Dynamics of Myo1c (Myosin-Iβ) Lipid Binding and Dissociation*

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Myosin-I is the single-headed member of the myosin superfamily that associates with lipid membranes. Biochemical experiments have shown that myosin-I membrane binding is the result of electrostatic interactions between the basic tail domain and acidic phospholipids. To better understand the dynamics of myosin-I membrane association, we measured the rates of association and dissociation of a recombinant myo1c tail domain (which includes three IQ domains and bound calmodulins) to and from large unilamellar vesicles using fluorescence resonance energy transfer. The apparent second-order rate constant for lipid-tail association in the absence of calcium is fast with nearly every lipid-tail collision resulting in binding. The rate of binding is decreased in the presence of calcium. Time courses of myo1c-tail dissociation are best fit by two exponential rates: a fast component that has a rate that depends on the ratio of acidic phospholipid to myo1c-tail (phosphatidylserine (PS)/tail) and a slow component that predominates at high PS/tail ratios. The dissociation rate of the slow component is slower than the myo1c ATPase rate, suggesting that myo1c is able to stay associated with the lipid membrane during multiple catalytic cycles of the motor. Calcium significantly increases the lifetimes of the membrane-bound state, resulting in dissociation rates \(< 0.001 \text{ s}^{-1}\).

Myosin-I is the widely expressed single-headed member of the myosin superfamily. Myosin-I isoforms play roles in membrane dynamics, cell structure, and mechanical signal transduction (1). Subcellular fractionations of vertebrate cells indicate that most myosin-I is associated with the membrane and cytoskeleton fractions with only a small soluble pool (2, 3). Myosin-I is dynamically localized to cell membranes (4–6), suggesting regulated membrane association. However, very little is known about the control of myosin-I localization and membrane association, though it has been proposed that calcium-calmodulin and phosphorylation play roles in modulating membrane attachment (7).

Electrostatic interactions play an important role in the binding of myosin-I to membranes. Positively charged tail domains of myosin-I bind to acidic phospholipids without any apparent specificity toward the acidic head groups (8–10). For example, myo1a (brush border myosin-I) binds tightly to vesicles composed of phosphatidylserine and phosphatidylglycerol but binds very weakly to phosphatidylcholine (8). Although myo1-I tail binding proteins have been identified in lower eukaryotes (11–15), none have been identified in vertebrates. Additionally, if is not known if these proteins play a role in anchoring myosin-I to the membrane.

Myo1c (also known as myr2 and myosin-Iβ) is widely expressed in vertebrates, is enriched in the perinuclear regions and dynamic cell margins, and is found concentrated in the tips of stereocilia of inner-ear hair cells (for a review, see Ref. 1). Chromophore-assisted laser inactivation (16) and chemical inhibition (17) studies have identified roles for myo1c in lamellipodial structure and mechanical signal transduction. To better understand the dynamics and regulation of myo1c membrane association, we determined the lipid association and dissociation rate constants using a recombinant protein construct containing the three IQ domains and the C-terminal tail region of myo1c (myo1c-tail). Membrane association was detected by fluorescence resonance energy transfer from myo1c-tail tryptophans to dansyl-labeled phosphatidylserine (18, 19).

MATERIALS AND METHODS

Reagents, Buffers, and Lipid Preparation—All experiments were performed in HNa100 (10 mM Hepes, pH 7.0, 100 mM NaCl, 1 mM EGTA, 1 mM DTT). Calcium concentrations were adjusted by adding CaCl2 to HNa100 and are reported as free calcium. All kinetic experiments were performed with 2 μM excess calmodulin.

