}, which exhibits the same slope of -1.3 as for F-actin. Nevertheless, at very low angles, the plot for G-actin with propanediol is no longer linear, and the intensity decreases with s to reach a constant value. Guinier plots, shown in Fig. 2, are bi-modal to a good approximation, which gives evidence of two scattering species, with corresponding radii of gyration calculated in the scattering vector domains $s < 0.00735$ Å^{-1} and $s > 0.00735$ Å^{-1}. R_G values (1) are 40 Å ± 0.5 and 29 Å ± 1 for G-actin with propanediol, to be compared with R_G of 35 Å ± 1 and 23 Å ± 1 found for G-actin without any organic solvent.

SAXS patterns from G-actin with glycerol or the mixture of glycerol and propanediol are similar to that obtained from G-actin alone (not shown in Fig. 1). The corresponding values of the radii of gyration are 37 Å ± 0.5 and 23 Å ± 1 respectively, in the same s ranges as defined above.

Polymerizing ionic conditions

In the presence of 40 mM KCl and 1 mM $MgCl_2$, the log-log plot of the scattering patterns appear in Fig. 1 for

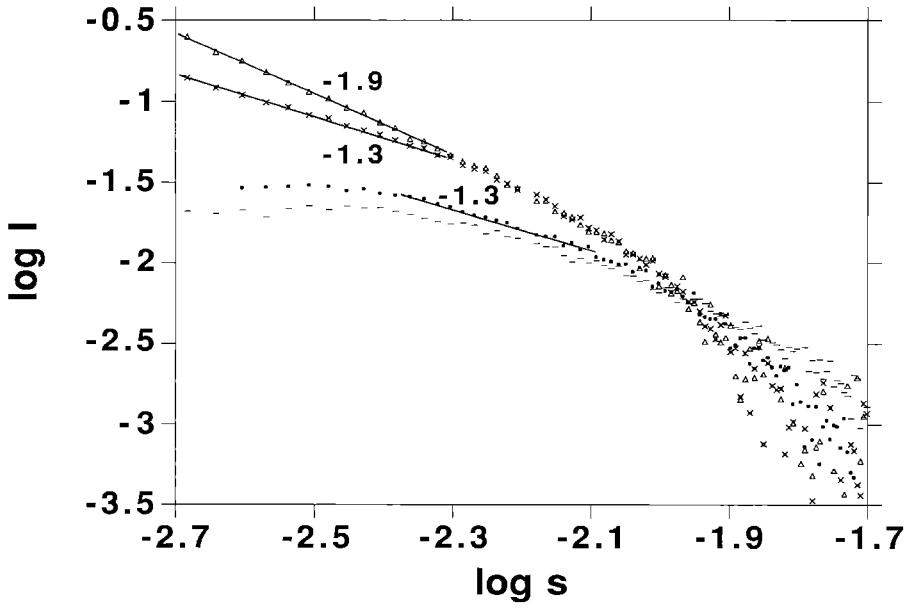

Fig. 1. Scattering patterns from G-actin solutions in buffer alone (- - -) or with propanediol 15% (.....), compared to F-actin solution without cryosolvent (× × ×) or with propanediol 15% (ΔΔΔ). Scattering of G-actin in the presence of propanediol exhibits a linear section in the range $0.0045\,Å^{-1} < s < 0.009\,Å^{-1}$ with a slope of −1 3. Scattering patterns from F-actin solutions in polymerizing buffer alone (× × ×) or with propanediol 15% (ΔΔΔ) provide slopes of −1.3 and −1.9 respectively, for $s < 0.005\,Å^{-1}$.

