# Electrophoresis and orientation of F-actin in agarose gels

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ABSTRACT F-Actin was electrophoresed on agarose gels. In the presence of 2 mM $MgCl_2$ and above pH 8.5 F-actin entered 1% agarose; when the electric field was 2.1 V/cm and the pH was 8.8, F-actin migrated through a gel as a single band at a rate of 2.5 mm/h. Labeling of actin with fluorophores did not affect its rate of migration, but an increase in ionic strength slowed it down. After the electrophoresis actin was able to bind phalloidin and heavy meromyosin (HMM) and it activated $Mg^{2+}$-dependent ATPase activity of HMM. The mobility of F-actin increased with the rise in pH. Acto-S-1 complex was also able to migrate in agarose at basic pH, but at a lower rate than F-actin alone. The orientation of fluorescein labeled F-actin and of fluorescein labeled S-1 which formed rigor bonds with F-actin was measured during the electrophoresis by the fluorescence detected linear dichroism method. The former showed little orientation, probably because the dye was mobile on the surface of actin, but we were able to measure the orientation of the absorption dipole of the dye bound to S-1 which was attached to F-actin, and found that it assumed an orientation largely parallel to the direction of the electric field. These results show that actin can migrate in agarose gels in the F form and that it is oriented during the electrophoresis.

## INTRODUCTION

In spite of the difficulties associated with the electrophoresis of native proteins due to their complex shapes and small charges, they can be fractionated by electrophoresis (or by isoelectric focusing; for review see Chrambach, 1980). In particular, native gel electrophoresis allows one to measure (during the fractionation) the catalytic activity of various enzymes (Siciliano et al., 1976; Moody and Dailey, 1983; Nagy and Simon, 1983), to observe the degree of polymerization of native polymers (e.g., tubulin; Kravit et al., 1984) and to fractionate isoenzymes (e.g., myosin; Hoh et al., 1976; d'Albis et al., 1979; Takano-Ohmuro and Kohama, 1987). If it were possible to carry out the electrophoresis of native F-actin (and of a complex of F-actin with myosin fragments), one could likewise measure the activity of acto–heavy meromyosin (HMM)[1] (or acto-S-1) during the electrophoresis and one could estimate the weight (or number) distribution of F-actin sizes. In addition, one should be able to measure the affinity of binding of F-actin to myosin fragments without centrifugation or labeling of the proteins (because the migration of myosin fragments should be different than that of their complex with F-actin). Finally, if the electrophoresis of acto-S-1 were possible, one could construct an experimental system where purified F-actin was immobilized (by the gel matrix in the absence of the field) and where it could interact with S-1 in the presence of ATP. The best studied system in which actin and myosin interact with each other in the presence of ATP is a muscle fiber, but specific labeling of proteins in vivo is difficult (Miyanishi and Borejdo, 1988). This has led to the development of an in vitro assay (Kron and Spudich, 1986), but its practical implementation is complex. With these objectives in mind, we looked for conditions under which the electrophoresis of F-actin would be possible.

F-Actin should be able to migrate in a gel by a process similar to that of another large polyanion: DNA. One explains the ability of the large molecular weight linear polymers with a mean end-to-end distance of the order of microns such as DNA to migrate through gels with a pore size of the order of hundreds of angstroms by postulating that they "reptate" through the matrix like a snake making way through the undergrowth (Lumpkin et al., 1985; Slater and Noolandi, 1986). The theory of reptation predicts that during electrophoresis the long axis of a polymer assumes approximately parallel orientation with respect to the direction of the electric field, and that the degree of orientation increases with the strength of the field (Lumpkin et al., 1985). In the case of lambda DNA the predictions of the theory have been examined using

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[1] *Abbreviations used in this paper:* CB, Coomassie Brilliant Blue R; DTT, dithiothreitol; EB, ethidium bromide; HMM, heavy meromyosin; 1,5-IAEDANS, 5-iodoacetamidoethylaminonaphtalene-1-sulfonic acid; 5-IAF, 5-iodoacetamidofluorescein; $NaN_3$, sodium azide; S-1, myosin subfragment 1; TRITC-P, tetramethylrhodamine isothiocyanate phalloidin.


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linear dichroism techniques and it was found that excellent DNA orientation was induced by even small field gradients (Holzwarth et al., 1987; Baase et al., 1988; Borejdo and DeFea, 1988; Borejdo, 1989). F-Actin, a linear polymer with large axial ratio, was expected to move through the gel by a process similar to a reptation and thus to orient itself during the electrophoresis with respect to the direction of the electric field.

In this paper we report a successful attempt to carry out the electrophoresis of F-actin and a measurement of the orientation of a complex of F-actin and S-1 during electrophoresis. The main ideas which made it possible were: (*a*) The electrophoresis was carried out using agarose gels, taking advantage of their large pore size. In fact large DNA (up to $80 \times 10^6$ D) can be resolved in such gels (Stellwagen, 1985; larger DNAs require the use of reverse field electrophoresis). (*b*) The electrophoresis was carried out at alkaline pH (consistent with not denaturing or depolymerizing F-actin), which imparted to the F-actin a net negative charge. Using this approach we were able to make native, polymerized F-actin enter 1% agarose gels. We showed that after the electrophoresis actin was still in the filamentous form (namely that it bound phalloidin), that it was not denatured, i.e., that it was able to bind heavy meromyosin (HMM) and that it activated $Mg^{2+}$-dependent ATPase activity of HMM. The fluorescein chromophore bound to F-actin was found to be quite mobile and the measurement of the orientation of the labeled F-actin in the gel was difficult. However, the same label attached more rigidly to S-1. Because acto-S-1 also migrated through the gel (albeit considerably slower than F-actin), we were able to measure the orientation of the complex during the electrophoresis. We found that the complex was oriented and that the absorption dipole of fluorescein-labeled S-1 which was bound to F-actin assumed orientation largely parallel to the electric field.