Phosphatidylserine (PS), phosphatidylcholine (PC), and 1,2-dioleoyl-sn-glycero-3-phospho-L-serine-5-dimethylamino-1-naphthalenesulfonyle (dPS) were purchased as chloroform stocks (Avanti Polar Lipids). Large unilamellar vesicles (LUVs) were prepared by extrusion. Lipid components were mixed in the desired ratios in chloroform and dried completely under a stream of nitrogen. Lipids were resuspended in HNa100 to a total concentration of 2 mM and were subjected to five cycles of freeze-thaw. Lipids were then passed through 100-nm filters (11 times) using a mini-extruder (Avanti Polar Lipids). LUVs were discarded after 1 day. PS percentages reported throughout the text are the mole percentages of total PS (PS + dPS) with PC making up the remaining lipid. All fluorescent LUVs (dLUVs) contain 5% dPS. Lipid concentrations are given as total lipid (PC + PS + dPS).

Protein Expression and Purification—We cloned and expressed myo1c-tail (amino acids 690–1028), which consists of three IQ motifs, the tail domain, and an N-terminal HIS6 tag for purification. The cDNA for myo1c was kindly provided by D. P. Corey (Massachusetts General Hospital), and the appropriate bases were amplified with the following primers: 5’-ggggatctgtatcactacaactacaagggccagcaagacagctct-3’ and 5’-ggggatctactaagagaaatcggc-3’. The amplified fragment was cloned into baculovirus transfer vector pHBlueBac4.5 (Invitrogen). Recombinant baculovirus was generated using standard procedures and screened by plaque assay.

Myo1c-tail with bound calmodulin was purified from Sf9 cells that were co-infected with virus containing recombinant myo1c-tail and an N-terminal His6 tag for purification. The cDNA for myo1c was kindly provided by D. P. Corey (Massachusetts General Hospital), and the appropriate bases were amplified with the following primers: 5’-ggggatctgtatcactacaactacaagggccagcaagacagctct-3’ and 5’-ggggatctactaagagaaatcggc-3’. The amplified fragment was cloned into baculovirus transfer vector pHBlueBac4.5 (Invitrogen). Recombinant baculovirus was generated using standard procedures and screened by plaque assay.

Myo1c-tail with bound calmodulin was purified from Sf9 cells that were co-infected with virus containing recombinant myo1c-tail and calmodulin. Cells were suspended in 25 mM Tris, pH 7.5, 20 mMimid-
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Stopped-Flow Kinetics and Analysis—Transient kinetic measurements were made at 25 ± 0.1 °C with an Applied Photophysics (Surrey, UK) SX.18MV stopped-flow fluorometer. Trypsothan was excited with a monochromator set at 285 nm (2.3-nm slit width). A 440-nm long-pass filter (Oriel) was used to detect dansyl fluorescence. Experimental transients were fitted with software supplied with the instrument. All stated concentrations are those after mixing (i.e., the final reaction concentrations). The mixing ratio of the stopped-flow apparatus was 1:1, thus the concentrations in the stopped-flow syringes before mixing are twice those reported.

Association transients were obtained by mixing myo1c-tails with dLUVs. Two to eight association transients were averaged before nonlinear least-squares fitting. Dissociation transients were obtained by mixing pre-equilibrated myo1c-tail-dLUVs complexes with a large molar excess of unlabelled 80% PS LUVs. When mixed, myo1c-tails that dissociate from dLUVs have a much higher probability of rebinding to unlabelled LUVs resulting in decreased dLUVs fluorescence. Reported dissociation rates are the averages of four to eight transients from three different protein preparations.

RESULTS

Lipid Association—A fluorescence increase upon binding of myo1c-tail to dPS-containing LUVs (dLUVs) allowed us to monitor the rate of association of myo1c-tail with lipid. Fluorescence time courses of myo1c-tail binding to dLUVs in the absence (Fig. 2A) and presence of 250 μM free calcium (Fig. 2B) follow single exponential rates that depend linearly on the total lipid concentration (Fig. 2C). We did not detect lag phases, deviations from single exponential time courses, or fluorescence increases at longer time scales. However, a mixing artifact at high lipid concentrations (>100 μM) limited our ability to analyze the first 50 ms of the transient (Fig. 2, A and B).

