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Effect of organic cryosolvents on actin structures and actin/ α -actinin binding geometries

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Abstract. Cryopreservation procedures of living cells involve the use of organic solvents against freezing damage. An efficient cryoprotection might be achieved by obtaining a cytoplasmic gel able to reduce water flux and avoid crystallization. Small angle X-ray scattering experiments were performed at LURE (Orsay, France) to investigate the effects of 1,2-propanediol and glycerol on the structure of actin, a major cytoplasmic protein, and on the binding geometries of actin to α -actinin, an interconnecting protein. In the presence of 1,2-propanediol, monomeric G-actin exhibits oligomerization into short rod-like structures very close to that of salt-polymerized actin filaments (F-actin). In the presence of α -actinin, F-actin filaments aggregate into thick and tight bundles. The scattering pattern in the presence of propanediol suggests a "ladder-like" structure with a tilt angle of the α -actinin molecule relative to F-actin filaments. This angle is even larger with glycerol. The resulting loose structures favor the formation of a microporous network. All these results support previous electron microscopy observations and the structural mechanisms deduced from rheological measurements.

Introduction

Organic cryosolvents action upon actin-linked cytoskeleton may be critical for cryoprotection. Trapping water within the pores of a cytoplasmic gel could be a means to reduce water flux, avoid its crystallization, and even obtain vitrification. 1,2-propanediol induces shortening and bundling F-actin filaments [1-3]. Actin protomers within microfilaments undergo a conformational change which yields a conformation closer to that of monomeric actin [3]. Moreover, F-actin interaction with a number of actin-binding proteins is modified in the presence of 1,2-propanediol. α -actinin binding to F-actin is enhanced [3, 4]. Thick bundles of F-actin/ α -actinin determine a very coarse network without 1,2-propanediol, whereas its presence induces the formation of a microporous homogeneous network [3-6] able to vitrify by rapid cooling [5].

Small-angle X-ray scattering experiments were undertaken to study cryosolvents influence upon monomeric actin, and to examine any modification in the geometry of α -actinin binding to F-actin in the presence of cryosolvents which could account for the global structural changes observed.

Materials and methods

Actin and α -actinin were prepared as described elsewhere [4, 7, 8, 9]. Actin concentrations were 2mg/ml in the absence of polymerizing salts, 1mg/ml for F-actin polymerized with 40mM KCl and 1mM $MgCl_2$, and 1mg/ml in systems with α -actinin 0.2mg/ml. 1,2-Propanediol and glycerol concentrations were 15% (v/v) and 10% (v/v), respectively. All measurements were conducted at 4°C, where α -actinin/actin interaction is maximum [10, 11]. For experiments on F-actin or actin/ α -actinin mixtures, samples were allowed to polymerize for 2 hours at 4°C prior to scattering. Small-angle scattering data were collected on beamline D24 on the storage ring DCI at LURE (Orsay), at $\lambda=1.608\text{\AA}$ [12, 13].

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Results and discussion

Non-polymerizing ionic conditions [14]

Log-log plots of SAXS patterns (Fig. 1) exhibit a linear section for G-actin with propanediol in the range $0.0045\text{\AA}^{-1} < s < 0.009\text{\AA}^{-1}$ with a slope of -1.3 as in F-actin, which seems to indicate that propanediol initiates the formation of elongated structures. The curves for G-actin alone and in propanediol intersect at an isosbestic point around $0.011\text{\AA}$, as do G-actin and F-actin [15], another indication of actin association similar to that in F-actin.

Fig. 1 : Scattering patterns from G-actin solutions in buffer alone (---) or with propanediol 15% (....), compared to F-actin solution without cryosolvent (x x x) or with propanediol 15% ($\Delta \Delta \Delta$).

Fig. 2 : Guinier plots of the scattering patterns from G-actin solutions in buffer alone (---) or with propanediol 15% (....). The curve relative to buffer alone (---) is shifted one unit downwards in log I for purpose of readability.

