A Novel Method to Study the Electrodynamic Behavior of Actin Filaments. Evidence for Cable-like Properties of Actin

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ABSTRACT Actin, one of the most abundant intracellular proteins, forms long linear polyelectrolytic polymers in solution. A novel technique to handle single actin filaments in solution was developed that allows the study of ionic currents elicited along the surface of electrically stimulated actin filaments. Electrical currents were observed about the polymer's surface under both high (100 mM KCl) and low (1 mM KCl) ionic strength conditions. The data are consistent with a dynamic behavior of the counterionic cloud surrounding the actin filaments that support ionic movements along their longitudinal axis upon electrical stimulation. Counterionic waves were highly nonlinear in nature and remained long after the electrical stimulation of the actin filaments ceased. In this report therefore, we demonstrate that actin filaments can function as biological "electrical wires" and can thus be conceptualized as nonlinear inhomogeneous transmission lines. This ability of actin filaments to conduct electrical signals may have important implications in the coupling of intracellular signals.

INTRODUCTION

Actin filaments are abundant cytoskeletal structures of eukaryotic cells that play an important role in a variety of cell functions including cell shape and locomotion (Stossel, 1984). Actin filaments are one-dimensional polymers that display a high charge density (Kobayasi, 1964; Kobayasi et al., 1964; Cantiello et al., 1991a). This phenomenon is further manifested by the extensive changes in electric dipole moment observed in actin filaments oriented by shear flow (Kobayasi, 1964), the presence of an anomalous Donnan potential (Cantiello et al., 1991a) and a nonlinear electroosmotic response to weak osmotic stress (Cantiello et al., 1991a). The possibility thus exists for actin filaments to provide a suitable means for ionic movements as it was postulated for linear polyelectrolytes (Oosawa, 1970; Oosawa, 1971). No experimental evidence has heretofore been presented to prove the ability of actin filaments to conduct electrical signals. The ability of linear polymers such as actin to enable electrical currents in the form of ionic movements, nevertheless, may be of biological relevance in intracellular signaling mechanisms as we recently demonstrated that actin filaments are functionally coupled to membrane-imbedded ion channels (Cantiello et al., 1991b; Prat et al., 1993).

As charged polyelectrolytes, actin filaments may contain a proportion of their surrounding counterions in the form of a dense or "condensed" cloud about their surface which may be highly insensitive to large changes in the ionic strength conditions of the surrounding saline solution (Manning, 1969; Oosawa, 1970; Zimm, 1986). This tightly "bound" ionic cloud, provided that it is electrically shielded from the bulk solution (Parodi et al., 1985), will allow significant ionic movements along the polymer's length (Oosawa, 1970). The counterionic cloud about an actin filament could be, therefore, a highly conductive medium, a consequence of which is that electrically forced ions entering one end of the polymer will result in ions exiting the other; the ionic gradient lying along the polymer's length. Functionally, actin polymers may thus serve as biological "electrical wires" and can be modeled as nonlinear inhomogeneous transmission lines known to propagate nonlinear dispersive solitary waves (Kolosick et al., 1974; Ostrovskii, 1977). These waveforms can take the form of solitons (Noguchi, 1974; Lonngren, 1978).

In this report we studied the electrodynamic behavior of electrically stimulated single actin filaments in saline solution, specifically, their ability to conduct ionic currents. Data were obtained from single actin filaments using a variation of the "patch-clamp" technique applied to isolated actin filaments in solution. Electrical signals were observed in electrically stimulated actin filaments placed under both, high (physiological), and low ionic strength conditions thus proving that the actual conductive medium capable of enabling electrical signals was the condensed ionic cloud surrounding the polymer's surface. The nonlinear electrical signals elicited by electrically stimulated actin filaments may provide a novel means for intracellular ionic signals.

MATERIALS AND METHODS

Preparation and visualization of single actin filaments

Actin (Sigma Chemical Co., St. Louis, MO) was labeled with rhodamine-phalloidin (Molecular Probes Inc., Eugene, OR) and allowed to polymerize for at least 12 h in an actin-polymerizing solution containing, in millimolar, KCl, 100; $MgCl_2$, 5.0; ATP, 1.0; and 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes), 10, pH 7.4. The solution also contained 1% β-mercaptoethanol and 1 μM rhodamine-phalloidin (Yanagida et al., 1984). At the beginning of the experiment actin filaments were placed under a phase contrast microscope (MT2, Olympus Optical Co.) with a fluorescence attachment in a solution containing 100 mM KCl, 1 mM $MgCl_2$, 10 mM

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Hepes, pH 7.4. Before the experiment started, glucose-oxidase, 10 mg/ml; catalase, 1.8 mg/ml; and glucose, 2.3 mg/ml were added to remove O_2 (Kishino and Yanagida, 1988). This solution was purged under N_2 for 5 min to further reduce O_2 tension and thus prevent O_2-mediated photobleaching (Kishino and Yanagida, 1988). Fluorescent images under these conditions were stable for several minutes. Several criteria were followed to determine the presence of single actin filaments as previously indicated by Yanagida et al. (1984). Namely, the filaments were equally and uniformly bright under fluorescent light microscopy. Because only actin filaments deposited onto the bottom of the chamber were used for the experiments, and these were identified after the supernatant of the actin-containing solution was washed out to prevent the clogging of the pipettes, see below, the expected number of actin filaments for the seeding concentration was not taken as a determinant factor to assume the presence of single actin filaments. Actin filaments were first detected under fluorescent light and lower magnification, usually in clusters. Single actin filaments were "pulled up" as indicated in Results after having visualized them under higher, and phase-contrast magnification. Although the possibility exists that bundles instead of isolated actin filaments were used in our experiments, the even and always constant refringent width of the images under phase contrast was used as a main criterion for choosing the filaments.

