Osmotically induced electrical signals from actin filaments

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ABSTRACT Actin filaments, F-actin, a major component of the cortical cytoskeleton, play an important role in a variety of cell functions. In this report we have assessed the role of osmotic stress on the electrochemical properties of F-actin. The spontaneous Donnan potential of a polymerized actin solution (5 mg/ml) was -3.93 ± 1.84 mV, which was linearly reduced by osmotic stress on the order of 1–20 mOsm (0.28 ± 0.06 mV/mM). Calculated surface charge density was reduced and eventually reversed by increasing the osmotic stress as expected for a phase transition behavior. The electro-osmotic behavior of F-actin disappeared at pH 5.5 and was dependent on its filamentous nature. Furthermore, osmotically stressed F-actin displayed a nonlinear electric response upon application of electric fields on the order of 500–2,000 V/cm. These data indicate that F-actin in solution may display nonideal electro-osmotic properties consistent with ionic "cable" behavior which may be of biological significance in the processing and conduction of electrical signals within the cellular compartment.

INTRODUCTION

The regions of the mammalian cytoplasm adjacent to the cellular membrane contain a high concentration of actin filaments (F-actin) in an apparent network structure (Stossel, 1984). Changes in the mechanical properties of actin networks can be affected by changes in either the length or cross-linking status of F-actin by a number of well-described and characterized proteins, leading to the hypothesis that changes in the mechanical properties of actin-containing structures, such as sol-gel transitions, are important in a variety of cell functions including phagocytosis, secretion, and locomotion.

Much of the biophysical research on F-actin systems has focussed on their mechanical properties. However, actin filaments are polyelectrolytes and therefore may have electrochemical properties of biological significance. This is suggested by the previous observations that F-actin has a very large electric dipole moment and exhibits electric birefringence properties (Kobayasi et al., 1964; Kobayasi, 1964). The relationship between electrical events and F-actin integrity is further suggested by the fact that changes in ionic strength bring about the well-known polymerization of actin monomers into polymers (Bárány et al., 1962; Strzelecka-Gòlaszewska et al., 1978*a*, *b*; and Meggs, 1990). The studies in this report were undertaken to ascertain whether the F-actin gel matrix displays electric-charge changes upon an osmotic stress in the presence or absence of electric fields.

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MATERIALS AND METHODS

Dialysis bag experiments

Monomeric actin (G-actin) was prepared from rabbit skeletal muscle by the method of Spudich and Watt (1971). Dextran (average molecular weight 40,000 from Sigma Chemical Co., St. Louis, MO) was used without any further purification. Dialysis bags (3 cm length, 1.5 cm internal diameter, 12–14 kD cut-off) were washed overnight in distilled-deionized water at 4°C and rewarmed at room temperature for at least 3 h before conducting the experiments. Each bag was then pre-equilibrated with the buffer solution in millimolar: 10 imidazole-HCl, 0.2 $CaCl_2$, 0.1 ATP, 140 KCl, 1 $MgCl_2$, pH 8.0. Actin was kept at -70°C in a low ionic buffer until the time of the experiment. The F-actin solution (1–10 mg/ml) was allowed to polymerize for 1–2 h before the actual experiment inside the dialysis bag. The other end of the bag (internal volume ~ 1 ml) was closed with an agar bridge (3% in 100 mM KCl) connected to a chloride-plated silver electrode (World Precision Instruments Inc., New Haven, CT). The bag was placed into a 150-ml beaker filled with the same saline solution in the absence of actin. The beaker contained a twin bridge electrode (reference). The matched set of electrodes was used only if the electric potential difference between them was < 0.5 mV. The circuit was fed to a dual voltage clamp amplifier (Bioengineering, University of Iowa, Iowa City, IA). Continuous recordings were followed with a chart recorder. In some cases, as shown in Fig. 1 *A*, the entire process was followed with a Nicolet 310 (Nicolet Instrument Corp., Madison, WI), memory storage oscilloscope, and plotted on a HP7075 X-Y plotter. Voltages are expressed with reference to the internal solution, i.e., where F-actin is located.