Guinier plots from G-actin in solution with or without propanediol (Fig. 2) are bi-modal, which gives evidence of two scattering species [16], with radii of gyration of $35\text{\AA} \pm 1$ (49% relative amount) and $23\text{\AA} \pm 1$ (51%) in buffer, $40\text{\AA} \pm 0.5$ (45% relative amount) and $29\text{\AA} \pm 1$ (55%) in propanediol. SAXS patterns from G-actin with glycerol or the mixture propanediol/glycerol (not shown) are similar to that in buffer, with radii of gyration of $37\text{\AA} \pm 0.5$ and $23\text{\AA} \pm 1$. The radius of gyration of $23\text{\AA} \pm 1$ found in all solutions studied devoid of propanediol is in excellent agreement with the literature [15, 17-19]. The radii of gyration of $35\text{\AA}$ and $37\text{\AA}$ found in G-actin in buffer, glycerol, or glycerol/propanediol indicate aggregation, may be due to the lack of a gel filtration step immediately prior to the measurements, consistent with formation of actin dimers or oligomers in an end-to-end arrangement [20]. With propanediol, the monomeric radius of gyration of $23\text{\AA}$ is no longer observed : the monomer is no longer the elementary subunit. The values of $29\text{\AA}$ and $40\text{\AA}$ may result from the formation of side-to-side dimers subunits which then associate end-to-end, giving rise to linear but limited structures [20]. Propanediol is a nucleating agent for G-actin, mimicking the early steps of salt-induced polymerization, leading to the formation of short rod-like oligomers. Glycerol and propanediol/glycerol mixture yield no change, suggesting a specific interaction of propanediol with monomeric actin, prevented by the presence of glycerol as a co-solvent.

Since actin polymerization with salts requires a conformational change of monomeric actin,

propanediol effect on G-actin could also be ascribed to a conformational modification of monomeric actin, possibly due to perturbation of actin hydration shell by preferential exclusion of propanediol [21]. Further addition of salts to the oligomers formed with propanediol could stabilize and elongate the structures to obtain the same final state as by direct salt-polymerization in the presence of propanediol.

F-actin/ α -actinin binding

The small s - range of log-log plots of SAXS patterns can be linearly fitted with slopes of -0.4 for F-actin/ α -actinin, -0.8 with propanediol, -1.1 with glycerol or the mixture propanediol/glycerol (Fig. 3). A semi-logarithmic representation of sI versus s^2 (Fig. 4) exhibits local maxima at $s_{\max}$ leading to values of $1/s_{\max}$ of 164Å, 222Å and 208Å with propanediol, glycerol, and propanediol/glycerol mixture respectively, corresponding to the occurrence of characteristic distances within these systems. For F-actin/ α -actinin in buffer alone, no such maximum arises over the whole s -range.

Fig. 3 : Scattering patterns from F-actin/ α -actinin solutions in buffer alone (---), propanediol 15% (.....), glycerol 10% (x x x), and propanediol 15% + glycerol 10% ($\Delta \Delta \Delta$).

Fig. 4 : Semi-logarithmic plot of sI versus s^2 for F-actin/ α -actinin solutions in propanediol 15% (.....), glycerol 10% (x x x), and propanediol 15% + glycerol 10% ($\Delta \Delta \Delta$). Curves are shifted vertically one relative to the other. Full scale along the Y-axis is one logarithmic unit.

α -Actinin is a dumbbell-shaped homodimer (30- to 40-Å wide by 348Å long) [22, 23], binding to F-actin filaments with each end, forming bundles or networks [24]. Within tight bundles, α -actinin molecules are oriented parallel to the F-actin filaments. Lowering α -actinin/actin molar ratio induces loose bundles, with α -actinin molecules switching to a near-perpendicular orientation relative to filaments (ladder-like structures), with obliquely-oriented intermediates (Fig. 5). In this study, α -actinin/actin molar ratio value of 1/24 favors tight bundles with no spacing in the absence of cryosolvents.

The characteristic distances appearing in the presence of cryosolvents suggest a modification in F-actin/ α -actinin binding geometry. The angular tilt of α -actinin "rungs" relative to filaments in 10% glycerol ($\theta = 38^\circ$) is slightly higher than in 15% propanediol ($\theta = 27^\circ$). The results achieved in 10% glycerol + 15% propanediol ($\theta = 35^\circ$) tend to show that glycerol effect is predominant.