Preparation of myosin-containing pipettes

Patch-pipettes with an average diameter of 2–4 μm were prepared as previously reported (Cantiello et al., 1989). Pipette tips were filled by vacuum suction with a myosin-containing solution, 1 mg/ml. Myosin (Sigma Chemical Co.) was used to chemically attach the actin filaments to the pipettes as previously described (Yanagida et al., 1984). Myosin was solubilized in a buffer containing 600 mM KCl and 10 mM Hepes, pH 7.0. Pipettes were back-filled with the same, but myosin-free, solution.

Measurement of electrical signals mediated by single actin filaments

Electrical data were collected with a modified dual "patch-clamp" setup, consisting of two independent patch-clamp amplifiers, one to stimulate a single actin filament and the other to collect current signals. Both signals were simultaneously collected by the two channel-analog input of an A/D converter (TL-1 DMA Interface; Axon Instruments, Inc., Foster City, CA). The sampling interval was 8.0 μs, and the signal length was 400 samples. Signals were Gaussian-filtered at a cutoff frequency of 1.22 kHz. The two pipettes were immersed in a 100 mM KCl solution (see Figs. 2–4). Another set of electrical measurements was also conducted in a low ionic strength (1 mM KCl) conditions with previously polymerized, and phalloidin-labeled actin filaments to confirm that the electrical coupling was indeed mediated by the "adsorbed" ionic cloud about the surface of the polymer (Fig. 5). The myosin-containing pipette tips were connected to the electrodes and headstage of respective patch-clamp amplifiers. A closed circuit was achieved by two silver-chloride plated silver ground electrodes in solution far away (1–2 cm) from the pipette tips.

RESULTS

Mechanical setup to handle isolated actin filaments

The patch-clamp (Hamill et al., 1981) and a recently reported technique to manipulate single actin filaments (Kishino and Yanagida, 1988) were combined to obtain the desired electrodynamic information. Actin labeled with rhodamine-phalloidin was allowed to polymerize overnight at room temperature in an actin-polymerizing buffer enabling long rigid filaments to form (>10 μm) (Kishino and Yanagida, 1988). Actin filaments were placed in a chamber where fluorescent imaging of rhodamine-phalloidin-labeled actin was first used to select isolated filaments (Fig. 1 *A*), which were then visualized under phase-contrast optics (× 198 at the bottom of a chamber, Fig. 1 *B*). The patch-pipettes were placed close to the cluster of actin filaments (Fig. 1 *C*). A single actin filament was then secured at the tip of a patch-clamp pipette under higher magnification (× 298, Fig. 2 *A*). A second pipette was approached (Fig. 2 *B*) and secured to the other end (Fig. 2 *C*). Pipette tips were loaded with a myosin-containing solution allowing for a stable "biochemical" interface as well as mechanically secure connections between actin and the pipette tips (Kishino and Yanagida, 1988). The single actin filament could then be lifted from the bottom of the chamber and handled by the two pipettes in free saline solution (Fig. 2 *D*). Single actin filaments were connected to respective patch-clamp amplifiers through the two pipettes as indicated in the model (Fig. 3 *A*). The mechanical stability of the connection and the rigidity of the actin filament were observed when the actual connection between the actin filament and the pipette was broken by pulling the pipette away from the polymer (Fig. 2 *E*), usually at the end of the experiment.