Micropipette experiments

F-actin was prepared and allowed to polymerize in a solution containing in millimolar: 10 imidazole-HCl, 0.2 $CaCl_2$, 0.1 ATP, 140 KCl, 1 $MgCl_2$, pH 8.0. In some experiments the solution was modified to contain 10 mM Hepes, pH 7.4 instead of imidazole. No differences were observed in the electrodynamic response of actin between pH 7.4

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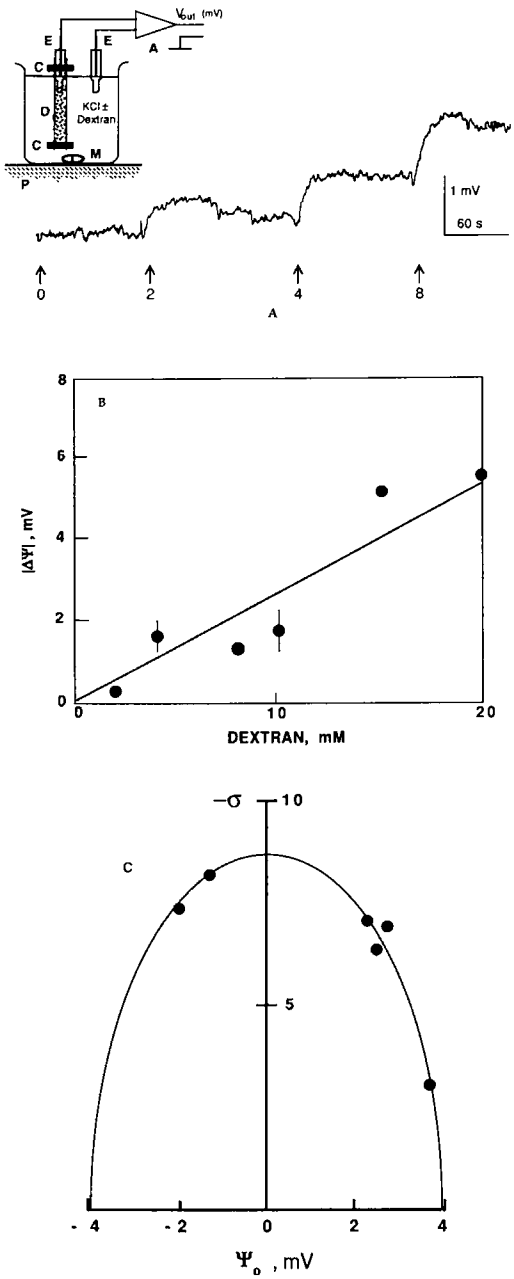

FIGURE 1 (*A*) Electrical measurements from the dialysis bag system. (*Top left*) *D*, dialysis bag containing the F-actin solution; *C*, clamps; *E*, matched electrodes: two different kinds of electrodes were used; first, the saline solution used to polymerize actin was used to gelify agar, 4%, as an ionic interface, the actual electrode, solid bar inside, is an AgCl/Ag interface which was connected to the input of the dual voltage clamp amplifier, *A*, as described in Methods; second, in some cases the AgCl/Ag electrodes were directly used instead. *M*, magnetic stirrer driven by a magnetic plate, *P*. Effect of osmotic gradients on the electrical potential developed by 5 mg/ml actin in solution. External osmotic pressure was altered by addition of dextran at arrows to 0, 2, 4, 8 mOsm. (*B*) Effect of various concentrations of external dextran on the module of the spontaneous Donnan potential, $|\Delta\Psi|$, from plateaus similar to Fig. 1 *A*, values were fitted to a linear regression curve as indicated in the text. The actual value of the potential (considering its sign with reference to the solution containing actin) decreased almost linearly. (*C*) Change in average charge density as a function of the Donnan potential after osmotic stress. Experimental values are indicated as filled circles. The solid line is the best fit of the hyperbolic integration of $d\Psi/dx$ from the plateaus at different osmotic stresses, i.e., different Donnan potentials. Maximal $\Delta\Psi$ was 3.93 mV.

and 8.0. Glass pipettes were pulled from borosilicate glass (WPI) with a Narishige vertical puller to a final tip diameter of < 1 μm. Each micropipette was placed into a vacuum line and allowed to fill a length of ~10–50 μm from the rim with F-actin containing solution. The pipette was then back filled with the "high K" solution and mounted onto a pipette holder connected to the headstage of a PC-501 patch clamp amplifier (current-voltage converter with a feedback resistor of 1 Ω; Warner Inst. Corp., Hamden, CT). The tip of the pipette was then placed in a drop of K^+-containing solution connected through a chloride-plated silver electrode to the ground of the headstage. Usually tip resistance was in the order of 10–50 MΩ in free solution. Signals were filtered with an 8-pole Bessel filter (Frequency Devices, Haverhill, MA) at 100 Hz. Data were displayed on the screen of a Nicolet 310 storage oscilloscope and stored in a desktop personal computer after being digitized with a Tecmar TL-1 board A/D converter. Voltage steps, storage of data, and further analysis of currents were performed with PClamp 5.03 (Axon Instruments, Foster City, CA).

