Water in Actin Polymerization

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ABSTRACT We have addressed the question whether water is part of the G- to F-actin polymerization reaction. Under osmotic stress, the critical concentration for G-Ca-ATP actin was reduced for six different osmolytes. These results are interpreted as showing that reducing water activity favored the polymerized state. The magnitude of the effect correlated, then saturated, with increasing MW of the osmolyte and suggested that up to 10–12 fewer water molecules were associated with actin when it polymerized. By contrast, osmotic effects were insignificant for Mg-ATP actin. The nucleotide binding site of the Mg conformation is more closed than the Ca and more closely resembles the closed actin conformation in the polymerized state. These results suggest that the water may come from the cleft of the nucleotide binding site.

INTRODUCTION

Actin is a dynamic polymerizing protein, found in almost all cells, that serves cellular locomotion. Its crystal structures have been determined recently (Chik et al., 1996; Kabsch et al., 1990; Lorenz et al., 1993) and its properties have been extensively studied. Globular actin (G-actin) aggregates into two-stranded helical polymer filaments (F-actin) consisting of up to thousands of monomers. Under physiological conditions, this polymerization process can be influenced by many different actin-binding proteins, by divalent cations, and by intrinsic ATPase activity (Korn et al., 1987; Tobacman and Korn, 1982).

Actin polymerization itself was modeled (Oosawa and Higashi, 1967, Oosawa and Kasai, 1971) analogous to a crystallization process. According to this scheme, shown schematically in Fig. 1, there exists at equilibrium a critical concentration of monomers coexisting with the polymers where there is no further growth of polymer, i.e., the rate of addition of monomers equals the rate of loss of monomers. This model for polymerization has since been complicated by the discovery that irreversible ATP hydrolysis is linked to, but is not necessary for, the addition of monomers (Korn et al., 1987; Carlier, 1991). Futhermore, the role of ATP hydrolysis is dependent on the particular metal ion bound to the nucleotide itself (Valentin-Ranc and Carlier, 1991).

In addition to the modification of actin polymerization by specific ligands, it is also possible that a change in macromolecular hydration occurs upon transition from one state to another, in this case, from monomer to polymer (see Fig. 1). This would cause the polymerization reaction to be sensitive to the surrounding water activity. In fact, Na and Timasheff (1981) have measured such an effect for an analogous process, polymerization of microtubules. Glycerol, as well as DMSO, polyethylene glycol, and dextran, in high concentrations, were shown to enhance tubulin self-assembly into microtubules. A detailed study (Na and Timasheff, 1981) was undertaken to examine the glycerol effect in terms of nonspecific thermodynamic interactions and, as a result, the solvent additives were referred to as "thermodynamic boosters".

A number of globular proteins have been shown to exclude neutral solutes close to their surfaces (Timasheff, 1993), the phenomenon of preferential hydration. Many macromolecules show strong mutual hydration repulsive forces (Leikin et al., 1993). The osmotic stress of neutral solutes that are excluded from more hydrated spaces has been used to measure water's participation in reaction equilibria in a number of very different systems. These include ion channel opening and closing, oxygenation of hemoglobin, DNA/receptor binding, enzyme activity and substrate binding, and hydration forces between all classes of biological molecules including lipid, protein, nucleic acid, and saccharide systems (Parsegian et al., 1995).

In this paper we investigate water's participation in actin polymerization. Early results of Kasai et al. (1965) suggest that actin polymerization, in the absence of nucleotide and divalent ion, is enhanced by high concentrations of sucrose. Using Mg-ATP actin, Drenckhahn and Pollard (1986) showed no change in monomer critical concentration in solutions containing sucrose, glycerol or ethylene glycol. Here we have examined the effects of six different solute molecules on the polymerization of both Mg- and Ca-ATP actin, and interpret the results in terms of changes in actin hydration. We estimate a value for a change in hydration upon binding of Ca-actin monomer to actin polymer.

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MATERIALS AND METHODS

Actin was extracted from rabbit leg and back muscle as described by Pardee and Spudich (1982). Sodium dodecyl sulfate gel electrophoresis revealed a single band. Concentration was determined by absorption at 290 nm using an extinction coefficient of 0.617 mg^{-1} ml cm^{-1} (Pantaloni et al., 1984).