The apparent second-order rate constant for the binding of myo1c-tail to 40% PS dLUVs (0.16 ± 0.02 μM⁻¹ s⁻¹) was the same as 60% PS dLUVs (0.18 ± 0.01 μM⁻¹ s⁻¹). However, the apparent rate of association was significantly reduced for both 40% PS (0.020 ± 0.01 μM⁻¹ s⁻¹) and 60% PS (0.049 ± 0.02 μM⁻¹ s⁻¹) dLUVs in the presence of 250 μM free calcium. Calcium alone does not affect the fluorescence of dLUVs.

Myo1c-Tail Lipid Dissociation—The rate of myo1c-tail dissociation from dLUVs was measured by mixing myo1c-tail-dLUVs with excess unlabelled 80% PS LUVs (see “Material and Methods”). After mixing, the starting fluorescence (F_start) decreased to the fluorescence level of dLUVs in the absence of tail (F_dLUV), indicating complete dissociation of tail from dLUVs (Fig. 3A). The signal-to-noise ratios of the dissociation curves (Fig. 3A) were much better than the association curves (Fig. 2) because the longer time scales allowed for longer signal integration times. Fluorescence time-courses of dissociation were best fit by a two-exponential rate function (Fig. 3A). The fast (k_fast) and slow (k_slow) rates were dependent on the mole fraction of total PS in the dLUVs at a single myo1c-tail concentration (Fig. 3B). The relative amplitude of the fast component (A_fast) decreased from ~70% of the total signal change at 20% PS to ~40% of the total signal change at 80% PS.

The dissociation rate was examined as a function of myo1c-tail concentration at a single 20% PS lipid concentration (Fig. 4A). At the lowest myo1c-tail concentrations tested (15–25 nM), fluorescence transients were adequately fit with a single-exponential rate function. At protein concentrations >25 nM, a two-exponential fit was required. The rate of k_fast increased significantly between 25 and 100 nM myo1c-tail and did not increase at >100 nM myo1c-tail. The rate of k_slow showed a small dependence on the myo1c-tail concentration (Fig. 4A).

The amplitude of the slow component (A_slow) reached its maximum level at ~100 nM myo1c-tail then slightly decreased, and A_fast reached its maximum level at ~200 nM myo1c-tail. The total amplitude of the fluorescence change (A_total = A_fast + A_slow) reached its maximum at 200 nM myo1c-tail, indicating that...
FIG. 2. Association of myo1c-tail with dLUVs. A, fluorescence time course of 75 nM myo1c-tail binding to 100 μM 40% PS dLUVs (35% PS, 5% dPS, 60% PC). The solid line is the best fit of the fluorescence transient to a single exponential function ($y = Ae^{-kt} + c$, where $A$ is the amplitude, $k$ is the observed rate, $t$ is time, and the start is $A + c$) with a rate of 13 s$^{-1}$. A residual obtained by subtracting the best-fit exponential function from the data is shown. B, fluorescence time course of 75 nM myo1c-tail binding to 100 μM 40% PS dLUVs (35% PS, 5% dPS, 60% PC) in the presence of 250 μM unlabeled LUVs. The solid lines are the best fits of the fluorescence transients to a two-exponential function ($y = A_{fast}e^{-kt_{fast}} + A_{slow}e^{-kt_{slow}} + c$) with $k_{fast} = 0.73$ s$^{-1}$ and $k_{slow} = 0.032$ s$^{-1}$ in the absence of calcium and $k_{fast} = 0.35$ s$^{-1}$ and $k_{slow} = 0.024$ s$^{-1}$ in the presence of calcium. The residuals obtained by subtracting the best-fit exponential function from the data are shown overlaid. The fluorescence levels in the absence ($F_{tail}$) and presence ($F_{tail + calcium}$) of myo1c-tail are shown on the $y$-axis. C, dependence of the dissociation rates, ($\bullet$) $k_{fast}$ and ($\bigcirc$) $k_{slow}$, of myo1c-tail from dLUVs as a function of the mole-fraction of PS in dLUVs in the absence of calcium. Final concentrations after mixing are 100 nM myo1c-tail, 12.5 μM myo1c-tail beads in a pull-down experiment in the presence of 250 μM free calcium (Fig. 1B).