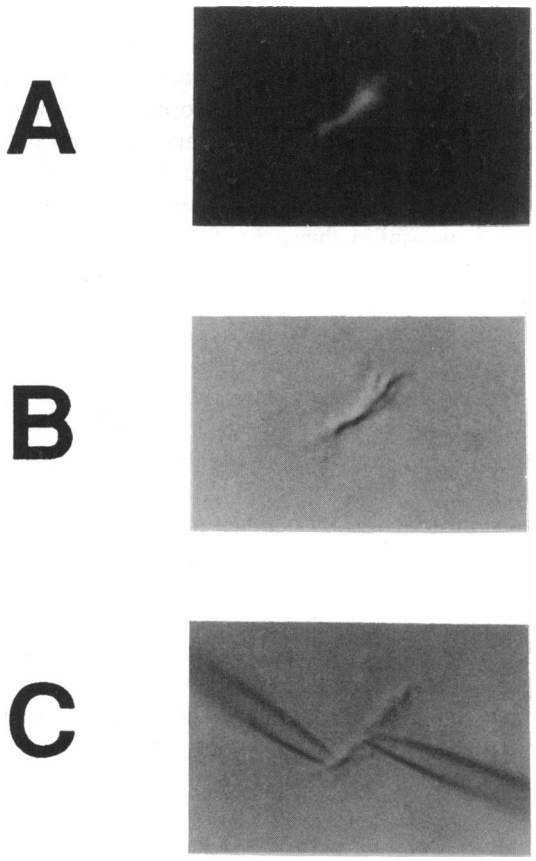

FIGURE 1 Phase contrast and fluorescent microscopy of isolated actin filaments. Preparation of single actin filaments. (*A*) Actin filaments were first visualized by the rhodamine-phalloidin staining under fluorescent light. (*B*) Actin filaments were then focused under phase contrast, × 198 before approaching the two patch-clamping pipettes as indicated in *C*. Actin was labeled with rhodamine-phalloidin and allowed to polymerize for at least 12 h prior to the actual experiment.

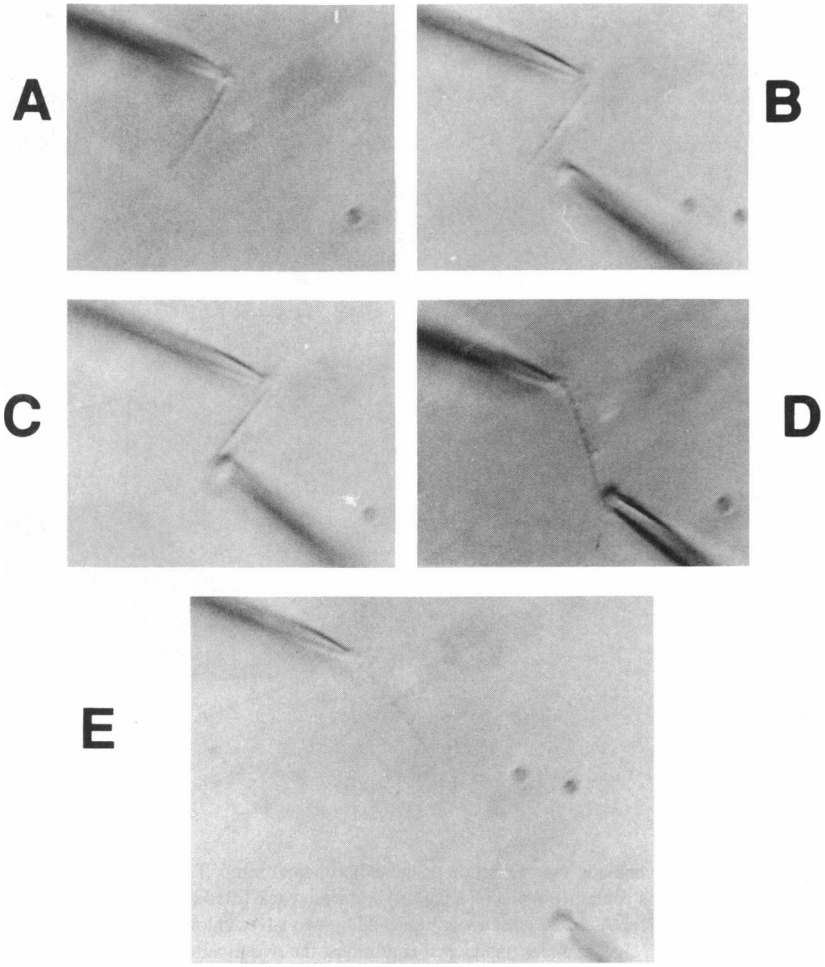

FIGURE 2 Mechanical interaction between a single actin filament and the myosin-containing pipette tips. Once visualized under lower phase contrast magnification, × 198, as in Fig. 1, the objective was switched to higher magnification, × 298 (DPlan Apo x60; Olympus Optical Co.), and an actin filament was singled out from a cluster (*A*) by touching the filament's end with a myosin-containing pipette tip under slight vacuum pressure. A second myosin-containing pipette (*B*) was then approached and attached to the other end of the filament (*C* and *D*). The mechanical stability of the myosin-actin interface was determined by pulling and stretching the actin filament now in free solution. The mechanical attachment between the pipette's tip, and the actin filament was eventually broken to indicate the stability of the connection at the end of the experiment (*E*).

Electrical characteristics of the experimental setup

The coupling characteristics of the two-electrode setup (Fig. 3 *A*) were first determined in the high ionic strength (100 mM), actin-polymerizing solution directly above a single actin filament without physical connection to the polymer. Upon imposing an input voltage pulse to the stimulating pipette (Fig. 4.1 *A*), a dissipated current signal was collected at the collecting pipette before attaching the actin filament to the patch-pipettes (Fig. 4.1 *B*). From the circuit model of the setup (Fig. 3 *B*), the bounds for the current transfer, i_3/i_1, between the collecting and stimulus pipettes, respectively, were calculated by varying the resistances over known possible values of both R_{grnd}, the ground connection of the respective amplifiers, and R_{med}, the saline solution between the pipettes. From the plot in Fig. 3 *C*, the theoretical current transfer in free solution expressed in percentage ranged between 0.03 and 3.9%, in agreement with the calculated value obtained from data of 10 collected signals, 2.63 ± 0.1%. In contrast, once the two pipettes were physically connected to an isolated actin filament at the same distance between pipettes as in the unattached set-up, a larger signal, 5.7 ± 0.6% ($p < 0.001$), was observed at the collecting pipette (Fig. 4.1 *D*) for the same input pulse and thus consistent with the hypothesis that actin filaments can support ionic movements in the form of nonlinear electrical currents. Whenever no voltage was imposed to the input pipette, no change in the collected signal was observed (Fig. 4.1 *C*).