## MATERIALS AND METHODS

### Materials

Tetramethylrhodamine isothiocyanate phalloidin (TRITC-P) was obtained from Sigma Chemical Co. (St. Louis, MO). Lambda DNA and the electrophoresis grade agarose were from BRL Laboratories (Gaithersburg, MD). 5-Iodoacetamidoethylaminonaphtalene-1-sulfonic acid (1,5-IAEDANS) and 5-iodoacetamidofluorescein (5-IAF) were from Molecular Probes Inc. (Eugene, OR).

### Solutions

Composition of solutions is shown in Table 1. Phalloidin staining solution contained 0.1 mg/ml fluorescent phalloidin in 50% methanol and 7.5% acetic acid. Coomassie Blue staining solution contained 50% methanol, 7.5% acetic acid, and 0.25% Coomassie Brilliant Blue R (CB; EM Science, Cherry Hill, NJ). Destaining solution contained 25% ethanol and 7.5% acetic acid.

**TABLE 1 Composition of solutions**

| Solution | KCl | $MgCl_2$ | Tris | ATP |
|---|---|---|---|---|
| G-buffer | 0 | 0 | 2 | 0.2 |
| F-buffer | 0 | 2 | 2 | 0.2 |
| KCl F-buffer | 50 | 2 | 2 | 0.2 |
| Tris F-buffer | 0 | 2 | 20 | 0.2 |
| −ATP F-buffer | 50 | 2 | 2 | 0 |
| +ATP F-buffer | 50 | 2 | 2 | 0.5 |
| (S-1)-actin* | 30 | 2 | 10 | 0 |

All concentrations in millimolar. All solutions contained 0.1 mM $CaCl_2$, 0.2 mM DTT, and 0.1 mM $NaN_3$. pH was adjusted with HCl to 8.8 at room temperature. *This solution had pH adjusted to 8.5 at room temperature.

### Actin preparation and labeling

Actin was prepared according to Spudich and Watt (1971). It was labeled at Cys-374 with 1,5-IAEDANS by incubating G-actin with 1.5 molar excess of the dye for 12 h on ice in the dark. Labeled actin was dialyzed overnight against large volume of a buffer containing 0.2 mM ATP, 0.1 mM $Ca^{2+}$, 0.2 mM DTT, 0.1 mM $NaN_3$, and 2 mM Tris-HCl buffer, pH 8.0, and centrifuged at 40,000 rpm in a type 50 rotor for 2 h to clarify depolymerized actin. It was then divided into two parts: one half was polymerized by the addition of 50 mM KCl, 2 mM $MgCl_2$, and the other was used as a G-actin control. Polymerized actin was centrifuged at 40,000 rpm in a type 50 rotor for 2 h, the supernatant containing any dye remaining after dialysis was decanted, and F-actin was resuspended by gentle homogenization in F-buffer. Actin was labeled with 5-IAF by incubating F-actin with 3 molar excess of 5-IAF for 6 h at room temperature in the dark. The reaction was stopped by adding 10 mM 2-mercaptoethanol; F-actin was centrifuged for 2 h at 40,000 rpm at 4°C, the supernatant was decanted, actin was resuspended in G-buffer at pH 8.0 by gentle homogenization and was dialyzed against G-buffer at pH 8.0. After dialysis actin was centrifuged at 40,000 rpm for 2 h at 4°C, the supernatant was retained and polymerized by the addition of 50 mM KCl and 2 mM $MgCl_2$.

### Preparation of HMM and S-1

Myosin was prepared from rabbit skeletal muscle by the method of Tonomura et al. (1966). HMM and S-1 were obtained by digestion of myosin according to Weeds and Pope, 1977, and Weeds and Taylor, 1975, respectively. HMM was labeled with 1.5 molar excess of 1,5-IAEDANS at $SH_1$ as previously described (Miyanishi and Borejdo, 1988). S-1 was labeled at $SH_1$ with 5-IAF by incubating it with 3 molar excess of the dye for 6 h in the dark at 0°C, stopping the reaction with 100 mM 2-mercaptoethanol and overnight dialysis against 50 mM KCl, 10 mM Tris, pH 8.

### Electrophoresis

This was carried out in a mini sub gel apparatus (Bio-Rad Laboratories, Richmond, CA). Unless otherwise stated, the strength of the electric field was 2.14 V/cm. For electrophoresis of F- and G-actins, 1 g of agarose was dissolved in 100 ml of F-buffer and G-buffer, respectively,

and the gels were cast at a thickness of 5 mm. G-Actin was run in a G-buffer and F-actin in an F-buffer. To prevent large change in pH during the electrophoresis, the running buffers were replaced with fresh solutions every hour.

## Polarization measurements

Steady-state polarization of fluorescence of 5-IAF labeled actin was measured using an SLM 8000 fluorometer (SLM Instruments, Urbana, IL) equipped with Glan-Thomson prisms. Excitation was at 488 nm and emission at 515 nm. A KV 500 filter (Schott Glass Technologies Inc., Duryea, PA) was used in the compensating channel (channel B).