Labeling with N-(1-Pyrenyl)iodoacetamide at the cys residue 373 was accomplished by a modification of the protocol of Kouyama and Mihashi

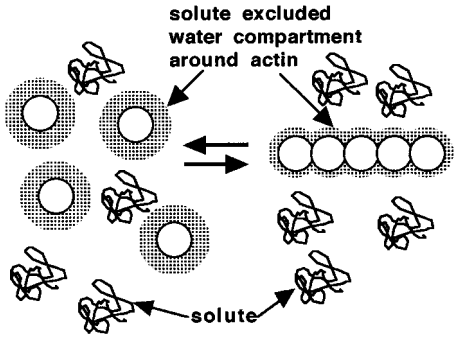

FIGURE 1 Schematic of the osmotic stress technique applied to the G-F equilibrium reaction of actin. The equilibrium is between G (monomer) and F (polymer) actin. Solutes that do not have access to aqueous compartments around either actin act osmotically on those compartments. Any difference in the size of those compartments between G and F actins results in the solute osmotically driving the equilibrium to the more dehydrated state.

(1981). The extraction protocol yielded about 22 ml G-actin, 3 mg/ml in G-buffer (5 mM Tris, 0.1 mM $CaCl_2$, 0.2 mM ATP, and 0.01% NaN_3). This was polymerized by adding 50 mM KCl, 2 mM $MgCl_2$, and 1 mM ATP. Immediately after these additions, excess label (1 ml of 2 mg/ml) in acetone was added. The mixture sat in the dark at room temperature for 2 days. Centrifugation pelleted the polymer along with unreacted probe. The pellet was homogenized and dialyzed against G-buffer overnight. After centrifugation, the supernatant contained the labeled actin monomers. The resulting actin was approximately 70% labeled as determined by absorbance at 344 nm, using an extinction coefficient of $2.2 \times 10^4\ M^{-1}$ for the labeled actin.

Fluorescence was monitored using a Perkin-Elmer LS50 Luminescence Spectrometer (Perkin-Elmer, Foster City, CA). Excitation was at 365 nm and emission was 387 nm. Slits were 2.5 nm and 4 nm, respectively. Typical spectra were obtained for the monomer (G-actin) and polymer (F-actin) forms (Kouyama and Mihashi, 1981). The polymerization process, triggered by the addition of Mg or Ca in the presence of ATP, could be monitored by observing a dramatic increase in fluorescence at 387 nm.

Determination of critical concentrations

Two methods were used to determine the critical concentration of G-actin for Mg or Ca. First, according to the traditional fluorescent method (Kouyama and Mihashi, 1981), various amounts of G-actin were added to equal volumes of buffer containing saturating levels of Mg or Ca. Samples were mixed well by repeated inversion and left to equilibrate at room temperature for 16–20 h (overnight). The equilibrium fluorescence was measured as a function of total actin concentration and the critical concentration determined by extrapolation to the actin concentration that gives zero polymer fluorescence.

This dilution method requires large amounts of actin for each determination. Consequently, a second method was used. For each fluorescent-labeled actin preparation, Mg-actin polymer was produced and used to calibrate fluorescence intensity with polymer concentration. This calibration could be used because of the very small and well-known critical concentration of Mg-actin, which itself did not change in any of our solutions, in agreement with results of Drenckhahn and Pollard (1986). In addition, our results showed that Ca and Mg polymer give identical fluorescence (Fig. 3). Critical concentrations for Ca-ATP actin were then determined from the difference between the total actin added and the observed polymer concentration.

We started with the Ca monomer. It has been shown (Carlier, 1991; Gershman et al., 1994) that Mg binds to actin with much lower affinity than Ca. The rapid polymerization rate on addition of 4 mM $MgCl_2$, can give polymers with a mixture of Ca-and Mg-bound actin, which Orlova et al. (1997) have shown to have two subsets of structure. In our conditions of overnight incubation and extensive monomer cycling, one might expect a Mg/Ca ratio close to that of the monomer, 9/1 (Gershman et al., 1994). In any case, such heterogeneous polymers have critical concentrations as low as those where polymerization began with Mg monomers (De La Cruz and Pollard, 1995). Therefore, our measure of critical concentration would be unaffected by the cationic heterogeneity of the polymers and indeed gives the same values as the classical dilution method.