RESULTS

Spontaneous electric potential of F-actin in solution

The spontaneous electric potential developed by F-actin (average length 2–4 μm) was observed by placing a chloride-plated silver electrode into a dialysis bag which contained an F-actin solution and a reference electrode into the external solution which bathed the dialysis bag. Ionic equilibrium between the inside and the outside of the dialysis bag was established before measurements were made. The spontaneous potential induced by a solution of polymerized actin, 5 mg/ml, was 3.93 ± 1.84 (9) mV, negative on the side containing the actin filaments and consistent with a Donnan potential. The equation of the Donnan potential, Ψ, for a solution containing an impermeant electrolyte, i.e., F-actin, is (Collins and Edwards, 1971):

$$\Psi = \frac{RT}{F} \ln\left(\sqrt{1 + \left(\frac{ZaCa}{2Co}\right)^2} + \frac{ZaCa}{2Co}\right), \qquad (1)$$

where Ca = actin concentration; Za = valence of actin; Co = concentration of ions in the external solution; R, T, and F have their usual meaning, so that $RT/F = 25$ mV at 20°C. Because the average molecular weight of a single monomer of actin is 42,000, the actin concentration in

the compartment is ~0.1 mM.

$$\text{Solving(1) for } ZaCa = 2Co \cdot \sinh \frac{\Psi F}{RT} \qquad (2)$$

a calculated $ZaCa$ = 31.6 mEq/l was obtained. Collins and Edwards (1971) obtained a value of 40 mEq/l at pH 7.5 for glycerol-extracted muscle in reasonable agreement with our results. The calculated number of charges per unit length of F-actin was calculated to be 1.65×10^5 e/μm, in close agreement with Elliott's previous report of 1.2×10^5 e/μm in intact muscle (Elliot, 1980; Elliot and Bartels, 1982; Pemrick and Edwards, 1974; Naylor, 1982).

Effect of an osmotic gradient on the electric potential of F-actin

The imposition of osmotic gradients, by the addition of various concentrations of dextran (MW ~ 40,000) to the external solution changed the Donnan potential in a concentration dependent manner (Figs. 1, *A* and *B*). The spontaneous Donnan potential decreased linearly as a function of increasing osmotic stress with a slope of −0.281 ± 0.061 (21) mV/mM (r = −0.7287). Furthermore, the polarity of the potential reversed when the external dextran concentration reached ~10 mM.

A plausible explanation of the observed changes in the potential of osmotically stressed F-actin at pH 8.0 is that the osmotic stress decreases the average distance between filaments thus altering the actual number of self-screened charges about the filaments. Anomalous Donnan behavior has already been postulated by Overbeek (1956) as a result of overlapping of the Gouy-Chapman double layer for highly charged nondiffusible micelles. We interpreted these findings as a quantitative change in the number of charges around the osmotically stressed actin filaments.

The actual electric field about the surface of a single polymer, Ψ_{max}, is given by the Poisson-Boltzmann equation (Overbeek, 1956; Philip and Wooding, 1970; Fixman, 1979; Fixman and Jagannathan, 1981). The macroscopic potential obtained in either the absence or presence of an osmotic stress is a mean Donnan potential which is somewhat lower than Ψ_{max} (Overbeek, 1956; Millman and Nickel, 1980). Despite the fact that Ψ_{max} and the average distance between the filaments are unknown, the overall change in charge density can be calculated from the ratio of absolute potentials before and after an osmotically induced change in Ψ occurs, by assuming that there is a relation between the potential at the surface of the polymer (x = 0), and the macroscopic potential at the average distance between two adjacent filaments (bulk solution) (Overbeek, 1956; Naylor, 1982). The change in charge density at a given osmotic challenge, i.e., a certain average distance, can then be calculated as the change in the number of charges as a function of the two potentials, before and after each osmotic stress, by an integration of the Poisson-Boltzmann equation such that (Overbeek, 1956):

$$d\Psi/dx = -\sqrt{\frac{8\pi cRT}{\xi}}\sqrt{2\cosh F\Psi_m/RT - 2\cosh F\Psi_o/RT}. \qquad (3)$$