## Dichroism measurements

Fluorescence-detected linear dichroism measurements of actin were done as previously described for DNA (Borejdo and DeFea, 1988; Borejdo, 1989), except that the data was collected using single photon counting and digital processing. The signal to the Pockels cell was provided by the MetraByte CTM-05 counter (MetraByte Co., Taunton, MA) and was amplified by a wide-band amplifier to 0-260 V. The discriminated photomultiplier pulses were counted synchronously with the signal provided to the Pockels cell by the same MetraByte counter, so that the detected fluorescence corresponded alternatively to a parallel and perpendicular excitation of the fluorescence dipole. The second channel of the MetraByte counter detected a suitably attenuated electrophoresis voltage which was converted to frequency by a home-made V/F converter. Dichroic ratio was computed as a ratio of the fluorescence intensity obtained when the gel was illuminated with the light polarized perpendicularly to the direction of the electric field and the fluorescence intensity obtained when the gel was illuminated with the light polarized parallel to the direction of the electric field. Therefore a dichroic ratio >1 signifies the orientation of the transition dipole of the dye more nearly in the direction perpendicular to the direction of the electric field and a ratio <1 signifies the orientation of the dipole more nearly in the direction parallel to the direction of the field. The dichroic ratio was computed 10 times a second and plotted on a Zeta plotter (Nicolet Co., Martinez, CA).

# RESULTS AND DISCUSSION

## Migration of actin in gels

G-Actin was labeled with 1,5-IAEDANS and electrophoresed on 1% agarose gels in G-buffer at pH 8.8 (Fig. 1 *A*). In Fig. 1 *B* the same actin has been first polymerized as described under Materials and Methods and then run in F-buffer. The migration time and the electric field were identical for the two (but the electric current during the electrophoresis of the G-actin was 2.3 times lower because the resistance of the G-buffer was correspondingly higher). When the origins of the two gels were aligned it was clear that the F-actin migrated 3.8 times slower then G-actin. Fig. 1, *C* and *D*, shows the electrophoretic pattern of the same actin visualized by staining with Coomassie Blue.

The mobilities of 1,5-IAEDANS modified and of native F-actins were identical showing that the negative charge of 1,5-IAEDANS didn't affect the rate of migration of F-actin. The same was true for F-actin modified with 5-IAF. This finding allowed us to routinely use labeled actin to monitor electrophoresis during the run and to measure the orientation of F-actin in the gels.

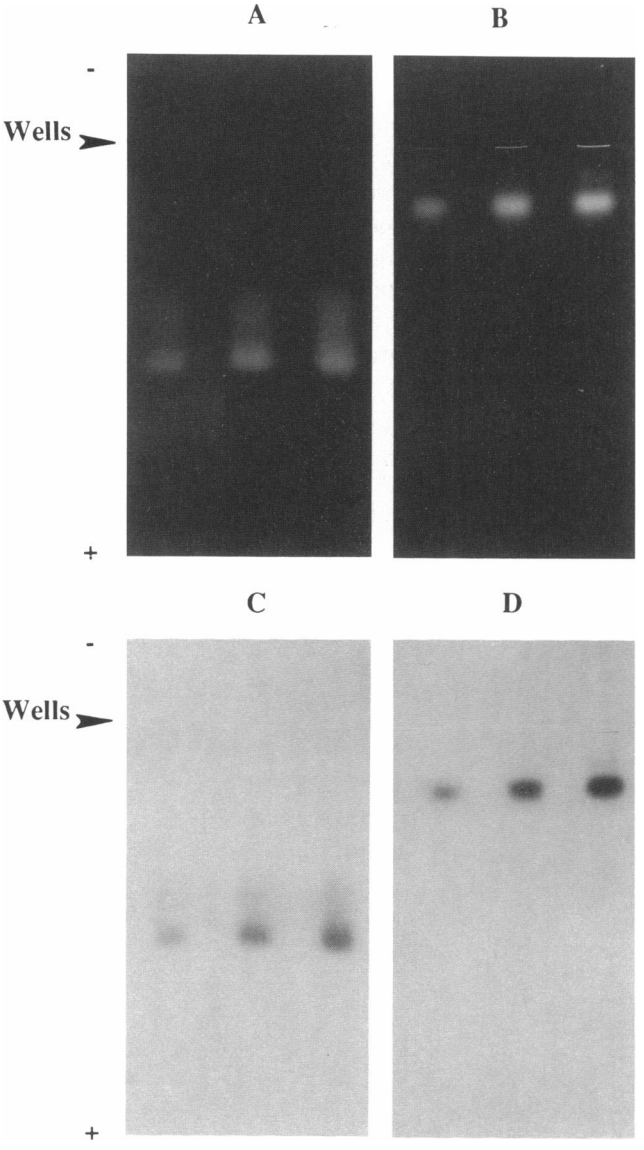


FIGURE 1 Electrophoresis of fluorescently labeled G- and F-actin on agarose gels. 1% agarose gels, 5 mm thick, electrophoresis at 4°C for 4 h. (*A*) 1,5-IAEDANS-labeled G-actin in a G-buffer pH 8.8. (*B*) 1,5 1,5-IAEDANS-labeled F-actin in an F-buffer of the same composition as G-buffer but containing in addition 2 mM $MgCl_2$. (*C*) As in A but stained with Coomassie Blue for 3 min and destained for 3 d. (*D*) As in B but stained with Coomassie Blue as in *C*. The amount of protein applied to each gel was (from left to right): 10, 15, and 20 μg. The electric field was 2.14 V/cm; the current was 3 mA in *A* and *C*, 7 mA in *B* and *D*. The cathode (−) was on the top, and anode (+) on the bottom of gel. Arrow indicates the position of the wells.

## Weight and number distribution of actin lengths

An experimental scan of the fluorescence intensity versus the distance of migration for a typical F-actin after 2 h electrophoresis in a KCl F-buffer (Table 1) is shown in Fig. 2 A. A question immediately arises as to why we see a relatively homogenous mobility in a sample which contains F-actin filaments of heterogenous lengths (strictly speaking an exponential distribution of lengths; Kawamura and Maruyama, 1970, 1972). The answer is that because we measure the intensity of fluorescence, the observed distribution is a weight distribution of F-actin sizes while the measurements of the length of F-actin yielded a number distribution. In fact the migration of F-actin as a bell-shaped, discrete band is expected from the exponential number distribution of filament lengths: when each experimental point of number distribution (characterized by an exponential) is multiplied by its abscissa to obtain a weight distribution (Tanford, 1961), the resulting curve will be bell-shaped.