Fig. 5 : Schematic model of F-actin (—●—)/ α -actinin (—○—) interaction in bundles or networks. (a) Tight bundles, (c) Loose bundles, (b) Intermediate configurations (from [24] with permission)

The slight slopes of $\log I$ versus $\log s$ with either solvent agree with the presence of such interstitial space in F-actin/ α -actinin assemblies. In propanediol, a slope of -0.8 indicates rather short and loose structures in agreement with rheological experiments [6]. In glycerol, associations seem more elongated, with a slope of -1.1. Glycerol does not shorten actin filaments [1], nor promote bundle formation of F-actin alone [14], neither α -actinin binding [25] - it rather stabilizes native proteins [26, 27]. Few parallel associations of F-actin interconnected by α -actinin may thus form, and they likely involve only two filaments. The observed slope of -1.1 is in agreement with these observations, even if associations are looser than in propanediol ($\theta = 38^\circ$). With both solvents present, a slope of -1.1 suggests loose elongated objects, as with glycerol alone, which has a predominant effect. In buffer, no elongated structures may be inferred whatsoever, since thick F-actin/ α -actinin bundles involving many filaments occur [3, 5].

Propanediol thus modify α -actinin/actin binding by increasing its efficiency, favoring network formation, but also at a geometrical level, loosening the remaining bundles. The whole process favors formation of a water-trapping gel able to vitrify at low temperature.

References

- [1] VINCENT, C, et al., *Cryo-Lett.* 8 (1987) 356
- [2] VINCENT, C, et al., *Cryobiology* 27 (1990) 9
- [3] NGUYEN, E, University Thesis, Paris VII (1991)
- [4] NGUYEN, E, et al., *C. R. Acad. Sci. Paris* 307 (1988) 93
- [5] PRULIÈRE, G, DOUZOU, P, *Biophys. Chem.* 34 (1989) 311
- [6] PAJOT-AUGY, E, AXELOS, MAV, *Cryobiology* 29 (1992) 563
- [7] SPUDICH, JA, WATT, S, *J. Biol. Chem.* 246 (1971) 4866
- [8] MACLEAN-FLETCHER, S, POLLARD, PD, *Biochem. Biophys. Res. Com.* 96 (1980) 18
- [9] FERAMISCO, JR, BURRIDGE, K, *J. Biol. Chem.* 255 (1980) 1194
- [10] HOLMES, GR, et al., *Biochim. Biophys. Acta* 253 (1971) 240
- [11] JOCKUSCH, BM, ISENBERG, G, *Proc. Natl. Acad. Sci. USA* 78 (1981) 3005
- [12] DEPAUTEX, C, et al., *LURE Annual Report*, Paris (1987)
- [13] BOULIN C, et al., *Nucl. Instr. and Meth. A* 249 (1986) 399
- [14] PAJOT-AUGY, E, AXELOS, MAV, *Eur. Biophys. J.* 21 (1992) 179
- [15] MATSUDAIRA, P, et al., *Proc. Natl. Acad. Sci. USA* 84 (1987) 3151
- [16] GUINIER, A, FOURNET, G, Wiley, New York, Chap 2 and 4 (1955)
- [17] PATKOWSKI, A, et al., *Biopolymers* 30 (1990) 1281
- [18] SAYERS, Z, et al., *Eur. Biophys. J.* 13 (1985) 99
- [19] SUCK, D, et al., *Proc. Natl. Acad. Sci. USA* 78 (1981) 4319
- [20] GODDETTE, DW, et al., *J. Biol. Chem.* 261 (1986) 2605
- [21] ARAKAWA, T, et al., *Cryobiology* 27 (1990) 401
- [22] BLANCHARD, A, et al., *J. Muscle Res. Cell Motil.* 10 (1989) 280
- [23] POBUBNAYA, ZA, et al., *J. Mol. Biol.* 92 (1975) 357
- [24] MEYER, RK, AEBI, U, *J. Cell Biol.* 110 (1990) 2013
- [25] AXELOS, MAV, PAJOT-AUGY, E, In : *Theoretical and Applied Rheology*, Moldenaers & Keunings, eds., Elsevier Science Publishers B.V. (1992) 729
- [26] GEKKO, K, TIMASHEFF, SN, *Biochem.* 20 (1981) 4667
- [27] GEKKO, K, TIMASHEFF, SN, *Biochem.* 20 (1981) 4677