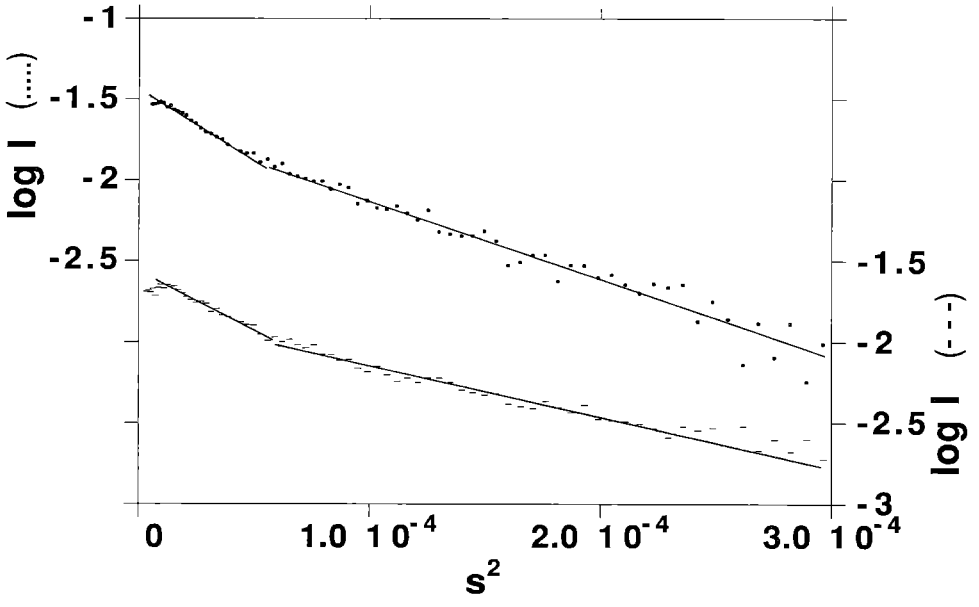

Fig. 2. Guinier plots of the scattering patterns obtained from solutions of G-actin in buffer alone (- - -) or with propanediol 15% (.....). The curve for buffer alone (- - -) is shifted one unit downwards in $\log I$ for clarity. Radii of gyration of 23 Å and 35 Å are calculated for $s < 0.00735\,Å^{-1}$ and for $s > 0.00735\,Å^{-1}$ for G-actin in buffer, as compared to 29 Å and 40 Å in the same regions for G-actin in propanediol

F-actin alone or with propanediol. The two curves exhibit the same intensities for $s > 0.005\,Å^{-1}$. In the range $s < 0.005\,Å^{-1}$, F-actin alone presents a slope of −1.3, compared to −1.9 for F-actin in the presence of propanediol. Plots of $\log(sI)$ versus the square of the scattering amplitude s^2 provide the radius of gyration of the cross-section R_C of the rod-like scattering elements ((2); Fig. 3). In this representation, both scattering curves are identical straight lines over the range $0.0065\,Å^{-1} < s$, which provides an R_C value of 25. 5 Å ± 1. With F-actin alone, this slope extends down to small scattering angles. In contrast, with propanediol the slope of the plot increases steadily with decreasing scattering angle below $0.0065\,Å^{-1}$, and no cross-section radius of gyration can be calculated in this range.

The presence of glycerol in F-actin solutions induces no modification of the scattering pattern compared to F-actin alone. No modification is observed either when glycerol is added to F-actin solutions containing propanediol.

Discussion

Previous studies of the action of cryosolvents on F-actin have indicated two effects of propanediol: 1) in the presence of 2 to 6 M propanediol, shortening and bundling of actin microfilaments is observed (Vincent et al. 1987, 1990; Nguyen 1991). Glycerol has no such effect. 2) Increasing concentrations of propanediol modify the fluo-