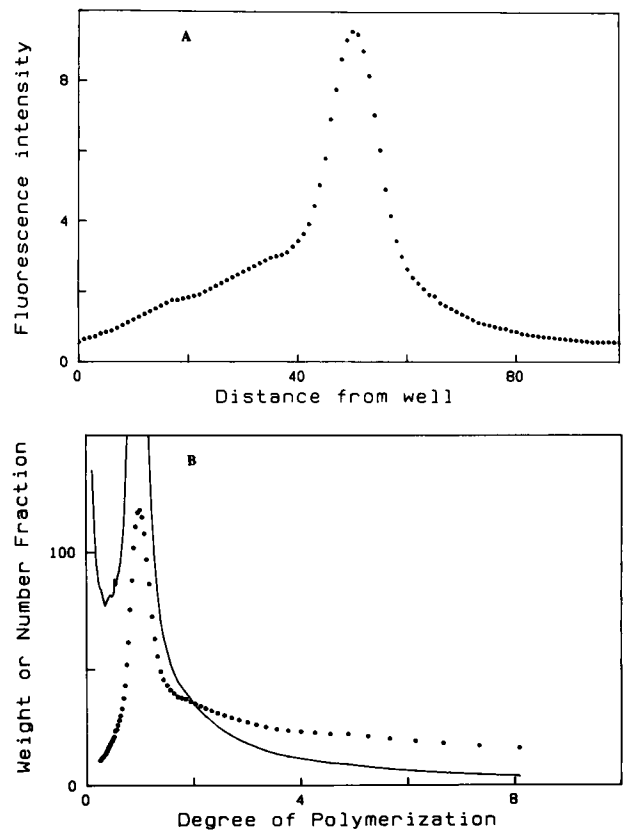


FIGURE 2 (*A*) Typical scan of the fluorescence intensity of the 1,5-IAEDANS labeled F-actin band. The gel was mounted between two parallel glass plates in a Shimadzu CS-930 gel scanner. A Xe lamp produced a rectangular light beam (6 × 0.05 mm) at 370 nm which was focused by 1.5-mm-thick TLC plate set in the sample holder. The beam was incident on the 10 μg of 1,5-IAEDANS labeled F-actin band electrophoresed for 2 h at 2.1 V/cm in KCl F-buffer. Ordinate (arbitrary units): measured fluorescence intensity which is proportional to the weight fraction of F-actin present in the band. Abscissa: distance from the well, 1 mm on a gel corresponds to 53 divisions on the scanned profile. (*B*)*(Open circles)* Measured weight fraction of actin polymers plotted vs. the inverse of the distance from the well which is proportional to the degree of polymerization. *(Continuous line)* Number fraction of actin polymers calculated from weight fraction by dividing each experimental point by its abscissa. The scales are in arbitrary units.

Assuming that the mobility of F-actin was inversely proportional to its molecular weight (see below), we computed the number distribution associated with the observed fluorescence intensity profile: in Fig. 2 *B (circles)* the measured weight fraction of actin polymers is replotted vs. the inverse of the distance from the well (distance from the well is proportional to the mobility). In turn, mobility of large polyanion such as F-actin in a gel matrix is most likely a result of reptation, and hence for low fields it should be inversely proportional to the size of the molecule (Lumpkin et al., 1985). The inverse of the distance traveled is therefore proportional to the size of the polymer (degree of polymerization) and it is thus labeled in Fig. 2 *B*. From the experimental distribution of the weight fraction, a number distribution can be calculated (by dividing each experimental point by its abscissa). This distribution (Fig. 2 *B*, lines), is no longer bell-shaped, but increases at small degrees of polymerization. This feature is characteristic of the theoretically predicted exponential (number) distribution of lengths of a linear helical aggregate of identical monomers (Oosawa, 1970; Oosawa and Asakura, 1975) and of the experimentally observed distribution of F-actin sizes in solution (Kawamura and Maruyama, 1970, 1972).

The peak in the number distribution is not predicted by the theory of a random condensation polymerization. We do not think, however, that the presence of a peak suggests a different mechanism of polymerization. The reason for this is that to obtain a weight distribution from the fluorescent intensity profile of F-actin band (such as shown in Fig. 2 *A*), an assumption has to be made regarding the relationship between the mobility and the size of the molecule: it was assumed above that the mobility of F-actin was inversely proportional to the size of the molecule. In reality this is an oversimplification. Firstly, short F-actin filaments may move through the gel by a mechanism characteristic of free electrophoresis (where mobility is well known to be independent of chain length). Secondly, according to a reptation model (Lumpkin et al., 1985), at higher fields the expression for mobility contains a term that is independent of the chain length. Thirdly, the prediction of the reptation model that the mobility is inversely proportional to the size has been tested only for the DNA (Stellwagen, 1985); we don't know to what extent it is applicable to F-actin.

## Electrophoresis in intermediate ionic strength buffers

The total ionic strength of the solutions used in our experiments was low. There were three reasons for this: (*a*) We wanted to keep the total ionic strength (and therefore electrophoretic current) low to prevent the gels from heating up. (*b*) We wanted to be able to apply large voltages (and therefore large orientating forces) without having to apply large currents to study the orientation of F-actin in the gels. (*c*) For induced dipoles the electric dipole moment (and therefore orientating force) increases with a decrease in ionic strength (Charney, 1988). A part of the F-actin dipole moment may be induced because if it were all permanent it would be difficult to explain a curious negative birefringence of F-actin (Kobayasi et al., 1964).