Electrical signals elicited by electrically stimulated actin filaments under high ionic strength conditions

Using the same experimental protocol for the input pulse, the collected signals for the actin-attached setup (Figs. 4.2, *A–F*) revealed the presence of a significant wavetrain long after the input pulse had ended. This was not the case in the unattached setup (Fig. 4.1 *B*). The wave patterns observed in electrically stimulated actin filaments are remarkably similar to recorded solitary waveforms from various experimental studies on electrically stimulated nonlinear transmission lines (Kolosick et al., 1974; Noguchi, 1974; Ostrovskii et al., 1974; Bogatyrev and Fainshtein, 1976; Lonngren, 1978; Stewart and Corones, 1978). Considering the actin filament's highly nonlinear complex physical structure (Pollard and Cooper, 1986) and the thermal fluctuations of the counterionic cloud from the average distribution (Oosawa, 1970; Oosawa, 1971), our observation of soliton-like ionic charge

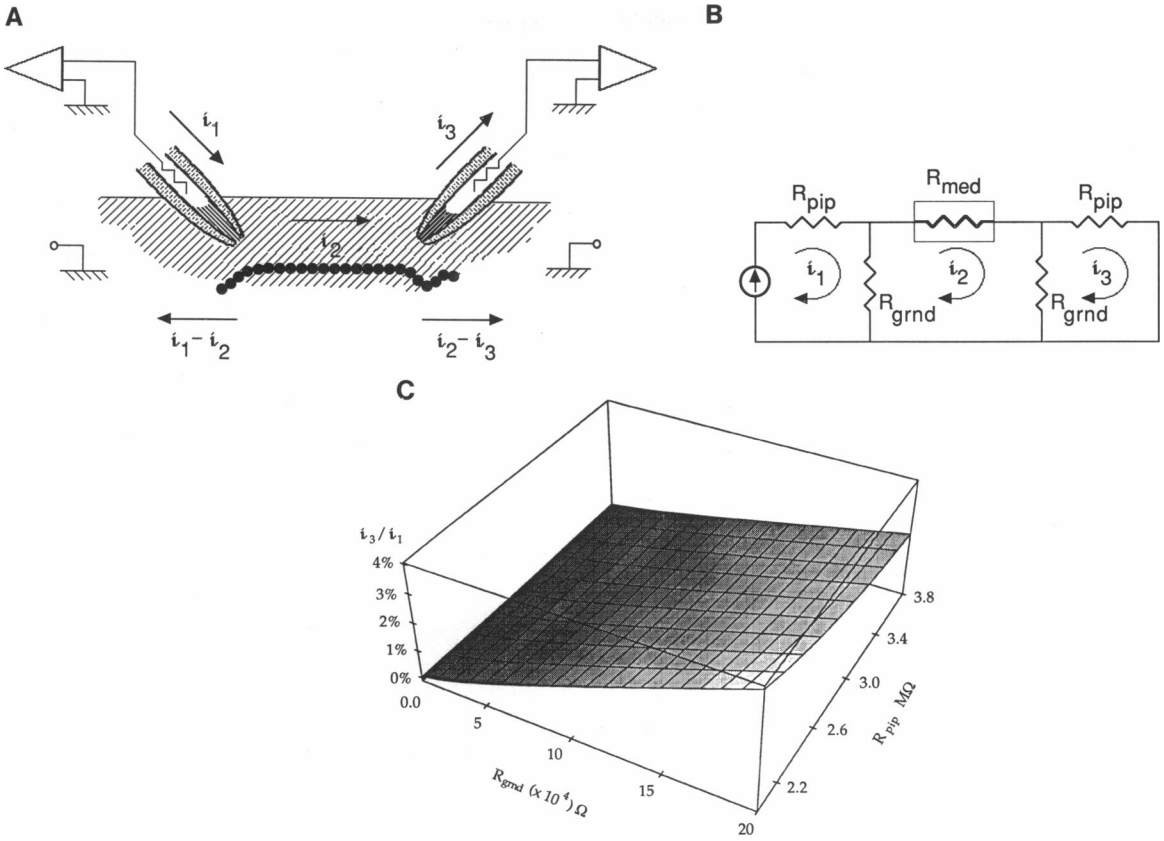

FIGURE 3 (*A*) Schematic representation of the experimental setup. The two pipettes were immersed in a 100 mM KCl solution as indicated in Fig. 2, *B–D*. The pipette tips contained myosin (1 mg/ml) and were back-filled with 600 mM KCl, connected to the electrodes and the headstage of the respective patch-clamp amplifiers. A closed circuit was achieved by two silver-chloride plated silver ground electrodes in solution far away (1–2 cm) from the pipette tips. After collecting signals under unattached conditions, the two pipettes moved downward "connecting" to a single actin filament indicated as a dotted line in filled circles. The currents shown are the following: i_1, the current coming out of the stimulus pipette and proportional to the input voltage pulse signal; i_2, the current coupling both pipettes in the free solution medium; and i_3, the incoming current to the collection pipette. The differences ($i_1 - i_2$), and ($i_2 - i_3$) represent leak currents to ground through their respective bridges. (*B*) Electrical circuit model of pipettes in free solution. The circuit model represents the current meshes obtained for the lumped circuits between the input and collection pipettes. R_{pip} is the pipette tip resistance of 2 – 3.8 MΩ as measured or previously reported (Schanne and Ruiz P.