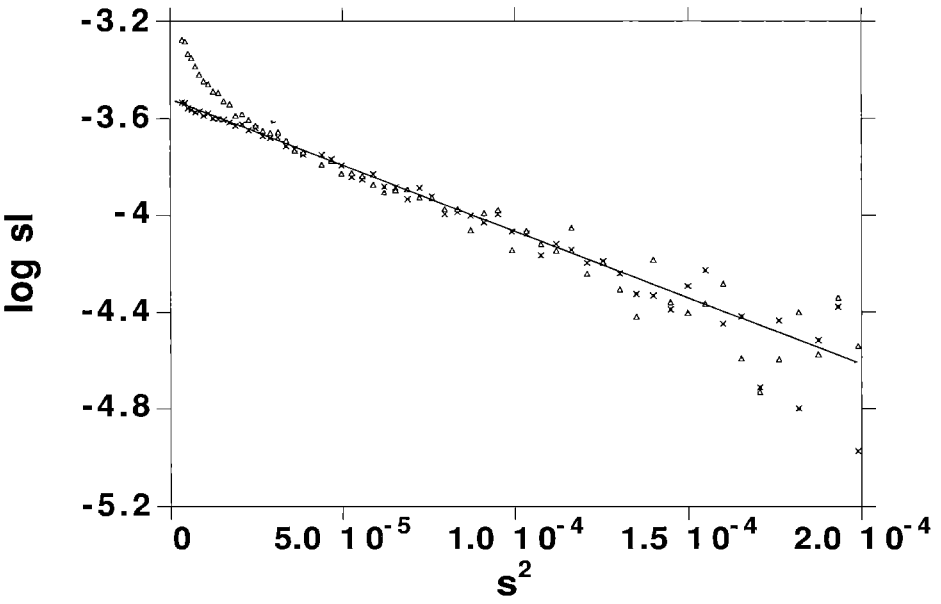

Fig. 3. Cross-sectional Guinier plots of the scattering patterns obtained from solutions of F-actin in buffer alone (× × ×) or with propanediol 15% (△△△). A cross-section radius of gyration R_C of 25.5 Å is calculated for $s<0.014\,Å^{-1}$ in buffer alone, but only for $0.0065\,Å^{-1}<s<0.014\,Å^{-1}$ with propanediol

rescence characteristics of solutions of F-actin labelled with N-(1-pyrenyl)-iodoacetamide (Nguyen 1991), so that the characteristics of the fluorophore become progressively closer to those of labelled G-actin. Kouyama and Mihashi (1981) and Cooper et al. (1983) have deduced that polymerization of actin brings about a conformational change of the actin protomer, which significantly influences the electronic environment of the pyrene label, and hence its fluorescence characteristics, providing a sensitive assay of actin polymerization. A possible depolymerization of microfilaments by propanediol was ruled out, since propanediol did not affect the critical concentration of actin, determined by pyrene fluorescence or a DNase 1 inhibition assay. The effect of propanediol was therefore interpreted as inducing an alteration of the conformation of actin protomer within the protofilament to a state closer to that of the actin monomer (Nguyen 1991). Since the detection of nuclei or short oligomers is difficult using fluorescence techniques, small-angle X-ray scattering has been used to examine possible modes of association of monomeric actin induced by propanediol.

X-ray diffraction patterns obtained on G-actin highlight the influence of propanediol upon monomeric actin behavior. In all solutions studied without the addition of propanediol, a radius of gyration of $23\,Å \pm 1$ was extracted from the Guinier plot, in excellent agreement with values found in the literature from dynamic light scattering studies (Patkowski et al. 1990), SAXS measurements (Sayers et al. 1985; Matsudaira et al. 1987) and with the three-dimensional actin structure (Suck et al. 1981). A slight degree of aggregation was detected in all samples, as indicated by the radii of gyration of 35 Å and 37 Å found respectively for G-actin alone, and for G-actin containing either glycerol or the mixture of glycerol and propanediol. This may be due to the lack of a gel filtration step immediately prior to the measurements, and may be consistent with formation of actin dimers in an end-to-end arrangement (Godette et al. 1986), but may also arise from other types of configurations involving higher order oligomers, but with the same end-to-end arrangement.

With propanediol, the main effect is the power-law decrease of scattering intensity as $s^{-1.3}$. The fact that a similar exponent is found in the case of F-actin seems to indicate that propanediol induces the onset of the formation of elongated structures. Intersection of G-actin and F-actin curves at an isosbestic point around 0.011 Å, as observed in Fig. 1, was documented by Matsudaira et al. (1987) from time-resolved patterns collected during the course of polymerization. The appearance of the same intersection between G-actin alone and in propanediol is another indication of actin association into the same type of structure as that present in F-actin. Moreover, both radii of gyration increase. The monomeric radius of gyration of 23 Å is no longer observed, which means that the monomer is no longer the elementary subunit in the aggregates. The values of 29 Å and 40 Å may result from the formation of side-to-side dimer subunits which then associate end-to-end, giving rise to linear but limited structures (Goddette et al. 1986). Propanediol thus proves to be a nucleating agent for G-actin, mimicking the early steps of salt-induced polymerization, leading to the formation of short rod-like oligomers, yet stopping short of producing typical full-grown filaments.