Because of the low ionic strength and relatively high pH, the conditions of our experiments did not favor polymerization. It was therefore important to test whether under these conditions actin was well polymerized. To this end we compared relative viscosity of actin under various conditions. Viscosity was measured in an Ostwald viscometer at 28°C. The flow times and relative viscosities are summarized in Table 2. The results show that 2 mM $MgCl_2$ is sufficient to induce nearly maximal polymerization at pH 8.8.

It should be noted, however, that low ionic strength was not absolutely necessary for electrophoresis of F-actin: in the buffers containing 50 mM KCl or 20 mM Tris (Table 1, lines 3 and 4) F-actin also migrated in 1% agarose, but the rate of migration was ~1.3 times slower than in the standard F-buffer. We speculate that this was due to the fact that a large concentration of counterions retarded the migration of protein (Tanford, 1961).

It is important to demonstrate that the migration in the electric field does not denature or depolymerize F-actin.

**TABLE 2 Viscosity of actin under various conditions**

| Solution | Flow time ± SD* | Relative viscosity |
|---|---|---|
| | *s* | |
| Water | 58.3 ± 0.2 | 1 |
| G-actin‡ in G-buffer | 61.2 ± 2.0 | 1.05 |
| F-actin‡ in 10 mM KCl, 2 mM $MgCl_2$, 5 mM Tris-HCl, pH 8.0 | 105.9 ± 1.2 | 1.82 |
| F-actin‡ in 10 mM KCl, 2 mM $MgCl_2$, 5 mM Tris-HCl, pH 8.8 | 98.2 ± 1.8 | 1.68 |
| F-actin‡ in 50 mM KCl, 2 mM $MgCl_2$, 5 mM Tris-HCl, pH 8.0 | 109.4 ± 3.1 | 1.88 |

*Mean ± SD of five measurements.
‡Actin was at 1 mg/ml.

To this end we have checked (*a*) the state of actin polymerization after the electrophoresis, (*b*) its ability to activate ATPase activity of HMM after the electrophoresis, and (*c*) its ability to bind HMM during and after the electrophoresis.

## State of polymerization

It is unlikely that electrophoresis causes F-actin to depolymerize, because the interaction energy involved in bonding actin monomers in a filament is large (12 Kcal/mol; Oosawa, 1977). It is conceivable, nevertheless, that the electrophoretic process somehow affects actin conformation so that actin in Fig. 1, *B* and *D*, is not in the F form even though it migrates slowly. To test for this possibility, after electrophoresis of native actin, the gels were stained with fluorescent phalloidin (TRITC-P). Phalloidin is known to bind only to F-actin (Wulf et al., 1979). To bring the pH to neutral after the electrophoresis, the F-actin containing gel was soaked in a solution containing 50 mM KCl, 2mM $MgCl_2$, 10 mM TRIS, pH 7.0, for 1 h, and G-actin containing gel in a solution containing 2 mM Tris, pH 7.0, for 1 h. 0.1 mg of TRITC-P was dissolved in 1 ml of 50% methanol, 7.5% acetic acid, and applied to gels to the position where the bands were known to be. After 15 min staining followed by 30 min washing in destaining solution, fluorescent bands in the position of F-actin (Fig. 3 *C*). but not in that of G-actin (Fig. 3 *B*) could be seen. This experiment, however, does not exclude the possibility that the fluorescent band corresponds to the assembly of large actin oligomers.

## Ability to activate ATPase of HMM

170 μl of 1 mg/ml unlabeled actin was applied to a well made by a preparative comb (one tooth, 2 mm thick) and electrophoresed in the F-buffer for 2 h at 30 V (current was 7 mA). At the end of the electrophoresis, actin bands were cut out and homogenized in a solution containing 20 mM imidazole, pH 7.0, 2 mM $MgCl_2$, and 1 mM ATP. 0.07 μM HMM was added to 0.7-ml aliquots of the homogenate which contained 1.3 μM actin (actin was present in 18 molar excess). Phosphate liberation was measured by the method of Fiske and Subbarow (1925). The activity of HMM activated by electrophoresed actin was 0.072 μmol $P_i$/mg HMM per min; the control activity of HMM activated by F-actin which was not electrophoresed was 0.093 μmol $P_i$/mg HMM per min.

## Ability to bind HMM

Binding of HMM was checked first during the electrophoresis. Fig. 3 *E* and *F*, show the electrophoretic bands