Because the boundary condition requires that $d\Psi/dx = 0$ halfway between two given filaments, the compensated average number of charges after an osmotic stress is given by:

$$-\sigma = \sqrt{\frac{\xi cRT}{2\pi}}\sqrt{2\cosh F\Psi_m/RT - 2\cosh F\Psi_o/RT} \qquad (4)$$

in arbitrary units as shown in Fig. 1 *C*, where Ψ_m is the average potential in the absence of an osmotic challenge and Ψ_o is the average potential in the presence of a given concentration of external dextran, each one of them with its own polarity, ξ is the dielectric constant of the solution and c is the ionic concentration in the bulk solution. Eq. 4 predicts that changes in the charge density will be observed after various osmotic stresses as shown in Fig. 1 *C*, such that as the external concentration of dextran is raised, the charge density σ decreases and eventually reverses its polarity.

The above explanation for the anomalous Donnan effect mediated by an osmotic stress on the average charge density is further evidenced by the fact that the change in potential with applied osmotic stress disappeared when the measurements were performed at pH 5.5 (Fig. 2). This lack of response at a pH close to the isoelectric point of actin in solution supports the notion that the electro-osmotic changes are mediated by the actual number of unscreened charges available at any given time (Collins and Edwards, 1971; Pemrick and Edwards, 1974).

The observed electrical properties appear to be a manifestation of the filamentous nature of F-actin in

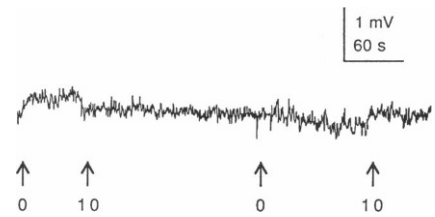

FIGURE 2 Effect of 10 mM dextran on the spontaneous Donnan potential of actin at pH 5.5. Conditions for measurements are similar to the ones in Fig. 1 *A*.

addition to its polyelectrolytic properties. Evidence for this, shown in Fig. 3, was obtained by performing experiments similar to those shown in Fig. 1 *A* substituting bovine serum albumin (a 65-kD globular protein) for F-actin. This protein is also negatively charged at physiologic pH, but does not form filaments. The Donnan potential induced by albumin was not modified by an increase in the osmotic pressure to a level sufficient to induce a 1.5-mV change with an equivalent concentration of actin.

Nonlinear electrical response of osmotically stressed F-actin exposed to an electric field

To further investigate the dynamics of interaction between the counterionic charges and the osmotic stress within the F-actin matrix, an experimental setup was devised to follow electric field-induced ionic movements (Fig. 4, *inset*). The experimental approach consisted of a polymerized actin solution filling the tip of a glass pipette (inner diameter of ~1 μm and a tip impedance between 1–10 MΩ in 140 mM KCl symmetric solutions). The pipette was immersed in a chamber whose content could be changed from normal saline to solutions containing varying concentrations of dextran (see Fig. 4, *inset*). The electro-osmotic response of F-actin upon imposition of a sequence of voltage steps and application of an osmotic stress was followed with a current-voltage converter with a feed-back resistor of 1 GΩ. The dynamic response of osmotically stressed F-actin to imposed electric fields could then be followed. The change in the tip potential of a 5-mg/ml F-actin solution after an osmotic stress in the absence of an electric field was 4.94 ± 0.34 (5) mV, whereas only 0.67 ± 0.24 mV under the same conditions in the absence of the polymer, i.e., the tip potential alone, $p < 0.001$. The value was also statistically different from the voltage induced by an equivalent concentration of BSA, 0.67 ± 0.06 (5) mV. This indicates that in the absence of an electric field the values obtained with the micropipette technique are in close agreement to the ones obtained with the dialysis bag. Different voltage steps were then applied from 20 to 80 mV (electric fields in the order of 670–2,670 V/m). The system was maintained at a steady state potential for 160 ms (fast enough not to induce electrophoretic changes in the actin distribution) after which the field was reversed to −80 mV (Fig. 4). The output current responses before osmotic stress as well as the actual "background" of the KCl solution in the absence of F-actin (measured before the tip was filled with actin) were subtracted from the curves after exposure to an osmotic stress. From the changes in the electric current after reversal of the electric field (and the subtraction of the RC component of the same pipette in the absence of actin) an indication was obtained of the nonlinear relaxation component of the transient current through F-actin. This response was not observed in the BSA-containing solution (Fig. 4). It is clear from the output currents obtained by a reversal of the electric field that the apparent conductance of the osmotically stressed F-actin containing solution was nonlinear.