Measurement of critical concentrations in various osmotic stress solutions

Critical concentrations under osmotic stress, using the second method above, were measured using G-buffers containing polymerizing cation and various amounts of osmolytes. Osmolytes used in the study were sucrose (BDH, Inc., Toronto), D-glucose (BDH), glycerol (Sigma, St. Louis, MO), sorbitol (Sigma), glucopyranoside (Sigma), and trimethylamine N-oxide (TMAO) (Sigma). Osmotic solutions were prepared by adding measured weight percents of osmolytes to prepared buffers already containing 4 mM Mg or 6 mM Ca. Osmotic pressures Π were measured using a vapor pressure osmometer (Wescor 5500, Mandel, Guelph, Ontario) or obtained at *http://aqueous.labs.brocku.ca/osfile.html*. Actin samples were prepared by pipetting equal volumes of buffer or osmotic solution into cuvettes and adding the same volume of actin to each cuvette, to a final concentration of 0.1 mg/ml actin. Samples were mixed well by repeated inversion and left to equilibrate overnight at 22°C. Repeated trials on separate actin preparations were carried out in the same manner. Although the final proportion of the actin that became fluorescently labeled varied slightly between preparations, each actin preparation was internally controlled through its own calibration.

The observed critical concentration can be equated to an equilibrium binding or dissociation constant for the simplified reaction:

$$G.\Delta N_{ew} + \text{F-actin}_{(n)} \leftrightarrow \Delta N_{ew} + \text{F-actin}_{(n+1)}$$

where ΔN_{ew} is the change in number of solute-excluded water molecules associated with an actin molecule when it joins the polymer, and $G.\Delta N_{ew}$ is the critical concentration C_c. Oosawa and Higashi (1967) have shown that for a general model of helical or tubular polymerization, the binding constant, $K_b = C_c^{-1}$. C_c would then be the dissociation constant K_d. The measurement we are making, shown graphically in Fig. 1, is the value of ΔN_{ew}.

The magnitude of the effect of osmotic pressure Π on K_d (observed experimentally as a change in critical concentration, from Cc_0 to Cc_s) will depend on ΔN_{ew}, and therefore, ΔN_{ew} can be determined from the relation:

$$\frac{d[kT\ln(K_d)]}{d\mu_w} = \Delta N_{ew} \quad (1)$$

where the change in chemical potential of water $d\mu_w$, is given by

$$-d\mu_w = \overline{\nu_w} d\Pi_{osm} \quad (2)$$

We take $\overline{\nu_w}$, the molecular volume of water, as 30 Å^3, and the molarity of pure water as 55.6. For a complete derivation of these thermodynamic relations see Parsegian et al. (1995).

RESULTS

Fig. 2 shows the degree of fluorescence or actin polymerization as it varies with Ca and Mg concentration. From this, 4 mM Mg or 6 mM Ca was used in subsequent experiments to measure critical concentrations.

Fig. 3 shows dilution curves for polymerization of ATP actin under various conditions. In buffer with saturating

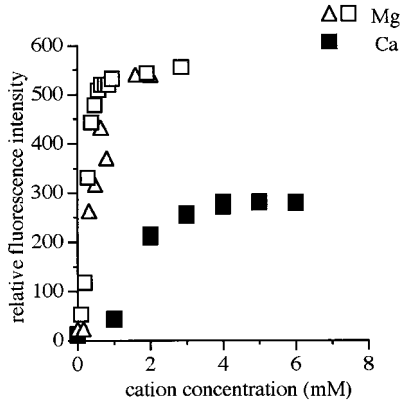

FIGURE 2 Fluorescence intensity reflecting F-actin concentration as it depends on divalent cation concentration. Open symbols represent two different actin preparations. From this data we established that using 4 mM Mg and 6 mM Ca in polymerizing reactions would not be limiting in divalent cation concentration when determining critical monomer concentrations.

levels of either $MgCl_2$ or $CaCl_2$, the resulting critical concentrations, 0.01 mg/ml in $MgCl_2$ and 0.04 mg/ml in $CaCl_2$, agree with those measured previously (Brenner and Korn, 1983; Detmers et al., 1981; Pantaloni et al., 1984). Note that the difference between the Mg and Ca fluorescence is maintained over the range of actin concentrations shown. The fluorescence corresponds to the concentration of polymerized actin, whether for Ca or Mg, and the determined critical concentrations of monomers add to give the known total actin concentration. Furthermore, the dilution curves for Mg-ATP actin in the presence and absence of 36 wt% glucose, show no difference in the critical concentration of the Mg-ATP actin within the detection limits of our experiments. This allows us to calibrate the fluorescence with concentration of polymerized actin, using a Mg sample. Ca-ATP actin, on the other hand, shows significantly different fluorescence levels in the presence of glucose (see below).