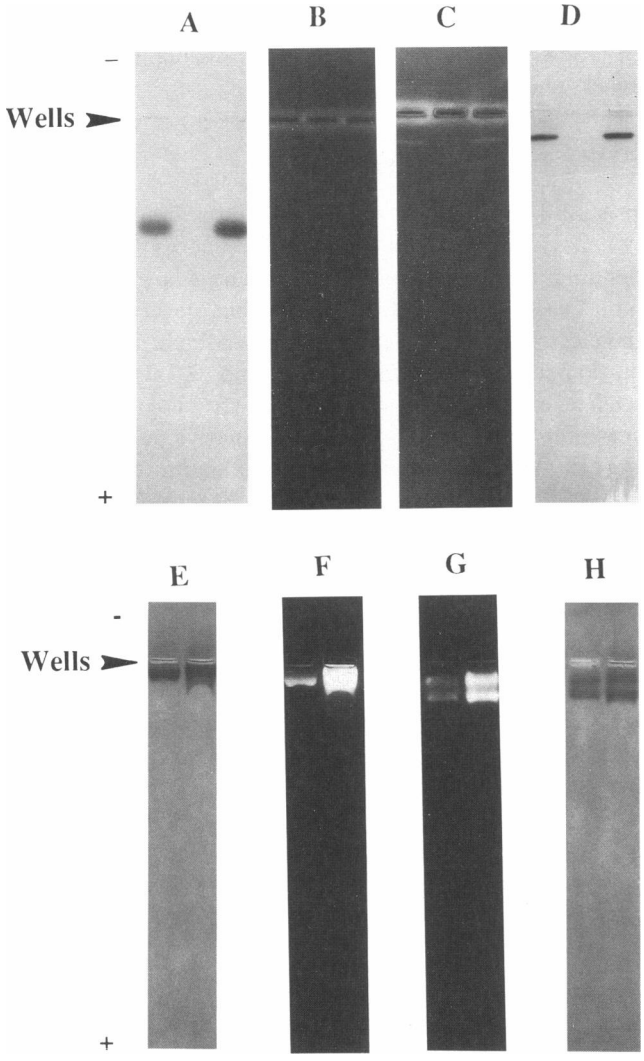


FIGURE 3 Staining electrophoresed actin with phalloidin (*A–D*) and co-migration of actin and HMM in the presence and absence of ATP (*E–H*). Phalloidin staining experiments were run under the same conditions as Fig. 1. The gels were stained with 100 μg/ml TRITC-phalloidin in 50% methanol for 15 min and destained for 0.5 h with destaining solution. The electric field was 2.1 V/cm and the current was 3 mA in *A* and *B* and 7 mA in *C* and *D*. (*A*) G-Actin in G-buffer stained with CB; (*B*) the same gel stained with TRITC-P; (*C*) F-actin in F-buffer stained with TRITC-P; (*D*) the same gel as in *C* but stained with CB. In co-migration experiment 0.5 mg/ml IAF-actin and 0.5 mg/ml 1,5-IAEDANS HMM were electrophoresed in *E*: −ATP F-buffer (Table 1), left and right, 10 and 15 μg of protein applied to the gel, respectively, stained with CB; (*F*) the same as *E* but photographed with UV illumination; (*G*) +ATP F-buffer (Table 1), left and right, 10 and 15 μg of protein applied to the gel, respectively, photographed with UV illumination; (*H*) the same as *G* but stained with CB. The electric field was 2.1 V/cm. All gels were electrophoresed at 4°C for 2 h. Anode is at the bottom. Wells are indicated by the arrow.

obtained when 0.5 mg/ml IAF-actin migrated in the presence of 0.5 mg/ml 1,5-IAEDANS-labeled HMM in the solution containing no added ATP (−ATP F-buffer, Table 1). Only one band was present, migrating at a rate slower than either actin or HMM, suggesting that actin and HMM formed a complex. In Fig. 3, *G* and *H*, the gels were electrophoresed in the presence of added ATP (+ATP F-buffer, Table 1). Here actin and HMM migrated separately: IAF-actin was identified as a top band (it had orange fluorescence) and IAEDANS-HMM as a bottom band (it had blue fluorescence).

Ability to bind HMM was also tested after the electrophoretic run: 170 μl of 1 mg/ml of unlabeled actin was applied to the well as above and electrophoresed in the F-buffer for 2 h at 30 V (current was 7 mA). At the end of the electrophoresis actin bands were cut out and homogenized in a small volume of a solution containing 50mM KCl, 2 mM $MgCl_2$, and 10 mM imidazole, pH 7.0. The suspension was centrifuged for 10 min in the desktop centrifuge to separate agarose from actin. Actin concentration was determined at this point as 43 μg/ml. Increasing concentrations of 1,5-IAEDANS-labeled HMM (between 0.25 and 2 μM) were added to the aliquots of actin. The complex was centrifuged at 40,000 rpm in type 50 rotor at 0°C for 2 h and the amount of fluorescence in the supernatant was measured in an SLM 8000 fluorometer using excitation at 365 nm and emission at 480 nm. In parallel, HMM was centrifuged alone (without actin) as a control. The difference between the fluorescence of control HMM and the fluorescence of HMM remaining in the supernatant after the centrifugation in the presence of actin was proportional to the HMM bound to actin. The difference increased progressively with increasing amount of added HMM and leveled off at 0.8 μM added HMM; the dissociation constant was estimated from the difference (by the method of Borejdo and Assulin, 1980) and was 0.54 μM.

As expected, the migration of actin in the gel was critically dependent on the pH of the running buffer. The more alkaline the pH, the more net negative charge is conferred on the F-actin and it migrates faster towards the anode. Fig. 4 compares the mobilities of F-actin at various pHs; between pH 7.0 and 8.0, F-actin did not enter the gel at all.

## Linear dichroism of IAF-actin

Having found the conditions under which native F-actin would migrate in the gels, we attempted to measure its orientation by the fluorescence detected linear dichroism method (Borejdo et al., 1982). In this method the sample is illuminated alternatively with vertically and horizontally plane polarized light. When the absorption dipole of the sample has a preferred orientation (but not 54.7° with