The dissociation transients in the presence of calcium were best fit to two-exponential rates at all myo1c-tail concentrations: $k_{fast}$ and $k_{slow}$ (Fig. 4C) and their associated amplitudes, $A_{fast}$ and $A_{slow}$ (Fig. 4D), showed a much smaller dependence on the myo1c-tail concentration than in the absence of calcium. The starting fluorescence levels of the dissociation transients in the presence of calcium ($F_{fast}$) were the same as those obtained in the absence of calcium (Fig. 3A), suggesting equivalent binding of myo1c-tail to dLUVs. However, the total amplitude change ($A_{fast} + A_{slow}$) of the transient after mixing with unlabeled LUVs in the presence of calcium was $>2$-fold less than the expected signal change ($F_{tail} - F_{tail + calcium}$) at almost every myo1c-tail concentration tested (Fig. 5), suggesting that $>60\%$ of myo1c-tail does not dissociate from the dLUVs under experimental conditions. A further amplitude change was not observed when the acquisition time was increased to 1000 s. A further decrease in the fluorescence signal was also not observed when the plus-calcium dissociation reaction was mixed with 10 mM EGTA to chelate the free calcium, thus indicating that the effect of calcium on myo1c-tail dissociation is not reversible on the time scale of the experiments (not shown). Similar calcium effects on the rates and amplitudes were observed with 40% PS dLUVs (data not shown).

FIG. 3. Dissociation of myo1c-tail from dLUVs. A, time courses of dissociation of lipid-bound myo1c-tail obtained by mixing myo1c-tail bound to 20% PS dLUVs (15% PS, 5% dPS, 80% PC) with 80% PS LUVs (80% PS, 20% PC) in the presence and absence of 250 μM free calcium. Final concentrations after mixing are 100 nM myo1c-tail, 12.5 μM dLUVs, and 250 μM unlabeled LUVs. The solid lines are the best fits of the fluorescence transients to a two-exponential function ($y = A_{fast}e^{-kt_{fast}} + A_{slow}e^{-kt_{slow}} + c$) with $k_{fast} = 0.73$ s$^{-1}$ and $k_{slow} = 0.032$ s$^{-1}$ in the absence of calcium and $0.35$ s$^{-1}$ and $0.024$ s$^{-1}$ in the presence of calcium. The residuals obtained by subtracting the best-fit exponential function from the data are shown overlaid. The fluorescence levels in the absence ($F_{tail}$) and presence ($F_{tail + calcium}$) of myo1c-tail are shown on the $y$-axis. B, dependence of the dissociation rates, ($\bullet$) $k_{fast}$ and ($\bigcirc$) $k_{slow}$, of myo1c-tail from dLUVs as a function of the mole-fraction of PS in dLUVs in the absence of calcium. Final concentrations after mixing are 25 μM 20–80% PS dLUVs, 125 nM myo1c-tail, 250 μM 80% PS LUVs. Lipid concentrations are reported as total lipid for dLUVs (PS + dPS + PC) and LUVs (PS + PC).