Fig. 4 shows the effect of increasing concentrations of sucrose in the polymerizing buffer to which equal concentrations of actin monomers are added. After equilibration there is little difference in fluorescence intensity (polymerization) for Mg-ATP actin at any concentration of sucrose. Similar results were obtained for Mg-ATP actin by Drenckhahn and Pollard (1986), who showed no change in critical concentration for sucrose, glycerol, or ethylene glycol solutions. Ca-ATP actin, however, shows increasing levels of polymerization with increasing sucrose concentration. Critical concentrations were calculated in these experiments by comparing the fluorescence of Ca- and Mg-ATP actin in tightly controlled samples as described in Materials and Methods.

Experiments similar to those shown in Fig. 4 were done using glucose, glycerol, TMAO, sorbitol, and glucopyranoside, giving changes in critical concentrations for each of these solutes.

Our results would suggest that adding a neutral solute to the polymerizing buffer for Ca-ATP actin changes the dissociation constant from Cc_0 (critical concentration with no osmolyte) to Cc_s (critical concentration with added osmolyte) and therefore changes the free energy of the actin-polymerizing reaction. In order to investigate further this difference in free energy, we plotted ln[critical concentration], (equivalent to ln[dissociation constant]) versus the osmotic pressure of the polymerizing solutions according to Eqs. 1 and 2. Data for the six different solute molecules are shown in Fig. 5. The observed dependence of ln[critical concentration] on osmotic pressure is consistent with the exclusion of these solute molecules being different for G- and F-actin (Garner and Rau, 1995; Parsegian et al., 1995). Raising the osmotic pressure favors the state with the lower volume of solute-excluding water. The decrease in critical concentrations for all solutes shows that Ca-ATP G-actin

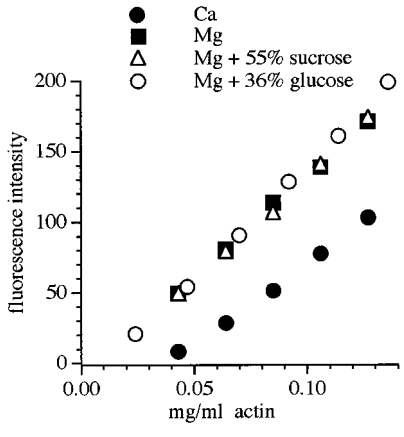

FIGURE 3 Dilution curves for the polymerization of ATP actin. Critical concentrations were determined by linear extrapolation to the *x* axis giving 0.01 mg/ml and 0.04 mg/ml for Ca and Mg, respectively. These agree well with previous studies. Glucose and sucrose did not change the critical concentration for Mg-actin. Their effects using Ca-actin were explored in subsequent experiments.

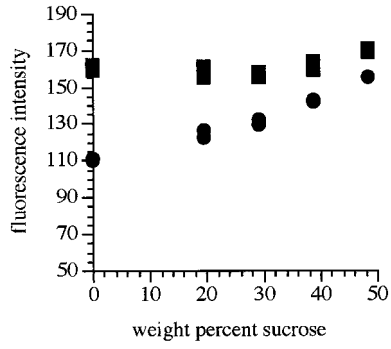

FIGURE 4 Fluoresence intensity, reflecting F actin concentration, as it is affected by added sucrose. Divalent cation concentrations, 4 mM Mg (*squares*) and 6 mM Ca (*circles*), were above the saturating levels shown in Fig. 2. Critical concentrations for each experiment were determined by the difference between total actin concentration and polymerized actin as described in the text. Mg-actin shows the independence of degree of polymerization as shown in Fig. 3. Ca-actin shows a dependence of degree of polymerization on sucrose concentration.