-Ceretti, 1978). R_{grnd} represents resistance to ground connected by either agar bridges or plated silver electrodes as above to the feedback loop of the patch-clamp amplifier. Values ranged from 10 to 200 kΩ (Schanne and Ruiz P.-Ceretti, 1978). R_{med} is the net resistance of the current pathway between the two pipettes prior to coupling to an actin filament, 80 kΩ (Schanne and Ruiz P.-Ceretti, 1978). The current source represents the current flowing out of the stimulus pipette, as indicated by *A* and measured in Fig. 4.1 *A*. (*C*) Maximum current transfer values for various R_{grnd} and R_{pip}. The electrical circuit in *B* was solved for the ratio i_3/i_1, the current transfer parameter. The relationship obtained was:

$$i_3/i_1 = R_{grnd}^2/((2{*}R_{grnd} + R_{med})(R_{pip} + R_{grnd}) - R_{grnd}^2).$$

This equation was plotted for different values of R_{grnd} and R_{pip}, with ranges selected as mentioned in Fig. 3 *B* and shown above. For the ranges of R_{grnd} and R_{pip} used, the current transfer expressed as percentage was between 0.03 and 3.9%.

waves is consistent with the idea of actin filaments functioning as biological transmission lines. This was also supported by the lack of waves in the attached conditions in the absence of an electrical stimulus (Fig. 4.1 *C*).

Electrical signals elicited by electrically stimulated actin filaments under low ionic strength conditions

The ability of actin filaments to conduct electrical signals was also determined in a low ionic strength solution containing 1 mM KCl, 5 mM ATP, and 1 mM Hepes, pH 7.4. Actin filaments were obtained and visualized as described before. The solution bathing the filaments was immediately replaced with the solution described above to decrease the bulk ionic strength. Electrical signals were then collected with the same electrical setup before and after the actin filaments were attached to the patch-pipettes. In the absence of a mechanical coupling between the patch-pipettes and the actin filament only the capacitive charge movements elicited by a 150 mV square pulse (*inset*) were observed (*dashed line*, Fig. 5, left). Instead, applying the same electrical protocol after attachment of the actin filaments to the pipettes revealed the presence of significant nonlinear wavetrains long after the input pulse had ended (Fig. 5, right).

DISCUSSION

Actin filaments can be considered one-dimensional polymers that display a very high charge density as we and others have