The dissociation transients in the presence of calcium were best fit to two-exponential rates at all myo1c-tail concentrations: $k_{fast}$ and $k_{slow}$ (Fig. 4C) and their associated amplitudes, $A_{fast}$ and $A_{slow}$ (Fig. 4D), showed a much smaller dependence on the myo1c-tail concentration than in the absence of calcium. The starting fluorescence levels of the dissociation transients in the presence of calcium ($F_{fast}$) were the same as those obtained in the absence of calcium (Fig. 3A), suggesting equivalent binding of myo1c-tail to dLUVs. However, the total amplitude change ($A_{fast} + A_{slow}$) of the transient after mixing with unlabeled LUVs in the presence of calcium was $>2$-fold less than the expected signal change ($F_{tail} - F_{tail + calcium}$) at almost every myo1c-tail concentration tested (Fig. 5), suggesting that $>60\%$ of myo1c-tail does not dissociate from the dLUVs under experimental conditions. A further amplitude change was not observed when the acquisition time was increased to 1000 s. A further decrease in the fluorescence signal was also not observed when the plus-calcium dissociation reaction was mixed with 10 mM EGTA to chelate the free calcium, thus indicating that the effect of calcium on myo1c-tail dissociation is not reversible on the time scale of the experiments (not shown). Similar calcium effects on the rates and amplitudes were observed with 40% PS dLUVs (data not shown).

**DISCUSSION**

Association Rates—Association transients follow a single exponential at all concentrations tested. The rate constants for the tail binding sites on the dLUVs were saturated at this concentration (Fig. 5).

**Dissociation and the PS/Tail Ratio**—To better understand the relationship between the dissociation rate and the PS content of LUVs, we measured dissociation as a function of myo1c-tail concentration (15–375 nM) from 20%, 40%, and 60% PS dLUVs (Fig. 6). The rates of the fast and slow components are plotted as a function of moles of PS/moles of myo1c-tail (Fig. 6). The dependence of the rate on the PS/tail ratio is nearly identical for dLUVs of the different PS percentages. Therefore, the dependence of the dissociation rate of the fast component on the percentage PS at a single myo1c-tail concentration (Fig. 3B) is due to the variation in the PS/tail ratio, and it is not a function of the percentage of PS or PC in a vesicle.

**Myo1c-Tail Lipid Dissociation in the Presence of Calcium**—The myo1c-tail dissociation rate from 20% PS dLUVs was examined as a function of myo1c-tail concentration in the presence of 250 μM free calcium. As shown previously for myo1c and other myosin-I isoforms (reviewed in Ref. 1), calcium weakens the affinity of calmodulin for myo1c-tails. This is seen by the partial dissociation of calmodulin from myo1c-tails bound to beads in a pull-down experiment in the presence of 250 μM calcium (Fig. 1B).

The dissociation transients in the presence of calcium were best fit to two-exponential rates at all myo1c-tail concentrations: $k_{fast}$ and $k_{slow}$ (Fig. 4C) and their associated amplitudes, $A_{fast}$ and $A_{slow}$ (Fig. 4D), showed a much smaller dependence on the myo1c-tail concentration than in the absence of calcium. The starting fluorescence levels of the dissociation transients in the presence of calcium ($F_{fast}$) were the same as those obtained in the absence of calcium (Fig. 3A), suggesting equivalent binding of myo1c-tail to dLUVs. However, the total amplitude change ($A_{fast} + A_{slow}$) of the transient after mixing with unlabeled LUVs in the presence of calcium was $>2$-fold less than the expected signal change ($F_{tail} - F_{tail + calcium}$) at almost every myo1c-tail concentration tested (Fig. 5), suggesting that $>60\%$ of myo1c-tail does not dissociate from the dLUVs under experimental conditions. A further amplitude change was not observed when the acquisition time was increased to 1000 s. A further decrease in the fluorescence signal was also not observed when the plus-calcium dissociation reaction was mixed with 10 mM EGTA to chelate the free calcium, thus indicating that the effect of calcium on myo1c-tail dissociation is not reversible on the time scale of the experiments (not shown). Similar calcium effects on the rates and amplitudes were observed with 40% PS dLUVs (data not shown).
myo1c-tail binding to 40% PS and 60% PS LUVs in the absence of calcium were not statistically different, suggesting that the association rate is not dependent on the PS percentages that we tested. If we assume that the molecular weight of an LUV is $1.2 \times 10^8$ (20), then the apparent second-order rate constant for binding in terms of LUV concentration is $-2 \times 10^{10} \text{M}^{-1} \text{s}^{-1}$ for 40% PS and 60% PS. This rate is on the same order as the collisional association rate ($1 \times 3 \times 10^{10} \text{M}^{-1} \text{s}^{-1}$) as estimated by the Smoluchowski equation (20, 21). Therefore, nearly every tail-vesicle interaction in the absence of calcium results in binding.