Glycerol alone or in combination with propanediol produces no change in the G-actin structure. This tends to suggest that propanediol interaction with monomeric actin is specific and is prevented by the presence of glycerol as a co-solvent.

Under polymerizing ionic conditions (40 mM KCl and 1 mM $MgCl_2$), the F-actin scattering pattern is that of elongated objects with a non-negligible thickness (Fig. 1, slope of -1.3 in a double logarithmic representation, and Fig. 3, $R_C = 25.5$ Å). This calculated value for the cross-section radius of gyration provides a corresponding diameter of 72 Å for the equivalent compact rod. This result is to be considered in the light of the molecular shape of

F-actin (Milligan et al. 1990; Holmes et al. 1990). The maximum diameter of the filament is between 90 and 100 Å. The two domains of the actin monomer (Milligan and Flicker 1987) are located at two distinct radii from the filament axis, and leave a low density region in the centre of the filament. The measured equivalent cross-section is compatible with this geometry to a first approximation, and is in good agreement with other studies (Matsudaira et al. 1987; Sayers et al. 1985). In the angular domain of determination of R_C, no structural change appears whatever solvent is present.

The slope increase up to -1.9 in the double-logarithmic plot of the X-ray diffraction pattern (Fig. 1), and the strong upward curvature of the cross-section plot at low scattering angle (Fig. 3) in the presence of propanediol alone or the mixture of propanediol and glycerol reveal that individual F-actin filaments aggregate into bundles with a distribution of cross-sections, which can themselves arrange into a loose network. This is in agreement with the bundling already observed in previous electron microscopy experiments (Vincent et al. 1990). The overall size distribution of the pores and the walls of this network cannot be estimated under the present experimental conditions. No conclusion may be drawn either about the shortening of F-actin filaments documented by Vincent et al. (1990). Only a statistical evaluation of the structures observed on a large number of electron micrographs or light scattering measurements may be used to determine changes in F-actin filament length and association into bundles. Viscometry measurements already performed on this system (Vincent et al. 1990) also confirm that bundling of F-actin filaments occurs in the presence of propanediol.

Glycerol alone, which is known for its stabilizing effect on proteins (Gekko and Timasheff 1981 a, b), induces no major alteration of the whole scattering pattern of F-actin. However, the lack of influence of glycerol on solutions of F-actin in propanediol shows that the propanediol effect upon filament association prevails over glycerol stabilization.

The results obtained by Nguyen (1991) under polymerizing ionic conditions show a propanediol-induced conformational change in actin protomers within actin filaments, leading to filament shortening and bundling. Since a conformational change of monomeric actin is necessary for actin polymerization with salts, it can be assumed that propanediol-induced nucleation of G-actin into short structures could also be ascribed to a conformational modification of monomeric actin induced by propanediol under non-polymerizing ionic conditions. Conformational changes of actin protomers may arise from the perturbation of the hydration shell by preferential exclusion of propanediol (Arakawa et al. 1990). Besides, propanediol was shown by several authors to induce an increase in the α-helical content of the secondary structure, and thus a conformational change, in α-actinin (Nguyen 1991), α_S-casein (Franks 1975), glucagon (Contaxis 1974), and bovine serum albumin at acid pH (Gekko and Koga 1984).

We suggest that conformations of actin protomers attained through the effect of propanediol in both F-actin and G-actin could present some similarity. It may be assumed that further addition of salts to the oligomers formed only by the propanediol effect would stabilize the structures formed and allow their elongation, so that a final state similar to that obtained by direct polymerization in the presence of propanediol might be achieved.

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