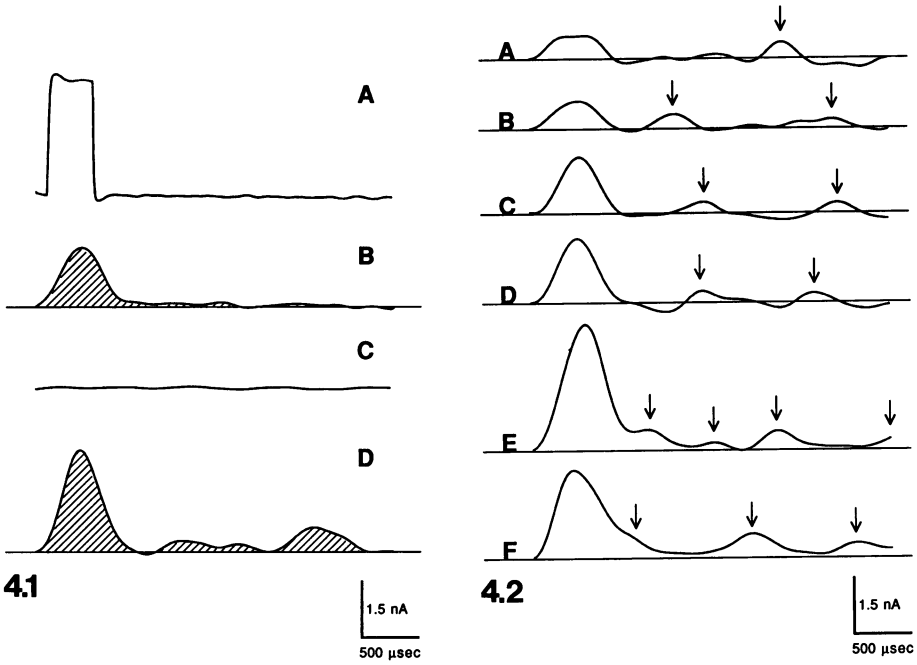

FIGURE 4 Part 4.1 (left): Electrical signals mediated by single actin filaments in a high ionic strength solution. Data were collected with a modified dual patch-clamp setup, consisting of two independent patch-clamp amplifiers, one to stimulate a single actin filament (input pipette and signal) and the other to collect current signals (collecting pipette and signal). (*A*) A representative current signal (scaled 1/20) at the stimulus pipette (i_1) as a response to a single square pulse of amplitude 199 mV, and a total duration 800 μs. The actual R_{pip} for the example depicted was 2.93 MΩ and increased to 4.4 MΩ after attachment to the actin filament. (*B*) A representative signal at the collecting end in an unattached experiment where the two pipettes were coupled solely by free solution and stimulated with input as shown in Fig. 4.1 *A*. A single actin filament was then attached to both pipettes. Fig. 4.1 *C* was collected in the absence of an input pulse; no signal was apparent. *D* was collected under same applied input (*A*) in the actin-attached experiment. *D* shows the presence of a complex nonlinear wavetrain of charges propagating from one pipette to the other. The shading in *B* and *D* represent the total charge transferred; integration was approximated by sample-by-sample summation yielding a total charge of 266.8 vs. 599 pC for the free solution and the actin attached, respectively. Part 4.2 (*right*): Effect of applied voltages on electrical signals mediated by single actin filaments. (*A–F*) collected signals similar to Fig. 4.1 *D* except that were obtained under varying voltage amplitudes for the input pulse. 50 mV were applied for *A* and *B*, 150 mV for *C* and *D*, and 199 mV for *E* and *F*. Signals show the large nonlinear characteristics of the current wavetrains. The arrows indicate trailing waves that are formed by the input pulse "fissioning" as it propagates down the filament. Although the number of these waves could not be predicted nor characterized with our available data, more and faster waves were observed as the input voltage pulse increased.

previously demonstrated (Kobayasi, 1964; Kobayasi et al., 1964; Cantiello et al., 1991a). The average charge density of isolated actin filaments, by assuming a linear distribution of counterions per unit length of polymer, was calculated to be 1.65×10^5 e/μm in our previous studies (Cantiello et al., 1991a), in close agreement with the original reported value of 75 Debye/monomer (which renders a value of approximately 5.02×10^5 e/μm) from birefringence studies of electric field oriented actin filaments (Kobayasi et al., 1964). Interestingly, much higher values have also been reported for actin filaments oriented by shear flow (Kobayasi, 1964). A charge density of this magnitude, at least 40 times higher than that expected for DNA (Zimm, 1986), is extremely high for a protein the size of the actin monomer (Holmes et al., 1990; Kabsch et al., 1990) and clearly no explanation for such a value can be obtained from the linear net charge surface distribution derived from the amino acids associated with the actin monomer. Because the available atomic models for the actin molecule are derived from the crystal structure of the monomer (Holmes et al., 1990; Kabsch et al., 1990), the possibility exists that other explanations for the seemingly high charge density of actin may arise from nonlinear interactions between the hydrated molecule with its surrounding counterions, which are not taken into account in the modeling of the molecule and that may play an important role in its electrodynamic properties (Rullman and van Duijnen, 1990). Due to the helical nature of the actin filament (Holmes et al., 1990), nevertheless, a nonuniform counterion distribution along the polymer may be expected with uneven electric fields arranged in peaks and troughs as originally postulated by Oosawa (1971). This uneven distribution of charges would entail both large changes in the local density of small ions around the polymer, and the consequent large dielectric discontinuity in the ionic distribution (Anderson and Record, 1990). This hypothesis, which awaits experimental proof, is consistent with the fact that both the highly charged amino and carboxyl termini of the actin monomer lie on the surface of the molecule (Kabsch et al., 1990). From an experimental viewpoint, the high charge density attributed to the actin filament is, nevertheless, supported by several phenomena associated with actin in solution, namely, the coexistence of different electric (Kobayasi, 1964; Kobayasi et al., 1964) and magnetic (Torbet and Dickens, 1984) dipole