Dissociation Rates—In most cases, dissociation transients are not described by single exponential functions (Fig. 3A), so dissociation can not be modeled as a single-step process limited by one rate constant. We propose a simple model in which the dissociation rate depends on the number of PS molecules bound to each tail: the myo1c-tail is in equilibrium between two states that differ in the number of tail-bound PS molecules; and the relative populations of the two states in the absence of calcium are determined in part by the PS/tail ratio.

At high PS/tail ratios, myo1c-tails do not compete with each other for PS, and a single slowly dissociating population predominates. We can estimate the effective number of PS molecules bound to the slowly dissociating population by examining the myo1c-dependent changes in $k_{\text{fast}}$ and $k_{\text{slow}}$. Fig. 6 shows that the rate of the fast component approaches the rate of the slow component at $100 \text{PS/tail}$. If 50% of the lipid is inaccessible due to its location within the inner leaflet of the lipid bilayer, then the effective number of PS molecules bound to each slowly dissociating tail is $50$.

At low PS/tail ratios, myo1c-tails compete with each other for PS, resulting in a fraction of tails with $<50$ bound PS molecules. Fewer electrostatic interactions between PS and tail result in an increased rate of dissociation. The fast component reaches its maximum rate at $100 \text{PS/tail}$ (Fig. 6), suggesting that the minimum number of PS molecules bound to a tail is $<13$. The rate of the slow component also increases at high...
myo1c-tail concentrations (Fig. 4A), which might be due to fewer PS molecules bound to the tail or to steric effects due to saturation of lipid with protein.

At the lowest PC/tail ratios in the absence of calcium, the slow component represents 20–30% of the total dissociation amplitude for dLUVs containing 20% PS (Fig 4B), 40% PS (not shown), and 60% PS (not shown). Therefore, there are factors other than the PS/tail ratio that define the relative population of the fast and slow dissociating components (22). These may include cooperative association of PS with membrane-bound tails or stable conformational states of the protein that do not bind less than 50 PS molecules.

Calcium Regulation—The rate of lipid association is decreased ~3-fold in the presence of 250 μM free calcium. The apparent second-order rate constants expressed in terms of dLUVs concentration (rather than total lipid) are 3 × 10^9 M^−1 s^−1 for 40% PS dLUVs and 8 × 10^9 for 60% PS dLUVs. Therefore, even in the presence of very high calcium concentrations, the rate of association of myo1c-tail with lipids is very fast. We do not know why the rate constant for lipid binding is decreased in the presence of calcium. However, possibilities include competition between calcium and myo1c-tail and PS as seen for other membrane-binding proteins (25).

As in the absence of calcium, dissociation transients are best fit to a two-exponential rate function in the presence of 250 μM free calcium. k_{fast} and k_{slow} do not show any dependence on the myo1c-tail concentration, and the total fluorescence amplitudes of the transients (A_{fast} + A_{slow}) are ~2-fold smaller than in the absence of calcium (Fig. 5). The starting fluorescence levels (F_{0-tail}) of the dissociation transients in the presence and absence of calcium are the same, so it is unlikely that the decreased amplitudes are due to fewer lipid-bound myo1c-tails. Rather, a large fraction of the myo1c-tails remain lipid-bound and do not dissociate on the time scale of our measurements. Qualitative lipid sedimentation experiments also suggest that calcium does not decrease myo1c-tail binding to LUVs (not shown). Experiments with myo1a brush border myosin-I suggest that calcium increases the number of myo1n-I molecules bound to lipid vesicles (7). However, the similar fluorescence levels (F_{0-tail}) in the presence and absence of calcium in the present study suggest that this is not the case for myo1c (Fig. 3). Highly stable protein-lipid interactions mediated by electrostatic interactions are not unique to myo1c. For example, protein kinase C has been shown to dissociate from PS-containing vesicles with a half-time >24 h (23).