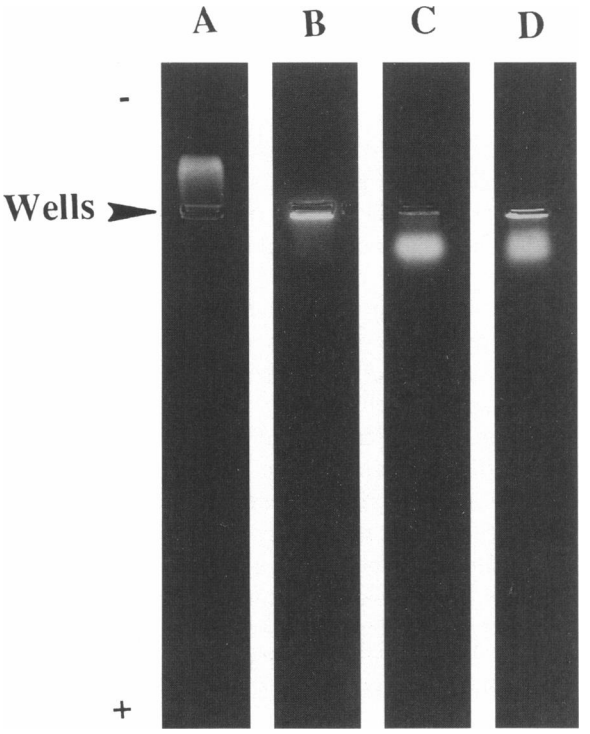


FIGURE 4 Electrophoresis of F-actin on agarose gels at different pHs. 1% agarose gels, 5 mm thick, illuminated with the broad UV lamp. 20 $\mu$g of 1,5-IAEDANS-labeled F-actin was applied to each gel. The electric field was 2.14 V/cm and the current varied between 7 and 9 mA. Gels were electrophoresed at 4°C for 2 h. (*A*) pH = 6.5; (*B*) pH = 7.5; (*C*) pH = 8.5; (*D*) pH = 8.8. Anode is at the bottom. Wells are indicated by the arrow.

respect to the vertical), the sample absorbs more light having one polarization than the other, i.e., it is dichroic. A comprehensive theory has been proposed relating the linear dichroism to the orientation of the absorption dipole of the label (Borejdo and Burghardt, 1987).

In the present experiments 1,5-IAEDANS could not be used as an F-actin label, because our microscope did not allow the use of the UV illumination (in any case 1,5-IAEDANS was flexibly attached to Cys-374 of actin [Takashi, 1979]. Dichroic measurements require a rigid attachment of the dye to the protein). We therefore tested whether 5-IAF could be used as a rigidly attached probe: F-actin was labeled with 5-IAF as described in Materials and Methods. To determine whether the fluorophore was rigidly attached to actin, we compared the polarization of fluorescence $p_0$ of 5-IAF immobilized with glycerol at low temperature with that of IAF-actin in solution. Excitation was at 488 nm, the emission at 515 nm. 10 $\mu$M 5-IAF in 99.8% glycerol at −20°C had $p_0 = 0.499$. 5$\mu$M solution of IAF-actin in 50 mM KCl, 2 mM $MgCl_2$, 2 mM Tris, pH 8.0, at 4°C had $p_x = 0.204$. The fact that the $p_x$ was small in comparison with $p_0$ suggested that 5-IAF was mobile on the surface of actin (P was not made small by rotations of F-actin, because the filaments are so large that they don't rotate during the lifetime of 5-IAF even in the absence of glycerol or agarose). The half angle of the cone in which the absorption dipole of 5-IAF can freely rotate on the surface of actin can be estimated from the ratio $p_x/p_0$ (Weber, 1966). It is ~37°. Like 1,5-IAEDANS, the dye was mobile probably because its attachment was through Cys-374 which was close to the unstructured COOH terminal of actin. Further, actin is hydrophilic (Borejdo, 1983) and for hydrophobic dyes such as 5-IAF the attachment to Cys-374 cannot be stabilized by an interaction between the ring and the surface of the protein. Binding of HMM stabilized the attachment of the dye, but even in the presence of equimolar HMM the polarization was still small. The other dye we tested (iodoacetamidotetramethylrhodamine) gave similar static polarization when bound to F-actin.

In view of the fact that the 5-IAF was mobile on the surface of F-actin we expected little dichroism even if F-actin was perfectly aligned by the electric field. We nevertheless measured the linear dichroism of IAF-actin during the electrophoresis in 1% agarose. For the small electric field strengths which we were able to use (between 2.1 and 10 V/cm) there was no dichroism regardless of where the laser beam was focused along the line of migration of IAF-actin. We could not apply larger fields, because the electrophoretic current heated the gel slab.

## Linear dichroism of (IAF S-1): native F-actin complex

In view of the fact that all dyes tested were mobile on the surface of actin, we used an alternative approach to measure the dichroism during electrophoresis. A chromophore was attached to a carrier, which was in turn rigidly attached to actin. S-1 can serve as such a carrier because 1,5 IAEDANS is known to be rigidly attached to $SH_1$ of S-1 (Borejdo, J. unpublished observations) and in rigor S-1 attaches rigidly to actin (Borejdo et al., 1982). To test whether 5-IAF was also rigidly attached to S-1 we measured $p_x$ of 5-IAF modified S-1 in the presence of unlabeled F-actin. A 1 $\mu$M solution of IAF S-1 in 50 mM KCl, 2 mM $MgCl_2$, 10 mM Tris, pH 8.0, in the presence of 2 $\mu$M unlabeled F-actin was illuminated at 488 nm in SLM fluorometer. The emission was at 515 nm and the temperature was 4°C. The polarization was $p_x = 0.309$, closer to the value for immobilized dye alone, showing that 5-IAF binds more rigidly to S-1 than to actin (but showing that 5-IAF, in contrast to 1,5 IAEDANS (Borejdo, J. unpublished observations) was not completely immobilized by binding to $SH_1$ on S-1.

F-Actin decorated with S-1 was still able to migrate in 1% agarose gels, although at a reduced rate. The linear

dichroism of (IAF S-1)-(F-actin) complex during the electrophoresis in 1% agarose was compared with that of lambda DNA. Fig. 5 *A* shows the time course of development and relaxation of dichroic ratio R for lambda DNA. This DNA consists of 48,502 base pairs and migrates in 1% agarose at pH 7.0 at ~8 mm/h. The measurements were done as described earlier (Borejdo and DeFea, 1988), except that the excitation was at 488 nm using a collimated beam of an argon ion laser; the laser beam was focused to a spot 7 $\mu$m in diameter. Emission was observed using a Zeiss fluorescein barrier filter LP 470. R was a ratio of the fluorescence intensity obtained when the gel was illuminated with the light polarized perpendicularly to the direction of the electric field, to the fluorescence obtained when the gel was illuminated with the light polarized parallel to the direction of the electric field. Significant dichroism developed when the electric field was turned on, and its sign showed that the dipole moment of EB was largely perpendicular to the direction of the field, i.e., that the helix axis of DNA was aligned along the direction of the electric field (EB intercalates with its phenanthridinium plane parallel to the plane of the bases which are approximately perpendicular to the helix axis). The magnitude of the dichroic ratio did not depend on the position of the laser spot within the fluorescent band.