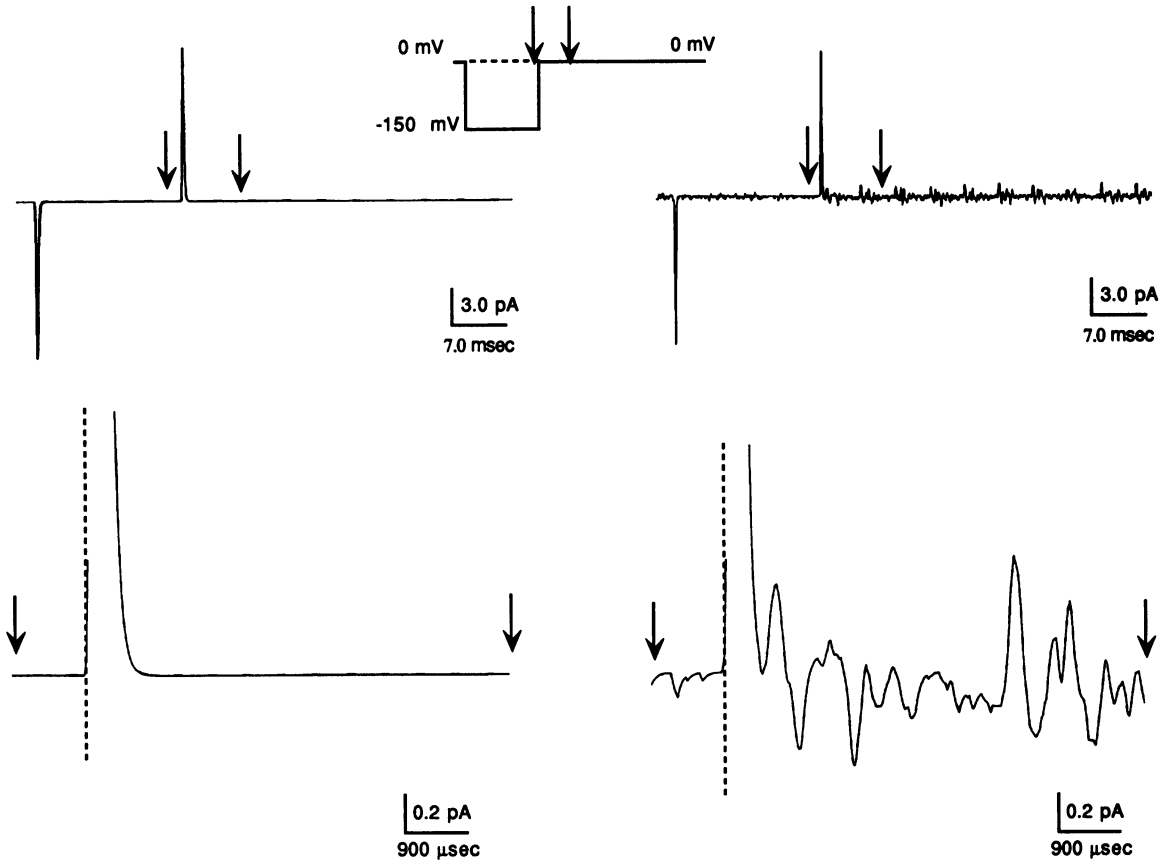

FIGURE 5 Electrical signals mediated by single actin filaments under low ionic strength conditions. Data were collected with the same experimental setup as per the experiments in Fig. 4, only modified in that the solution bathing the actin filaments only contained 1 mM KCl, 5 mM ATP, and Hepes, 1 mM, pH 7.4. Top: Representative current signals at the collecting pipette are shown in an unattached experiment where the two pipettes were coupled solely by free solution and stimulated with input, left, and after the pipettes were attached to an actin filament, right. Signals were collected as a response to a single square pulse of amplitude 150 mV and a total duration of 32 ms. A single actin filament was then attached to both pipettes and signals were obtained with the same applied input (*inset*). Bottom: Detail of the electrical currents collected on top as indicated in between the arrows. The left side of the dashed line indicates an applied voltage of −150 mV and to its right the current signal after the system went back to 0 mV. The presence of a complex nonlinear wavetrain of charges propagating from one pipette to the other was only observed after actin was attached to the patch-pipettes, right. Signals were filtered at 1.2 kHz with a Gaussian filter.

moments in actin filaments, the anomalous Donnan potential, and the highly nonlinear, osmotic (Ito et al., 1987), and electro-osmotic (Cantiello et al., 1991a) behavior of actin filaments in solution.

In order for linear polyelectrolytes to behave as molecular wires, the radial dissipation of counterions has to be constrained; thus self-screening has to be postulated (Parodi et al., 1985). This might be the case for actin filaments as well. Therefore, actin filaments may contain an uneven proportion of their surrounding counterions in the form of "condensed" clouds about their surface (Manning, 1969; Iwasa, 1977; Manning, 1978; Oosawa, 1970; Zimm, 1986). This can be supported. If we assume for simplicity a linear charge distribution about the actin polymer, the linear charge density parameter, ξ, calculated for the actin filament is much greater than $1/z$, where z is the valence of the counterions in solution, and ξ is defined as $e^2/4\pi\epsilon DkTb$ with e being the electron charge, ϵ the permittivity constant, D the dielectric constant of water, k the Boltzmann's constant, T the temperature, and b the average axial spacing of charges on the polyelectrolyte (Zimm, 1986). For actin filaments, ξ was calculated to be 110.2 (H^+ and K^+ with $z = 1$), and thus $\xi \gg 1/z$ (Cantiello et al., 1991a). Therefore, approximately 99% of the total counterionic population (Zimm, 1986) is predominantly constrained within a radius of 8 nm (two average radii) (Pollard and Cooper, 1986) around the polymer's radial axis (Zimm, 1986). Lower charge densities would only modify the radial interaction between the filaments and their condensed counterions, not its existence. Significant ionic movements will therefore occur within this radial ionic cloud resulting in a highly conductive medium (Oosawa, 1970) which is electrically shielded from the bulk solution (Parodi et al., 1985). A consequence of this behavior follows in that electrically forced ions entering one end of the polymer will result in ions exiting the other; the ionic gradient lying along the polymer's length. An experimental proof for this interaction is heretofore unavailable. Functionally, actin polymers may thus serve as biological "electrical wires" and can be modeled as nonlinear inhomogeneous transmission lines able to propagate nonlinear dispersive waves and solitons (Kolosick et al., 1974; Noguchi, 1974; Ostrovskii et al., 1974; Lonngren and Scott, 1978).