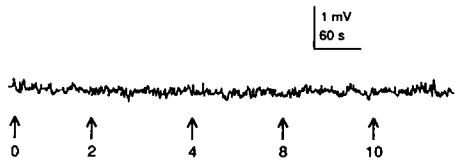

FIGURE 3 Effect of various concentrations of dextran (indicated in mOsm at arrows) on the spontaneous Donnan potential generated by bovine serum albumin, pH = 8.0. Measurements are similar to Fig. 1 *A*.

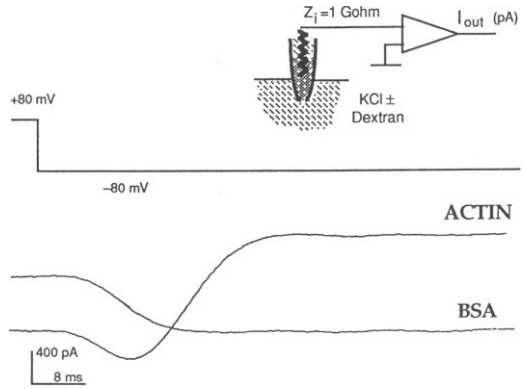

FIGURE 4 Current measurements from the micropipette system. Currents developed by osmotically stressed actin or bovine serum albumin (BSA) upon reversal of an applied electric field indicated on top from + to −80 mV. Details of the actual recording setup are summarized in Methods.

DISCUSSION

F-actin is an extremely long and rigid polymer molecule. These characteristics imply that a gel matrix composed of actin filaments will, under some conditions of length and concentration, be thermodynamically unstable and undergo a phase transition. We have previously demonstrated that changes in osmotic pressure can lead to a sudden change in the compressibility of an F-actin network which is consistent with a phase transition (Ito et al., 1986). An abrupt change in the structure of a system composed of rods, as in the case of F-actin, from

an isotropic to an anisotropic or bundled configuration is a well known phenomenon. While phase transitions induced by electric fields and/or ionic stress in synthetic polymers have been previously demonstrated (Tanaka et al., 1980; Tanaka et al., 1982; Tanaka and Ohmine, 1982), there is no information about these effects on biopolymers. It is reasonable to expect that such a phase transition may lead to alterations in the electro-osmotic properties of the actin gel.

The results in this report indicate that F-actin, upon exposure to an osmotic stress, modifies its spontaneous Donnan potential, at neutral pH but not at the isoelectric point. Furthermore, the electrodynamic behavior of the osmotically stressed gel indicates a nonlinear electric response to imposed electric fields. Because the electric fields imposed under the experimental conditions are not sufficient to induce an electrophoretic effect on the actin filaments, the system displays an extremely sensitive, osmotically induced electrical response that may be the expression of a favored route for counterionic movements in the vicinity of the filaments as compared with the bulk solution. This finding implies a possible role of electrically and/or osmotically directed signals through polymer gels in biological systems, and raises the interesting possibility that actin filaments may serve as intracellular ionic "wires." This behavior, based on the ionic condensation derived from the self-screening of charges about the polymer has been theorized for one-dimensional polymers such as DNA (Parodi et al., 1985).

The resting potential of a cell averages 60 mV across a membrane that is 80-Å thick, implying extremely large electric fields across the cell plasma membrane on the order of 100 kV/cm. Because very small electric fields, on the order of 10 mV/cm are capable of inducing cellular responses (Robinson, 1985), it is tempting to postulate that changes in the field strength at the membrane level may affect the structural status as well as functional properties of cortically located actin filaments (Luther et al., 1983). There is a great deal of evidence to indicate that plasma membrane-mediated responses may be directed into the cytoplasm by cytoskeletal structures including F-actin. For example, the ligand binding to a cellular membrane receptor could lead to the opening of a channel which, in turn, may lead to an electro-osmotic gradient, i.e., the net movement of ions and water, thereby increasing the local actin concentration to an unstable point, thus causing a phase transition. This transition leads to a transient electrical potential change which could act to close the channel and/or activate a membrane transporter such as the Na^+, K^+-ATPase thus ending the reaction. Alternatively, such a sequence of events could give rise to a cascade of reactions leading to a large collapse of the network structure, the consequences of which could be mechanical protrusion or the intracellular transport of vesicles within the cytoplasm.

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