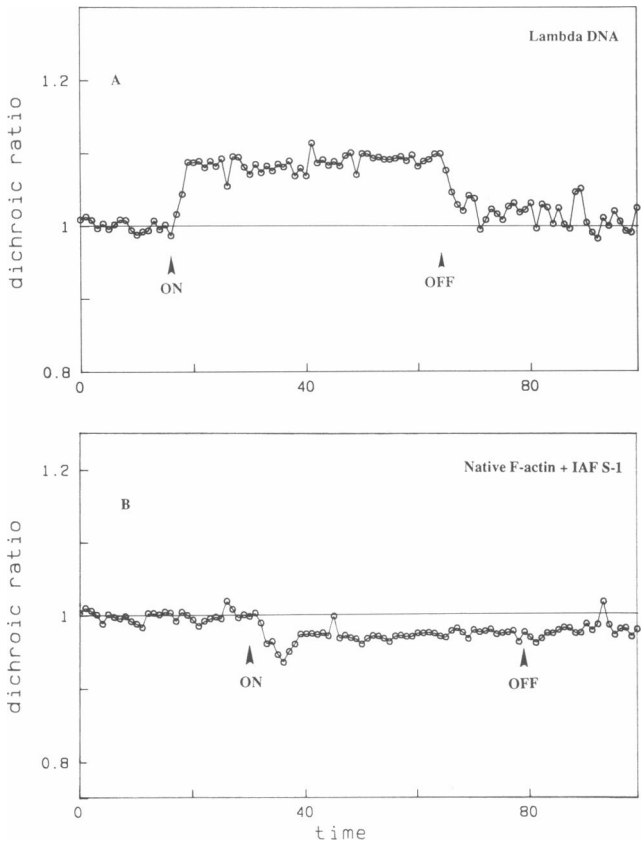


FIGURE 5 (*A*) Linear dichroism of the DNA and of F-actin decorated with 5-IAF labeled S-1. Fluorescent light emitted by the EB (*A*) and 5-IAF (*B*) is proportional to the absorption of light. (*A*) 10 $\mu$g of lambda DNA in the presence of 0.5 $\mu$g/ml ethidium bromide in 40 mM Tris-acetate buffer, pH 7.0, 1 mM EDTA was electrophoresed at room temperature at 2.2 V/cm (55 mA current). After 1 h the electrophoresis apparatus was transferred to the microscope. For measurements of dichroism 1 $\mu$W laser beam was focused to a spot 7 $\mu$m in diameter in the middle of the fluorescent DNA band. The arrows indicate when the electric field (6 V/cm, 150 mA current) was turned on and off. (*B*) 4.1 $\mu$M 5-IAF labeled S-1 was coelectrophoresed with 23 $\mu$M native F-actin in (S-1)-actin solution (Table 1, pH 8.5) at 4°C at 2.1 V/cm (current was 45 mA). After 1 h the electrophoresis apparatus was transferred to the microscope (at room temperature) and solution was replaced with a solution containing 10 mM KCl (to further decrease ionic strength). For measurements of dichroism 100 $\mu$W laser beam was focused on a spot 40 $\mu$m distant from the well (and not at the center of the fluorescent band). The arrows indicate when the electric field (20 V/cm, 150 mA current) was turned on and off. The ratio was computed every 200 ms as described in the text. 10 U in horizontal scale corresponds to 1 s.

The experiment was more complicated in the case of acto-S-1 complex. Here the dichroism depended on the position of the laser spot relative to the fluorescent intensity profile (such as shown in Fig. 2). The dichroic ratio of slowly migrating complex (i.e., when the measurement was done near the well) was always large and of the opposite sign to that of DNA. This means that the absorption dipole of 5-IAF labeled S-1 which was bound to F-actin assumed an orientation largely parallel to the field. The dichroic ratio decreased with the increasing distance from the well and became 1 when the distance exceeded ~90 $\mu$m. In particular, there was no dichroism at the peak of the fluorescent intensity of the band. A typical record of the time course of development of dichroism at a distance of 40 $\mu$m from the well is shown in Fig. 5 *B*. Orientation developed rapidly but relaxed only ~5 s after the electric field was turned off.

The dependence of the orientation on the distance of migration most likely results from the fact that long F-actins migrate more slowly (see e.g. Fig. 2). Long F-actins are expected to assume better orientation in the gel because this is what is experimentally observed in the case of DNA both in solution (Charney, 1988) and in the gel (Holzwarth et al., 1987; Borejdo and DeFea, 1988; Borejdo, 1989). We are at present studying the details of the dependence of orientation on the position across the profile of the fluorescent band and on the strength of the field.

We think that the orientation of the acto-S-1 complex reflected the orientation of F-actin itself, rather than the orientation of S-1 only. Although S-1 has a large dipole moment, it was completely random when its orientation was measured in the absence of F-actin. Much larger electric field gradients were necessary to orient it (Highsmith and Eden, 1986). Further, the dye on $SH_1$ of S-1 is rigid in relation to actin filaments (Dos Remedios et al.,

1972; Crowder and Cooke, 1984) and it is unlikely that the electric field could orient S-1 without orienting actin.

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