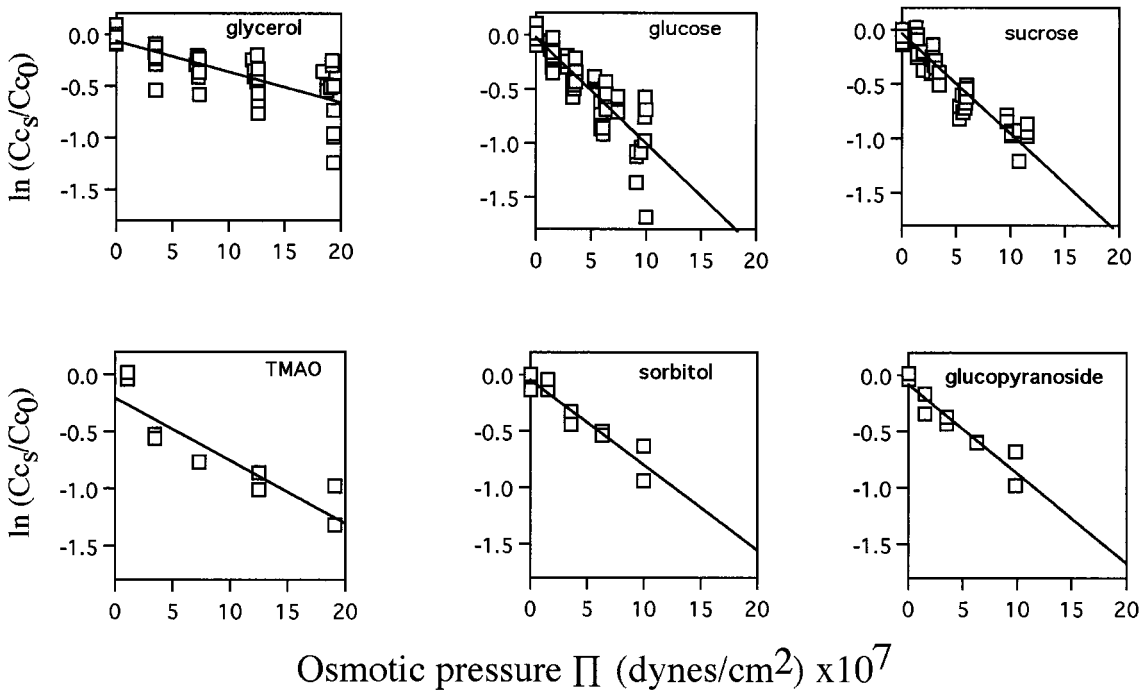

FIGURE 5 Relation between the change in critical concentration of actin as it varies with osmotic pressure produced by six different osmolytes. For ease of comparison, critical concentrations were normalized by the appropriate values with no osmolyte. The larger spread of data for glucose, sucrose, and glycerol likely results from the pooling of seven experiments using different actin preparations. The other solutes were used on a single actin preparation. Since $\ln(Cc_s/Cc_o)$ is equivalent to $\ln(K_d)$, the slope of the best fit line to the data is related to ΔN_{ew},the decrease in number of water molecules associated with the polymer compared to the monomer (Eqs. 1 and 2).

has more solute-excluding water than when it is associated in F-actin. This situation is similar to the polymerization of microtubules (Timasheff, 1993; Na and Timasheff, 1981).

From the best-fit slopes to the data in Fig. 5, the number of solute-excluding waters released on the binding of one actin monomer, ΔN_{ew}, are shown in Table 1.

DISCUSSION

Our data show a significant effect of several neutral solutes on the dissociation constant for actin polymerization by Ca- but, remarkably, not by Mg-actin. The Mg results agree with previous work (Drenckhahn and Pollard, 1986) and we address these results later. All six solutes enhance polymerization of Ca-ATP actin by lowering the critical concentration. These results are analogous to Timasheff's "thermodynamic boosters" for microtubule growth. Like the microtubule system, the effect requires high concentrations of solute, is similar for all solutes, and therefore is unlikely to result from specific solute binding (Na and Timasheff, 1981). This is in contrast to the specific binding of sugar phosphates, inositol phosphates, and phosphorylated amino acids to actin, which were shown to affect the critical concentration when present at concentrations of 0.5–5 mM (Gaertner et al., 1991). Therefore, we look at the change in equilibrium constant for actin polymerization as resulting from a change in actin-associated water.