It has been proposed for myo1a that calcium dissociates myosin-bound calmodulin unmasking positive charges that can bind acidic phospholipids (7). Because calcium weakens the affinity of calmodulin for myo1c and myo1c-tail (Fig. 1B; see Ref. 1 for review), we suggest that myo1c-tail follows a similar mechanism, resulting in a highly stable protein-lipid interaction. Such a mechanism might explain the data of Cyr et al. (24), which shows that the IQ motifs play a role in directing subcellular localization of myo1c. However, it remains to be determined if a calmodulin-disassociated state of myo1c is physiological. It is also possible that calcium mediates an electrostatic interaction between a cluster of negative charges on the tail and PS as seen for other membrane-binding proteins (25). In either case, the calcium effect is not reversible under our experimental conditions and time scales, so it is possible that other regulatory elements play a role in mediating membrane attachment/detachment in the presence of calcium.

Physiological Relevance of the Dissociation Rates—The steady-state actin-activated ATPase rate of myo1c under calcium-free conditions similar to the present experiments is 0.5 s^−1 (26), which is 10-fold faster than k_{slow} (0.02–0.06 s^−1). At high PS/tail ratios, most myo1c motors should be able to undergo several catalytic cycles before dissociating from the membrane. Therefore, an “anchoring” protein is not necessary to keep the motor associated with the membrane under these conditions. Remarkably, k_{slow} is nearly identical to the fluorescence-recovery-after-photobleach rate of green fluorescent protein-myo1a and green fluorescent protein-my1a-tail as measured in the brush border of kidney epithelial cells (5). Therefore, if membrane-associated tail-binding proteins are present in brush border microvilli, we predict that they do not increase the lifetime of membrane association.

The magnitude of k_{fast} (0.2–1.0 s^−1) is on the same order as the myo1c ATPase rate in the absence of calcium. So under conditions where the fast component predominates (i.e. low PS/tail ratios), the probability that myo1c will perform a power stroke while bound to a membrane is greatly decreased. The acidic phospholipid content in the membrane is regulated in both concentration and in spatial distribution (27). Therefore, the membrane dissociation rate of cellular myosin-I might be coupled directly to the regulation of lipid charge and acidic phospholipid density.

The motor domain of myo1n-I interacts preferentially with tropomyosin-free actin filaments (4, 28), so tropomyosin and spatially regulated actin polymerization play important roles in specifying the subcellular localization of myo1n-I (4). In the absence of a specific myo1n-I tail receptor, as in ruffling membranes (2, 4), we predict that myo1n-I will associate with acidic phospholipids adjacent to tropomyosin-free actin. Assuming a high acidic lipid/tail ratio, these membrane-linked motors can perform multiple power strokes as they move toward the barbed end of the actin filament. Because myo1n-I membrane interactions are relatively nonspecific, this model predicts that multiple myo1n-I isoforms will concentrate on the same cellular membranes. Overlapping localization of vertebrate myo1n-I isoforms has been shown (1, 2). However, it has also been shown that myo1n-I isoforms have distinct non-overlapping localizations in certain subcellular regions (2), thus it is likely that myo1n-I tails contain information for specifying localization to specific cellular regions (2, 4). Further experiments are required to better understand the mechanisms and regulation of myo1n-I targeting.

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