Actin filaments were observed and mechanically isolated in this report to enable the direct measurement of electrical signals from single polymers in solution. Electrically stimulated actin filaments were able to conduct highly nonlinear electrical signals both under high and low ionic strength conditions. The fact that electrical signals were also observed under limiting conditions for the bulk ions further supports the hypothesis that condensed ions around the filaments are the conductive medium. Interestingly, the wave patterns observed in the electrically stimulated actin filaments were remarkable similar to recorded solitary waveforms from various experimental studies on electrically stimulated nonlinear transmission lines (Kolosick et al., 1974; Noguchi, 1974; Ostrovskii et al., 1974; Bogatyrev and Fainshtein, 1976; Lonngren, 1978; Stewart and Corones, 1978). Nonlinear solitary waves, and in particular solitons, are novel physical phenomena already well established in several fields of physics and engineering (Lonngren and Scott, 1978; Dodd et al., 1982) and are particularly well characterized for nonlinear transmission lines modeled by discrete lumped circuits (Hirota and Suzuki, 1970; Hirota and Suzuki, 1973; Kolosick et al., 1973; Lonngren and Scott, 1978). Upon pulse excitation, an input square pulse will decompose into a finite number of solitons and a low amplitude oscillatory tail (Lonngren and Scott, 1978). The nondissipative nature of this decomposition adheres to current and voltage conservation according to the equations $\int I_n(t)\,dt$ = constant and $\int V_n(t)\,dt$ = constant, where I_n (V_n) is the current (voltage) measured at the nth nodal component along the line (Hirota and Suzuki, 1973). Adapting this discrete model to the actin filament entails that each actin monomer thus represents an electrical node, and the collecting pipette was "connected" to the nth one. By performing the above current integration (Fig. 4.1, *B* and *D*) on the two previous sets of 10 collected signals, the total charge that propagated from the stimulating pipette to the collecting one was calculated. For the unattached conditions, the total charge transferred was 237 ± 18.5 pC, compared to 545 ± 67.8 pC, ($p < 0.001$) for the actin-attached case, thus indicating that more net charge was transferred via actin filaments than via free solution. Interestingly, the ionic strength conditions of the saline solution (100 mM KCl), makes this coupling mechanism feasible in the intracellular milieu. Further proof for both ionic condensation about the polymer's surface and the ability of actin filaments to conduct nonlinear ionic waves was obtained when actin filaments were placed in a low ionic strength solution. Under these conditions only the "condensed" ionic cloud around the filaments could serve as the conductive medium for electrical coupling to take place. The particular characteristics of these waves including number and/or amplitude could not be predicted nor characterized with our available data. Nevertheless, an increase of number of faster waves was observed in electrically stimulated actin filaments as the input voltage pulse increased (Fig. 4.2, *A–F*), a property of the ionic waves which is consistent with solitary wave behavior (Lonngren and Scott, 1978; Miura, 1978).

Actin is a relevant intracellular protein involved in a variety of cellular functions including cell shape and locomotion, and in particular muscle contraction. A dynamic actin filament organization entails a potential diversity in the various cell responses. Actin can undergo changes in its polymeric status, such as G to F-actin (monomeric to polymeric) transformations, and, more importantly, a wide variety of cross-linked conformations mediated by relevant actin-binding proteins (Stossel, 1984; Pollard and Cooper, 1986). However, the electrodynamic properties of actin filaments observed in this report are based on their highly polarized behavior and effective electrical shielding that will remain stable throughout the various dynamic conformations. In solution, polyelectrolytes will tend to electrically repel one another (Oosawa, 1970; Oosawa, 1971) thus minimizing the "cross-talk" between neighboring polymers. Considering a calculated effective cloud radius around the actin filament of 8 nm, twice the average radius of the actin filament itself, it remains highly likely that actin filaments can exist in a complicated dynamic network and still preserve electrical screening from one another. One can envision an electric field fluctuation, such as that elicited by a single ion channel opening to which an actin filament can be attached (Cantiello et al., 1991b; Cantiello et al., 1993; Prat et al., 1993) as a novel and efficient long-range intracellular signaling mechanism whose significance will be dictated by the overall status of its organization. Therefore, based on our findings one may expect that electrically stimulated actin filaments generate soliton-like wave patterns based on the nonlinear wave behavior of ionic signals probably elicited by condensed ions around actin filaments. These ionic waves may play a relevant biological role as a novel intracellular signal transduction mechanism.

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