TABLE 1 Change in number of actin-associated water molecules, ΔN_{ew} (±SE) as measured with solutes of different molecular weights

Solute	MW	ΔN_{ew}
Glycerol	92.0	4.0 ± (0.4)
TMAO	74.0	7.4 ± (1.3)
Glucose	181.2	13.1 ± (0.7)
Sorbitol	182.2	10.2 ± (1.3)
Glucopyranoside	194.2	10.7 ± (1.3)
Sucrose	342.3	12.3 ± (0.5)

The osmotic pressure exerted by an osmolyte would favor the state that would release waters from actin, i.e., the more dehydrated state. In this case, the results show that water is released upon binding of monomer to polymer.

Such a purely osmotic effect should be nonspecific with respect to the chemical nature of the osmolyte as demonstrated here, but the magnitude of the effect may depend on osmolyte size. Our results show a similar qualitative effect for all solutes. Fig. 6 shows a correlation and then saturation of number of waters released with increasing solute molecular weight. Similar dependence of measured changes in hydration on solute size are reported for alamethicin channels (Bezrukov and Vodyanoy, 1993; Bezrukov et al., 1994; Vodyanoy et al., 1993; Bezrukov et al., 1996), for hexokinase (Reid, 1995), and for aspartate transcarbamylase (Li-Cata and Allewell, 1997). Such change in numbers of water molecules can be explained by the volume of solute exclusion being different for solutes of different sizes as they

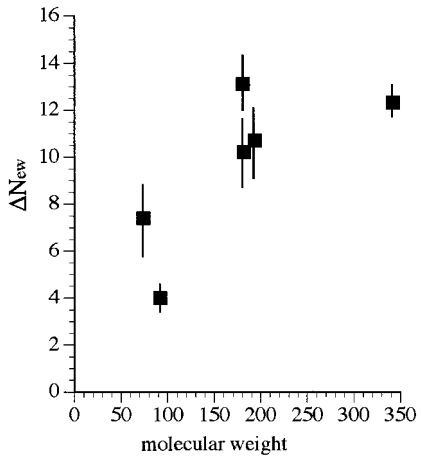

FIGURE 6 Relation between the change in number of solute-excluded water molecules ΔN_{ew} in the G-F actin polymerization reaction and the molecular weight of the osmolyte.

sterically partition into clefts or crevasses. In other words, the smaller glycerol and TMAO are excluded from a smaller volume than sucrose or glucose, and therefore have a smaller effect on polymerization. All solutes large enough (in this case, MW $>$ 200) are excluded from and act osmotically on the same large size aqueous compartment.

Our calculations indicate that the number of waters released on Ca-actin polymerization is relatively small compared to the changes in water associated with other conformational changes such as glucose binding to hexokinase (Reid and Rand, 1997) or oxygen binding to hemoglobin (Colombo et al., 1992). They are of the same order as those reported by Timasheff for microtubules, where 14 water molecules per monomer were estimated to be released on assembly (Na and Timasheff, 1981). Although it is not yet possible to determine how or where this net change in number of water molecules occurs, it is natural to think water leaves the contact sites between monomers. However, it has also been shown that polymerization can result in increased hydration (Kornblatt et al., 1993; Bhat et al., 1994; Na, 1986; Na et al., 1986).

A binding site within a cleft that closes around the substrate by a hinge connecting two rigid domains is a common structural feature (the actin fold) of hexokinase, actin, heat shock protein, and glycerol kinase (Kabsch and Holmes, 1995). Hexokinase binds glucose as well as nucleotide, and glucose binding is associated with the release of about 325 water molecules (Reid and Rand, 1997), far more than were observed here with actin. We interpret this to mean that solution conformations of hexokinase without bound substrate were far more open or flexible than the bound state. This would suggest that nucleotide bound actin is in a more closed state. It would be instructive to see if such large numbers of water molecules as were seen with hexokinase were also seen with nucleotide binding to actin, confirming the idea that substrate binding stabilizes more closed conformations.

It is remarkable that Mg-actin shows no osmotic dependence and therefore no change in associated water on polymerization, whereas Ca-actin does. There is one notable correlation between these results and actin structure. On the basis of molecular dynamics simulations, it has been shown that Mg-bound monomeric actin has a more closed nucleotide binding cleft than the Ca-bound state, and resembles more the closed state of actin in the polymer (Wriggers and Schulten, 1997). Our studies, confirming earlier results (Drenckhahn and Pollard, 1986), show that the Mg-actin, unlike Ca-actin, shows little change in solute-inaccessible water on polymerizing. Taken together, these suggest that the ΔN_{ew} measured here for Ca-actin may be largely related to the closure of the nucleotide cleft. In such a case, then, for both Ca- and Mg-actin, surface contacts made on polymerization either don't dehydrate significantly or any contact dehydration is compensated for by hydration elsewhere. Because hydration-dehydration reactions are energetically very high (Parsegian et al., 1995), the small net amount of water measured here means that the hydration energetics of actin polymerization-depolymerization reactions is small.

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REFERENCES

Bezrukov, S. M., and I. Vodyanoy. 1993. Probing alamethicin channels with water-soluble polymers: effect on conductance of channel states. *Biophys. J.* 64:16–25.

Bezrukov, S. M., I. Vodyanoy, R. A. Brutyan, and J. J. Kasianowicz. 1996. Dynamics and free energy of polymers partitioning into a nanoscale pore. *Macromolecules.* 29:8517–8522.

Bezrukov, S. M., I. Vodyanoy, and V. A. Parsegian. 1994. Counting polymers moving through a single channel. *Nature.* 370:279–281.

Bhat, T. N., G. A. Bentley, G. Boulot, M. I. Greene, D. Tello, W. Dall'Acqua, H. Souchon, F. P. Schwartz, R. A. Mariuzza, and R. J. Poljak. 1994. Bound water molecules and conformational stabilization help mediate an antigen-antibody association. *Proc. Natl. Acad. Sci. USA.* 91:1089–1093.

Brenner, S. L., and E. D. Korn. 1983. On the mechanism of actin monomer-polymer subunit exchange at steady state. *J. Biol. Chem.* 258: 5013–5020.

Carlier, M. 1991. Actin: protein structure and filament dynamics. *J. Biol. Chem.* 266:1–4.

Chik, J. K., U. Lindberg, and C. E. Schutt. 1996. The structure of an open state of beta actin at 2.65 Å resolution. *J. Mol. Biol.* 263:607–623.

Colombo, M. F., D. C. Rau, and V. A. Parsegian. 1992. Protein solvation in allosteric regulation: a water effect on hemoglobin. *Science.* 256: 655–659.

De La Cruz, E. M., and T. D. Pollard. 1995. Nucleotide-free actin: stabilization by sucrose and nucleotide binding kinetics. *Biochemistry.* 34:5452–5461.

Detmers, P., A. Weber, M. Elzinga, and R. E. Stephens. 1981. 7-Chloro-4-nitrobenzeno-2-oxa-1,3-diazole actin as a probe for actin polymerization. *J. Biol. Chem.* 256:99–105.

Drenckhahn, D., and T. D. Pollard. 1986. Elongation of actin filaments is a diffusion-limited reaction at the barbed end and is accelerated by inert macromolecules. *J. Biol. Chem.* 261:12754–12758.

Gaertner, A., G. W. Mayr, and A. Wegner. 1991. Binding of sugar phosphates, inositol phosphates and phosphorylated amino acids to actin. *Eur. J. Biochem.* 198:67–71.

Garner, M. M., and D. C. Rau. 1995. Water release associated with specific binding of gal repressor. *EMBO J.* 14:1257–1263.

Gershman, L. C., L. A. Selden, H. J. Kinosian, and J. E. Estes. 1994. Actin-bound nucleotide/divalent cation interactions. *Adv. Exp. Med. Biol.* 358:35–49.

Kabsch, W., and K. C. Holmes. 1995. The actin fold. *FASEB J.* 9:167–174.

Kabsch, W., H. Mannherz, D. Suck, E. Pai, and K. C. Holmes. 1990. Atomic structure of the actin: DNase complex. *Nature.* 347:37–44.

Kasai, M., E. Nakano, and F. Oosawa. 1965. Polymerization of actin free from nucleotides and divalent cations. *Biochim. Biophys. Acta.* 94: 494–503.

Korn, E. D., M. Carlier, and D. Pantaloni. 1987. Actin polymerization and ATP hydrolysis. *Science.* 238:638–644.

Kornblatt, J. A., M. J. Kornblatt, G. H. Bon Hoa, and A. G. Mauk. 1993. Responses of two protein-protein complexes to solvent stress: does water play a role at the interface? *Biophys. J.* 65:1059–1065.

Kouyama, T., and K. Mihashi. 1981. Fluorimetry study of N-(1-Pyrenyl)iodoacetamide-labelled F-actin. local structural change of actin protomer both on polymerization and on binding of heavy meromyosin. *Eur. J. Biochem.* 114:33–38.

Leikin, S., V. A. Parsegian, D. C. Rau, and R. P. Rand. 1993. Hydration forces. *Ann. Rev. Phys. Chem.* 44:369–395.

LiCata, V. J., and N. M. Allewell. 1997. Functionally linked hydration changes in *E. coli* aspartate transcarbamylase and its catalytic subunit. *Biochemistry.* 36:10161–10167.

Lorenz, M., D. Popp, and K. C. Holmes. 1993. Refinement of the F-actin model against X-ray fiber diffraction data by the use of a directed mutagen algorithm. *J. Mol. Biol.* 234:826–836.

Na, G. C. 1986. Interaction of calf skin collagen with glycerol: linked function analysis. *Biochemistry.* 25:967–973.

Na, G. C., L. J. Butz, D. G. Bailey, and R. J. Carroll. 1986. In vitro collagen fibril assembly in glycerol solution: evidence for a helical cooperative mechanism involving microfibrils. *Biochemistry.* 25:958–966.

Na, G. C., and S. N. Timasheff. 1981. Interaction of calf brain tubulin with glycerol. *J. Mol. Biol.* 151:165–178.

Oosawa, F., and S. Higashi. 1967. Statistical thermodynamics of polymerization and polymorphism of protein. *Prog. Theor. Biol.* 1:79–164.

Oosawa, F., and M. Kasai. 1971. Actin. *In* Biological Macromolecules. Vol. 5, Subunits in Biological Systems. S. N. Timasheff and G. D. Fasman, editors. Marcel Dekker, New York. 261–322.

Orlova, A., X. Chen, P. A. Rubenstein, and E. H. Egelman. 1997. Modulation of yeast F-actin structure by a mutation in the nucleotide-binding cleft. *J. Mol. Biol.* 271:235–243.

Pantaloni, D., M. Carlier, M. Coue, A. A. Lal, S. L. Brenner, and E. D. Korn. 1984. The critical concentration of actin in the presence of ATP increases with the number concentration of filaments and approaches the critical concentration of actin-ADP. *J. Biol. Chem.* 259:6274–6283.

Pardee, J. D., and J. A. Spudich. 1982. Purification of muscle actin. *Meth. Cell Biol.* 24:271–289.

Parsegian, V. A., R. P. Rand, and D. C. Rau. 1995. Macromolecules and water: probing with osmotic stress. *Methods Enzymol.* 259:43–94.

Reid, C. 1995. The influence of water on the glucose affinity of hexokinase. Thesis. Biological Sciences. Brock University, St. Catharines, Canada. 162 pp.

Reid, C., and R. P. Rand. 1997. Probing protein hydration and conformational states in solution. *Biophys. J.* 72:1022–1030.

Timasheff, S. N. 1993. The control of protein statility and association by weak interactions with water: how do solvents affect these processes? *Ann. Rev. Biophys. Biomol. Struct.* 22:67–97.

Tobacman, L. S., and E. D. Korn. 1982. The regulation of actin polymerization and the inhibition of monomeric actin ATPase activity by *Acanthamoeba* profilin. *J. Biol. Chem.* 257:4166–4170.

Valentin-Ranc, C., and M. Carlier. 1991. Role of ATP-bound divalent metal ion in the conformation and function of actin. *J. Biol. Chem.* 266:7668–7675.

Vodyanoy, I., S. M. Bezrukov, and V. A. Parsegian. 1993. Probing alamethicin channels with water-soluble polymers: size-modulated osmotic action. *Biophys. J.* 65:2097–2105.

Wriggers, W., and K. Schulten. 1997. Stability and dynamics of G-actin: back-door water diffusion and behavior of a subdomain 3/4 loop. *Biophys. J.* 73:624–639.