Myosin Thick Filaments and Subunit Exchange: A Stochastic Simulation Based on the Kinetics of Assembly†

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ABSTRACT: Subunit exchange between groups of myosin filaments at equilibrium in a volume similar to a sarcomere is simulated using Monte Carlo (probabilistic) methods. Five published kinetic parameters (three rate constants and two cooperativity parameters) which govern the assembly of thick filaments from purified myosin at pH 8.0 are used for the computations. Filament length distributions equivalent to those measured experimentally in the electron microscope result. Distinctive patterns of exchange emerge because cooperativity in myosin assembly is not confined to nucleation but functions throughout growth. Fluctuations in filament size, first apparent in the millisecond time domain, mediate exchange which first occurs at the tips of the filaments and then gradually progresses inwards toward the central bare-zone. Exchange rates decreased by an approximate factor of 10 per decade of time: full exchange takes years, 50% takes 28 h, and 10% takes a brief 100 ms. These data represent the fastest possible rates of exchange because synthetic myosin filaments lack the overall stabilizing influence of the copolymerizing proteins of native filaments. Exchange at equilibrium is therefore too slow to explain, for example, the much faster rates recorded in vivo for the complete replacement of one myosin isoform by another. Facilitated exchange where partial or complete filament dissociation is followed by the introduction of new subunits during reassembly offers a means of accelerating exchange. In this context, it is shown that the requisite disassembly and reassembly of myosin thick filaments can be completed in a minimum of a few seconds.

The question addressed in this paper is whether mechanisms of exchange at equilibrium provide a sufficient basis for the changes in the subunit composition of myosin filaments observed experimentally in vitro and in vivo. True subunit exchange occurs in systems at equilibrium where the average concentration of both filaments and dissociated subunits remains essentially constant. The process is mediated by a kinetically regulated flux of subunits between existing filaments and subunits free in the surrounding medium. Spontaneous fluctuations in filament size drive subunit exchange which is a passive process. Exchange of this type is quite distinct from mechanisms in which externally imposed changes in the concentration of free subunits or in the strength of binding of subunits to filaments result in disassembly followed by reassembly and the incorporation of new subunits. Mechanisms like this, in which facilitated exchange occurs, require the expenditure of energy in order to function.

Intuitively, exchange at equilibrium has particular appeal for the turnover of myosin in muscle because the subunit composition of myosin filaments would simply reflect the isoform composition of dissociated myosin in the sarcoplasm. On the other hand, the mechanism in its simplest form does not allow for the selective removal of old subunits and their replacement by new subunits. If some mechanism based on facilitated exchange functions, large numbers of thick filament subunits have to be removed and replaced by a process of disassembly followed by reassembly over a short period of time without overly compromising muscle function. No doubt, exchange at equilibrium occurs to some extent in all noncovalently bonded systems; the question is whether it is fast enough and extensive enough to be biologically relevant. To investigate these issues, I have explored the dynamics of exchange in the simplest and least stable experimental system available, namely, the pH 8.0 class of synthetic thick filaments formed from purified myosin [reviewed by Davis (1988a)].

In general, an investigation of the dynamics of exchange of subunits into a homopolymer would be of marginal relevance to the process in vivo. This is because most biopolymers assemble via relatively simple mechanisms in which an energetically unfavorable nucleation event is followed by the addition of many subunits in a series of like, energetically favorable, reaction steps. Myosin assembly is quite different as cooperativity is not confined to the nucleation event alone but is manifest throughout the course of assembly [see review by Davis (1988a)]. Unusual patterns of exchange are therefore anticipated, and it seems likely they will make a distinctive imprint on the process both in vitro and in vivo. These aspects are explored in detail in this paper.

Relevant aspects of the kinetic mechanism of myosin thick filament growth used for the quantitative analysis of subunit exchange described in this paper are illustrated in schematic form in Figure 1. The main features of the mechanism are a diffusion-controlled "on" rate for the addition of dimer to the filament that is independent of the changing length of the polymer (Davis, 1981b) and an "off" rate that increases exponentially with length in two phases linked to the dissociation of parallel- and antiparallel-packed myosin (Davis, 1981b, 1985). Growth ceases and length is regulated at the point where the "on" and "off" rates are equal. The net result is the formation of a narrow length distribution characteristic of synthetic myosin thick filaments at equilibrium. It is qualitatively evident from the kinetic mechanism that subunit exchange should first occur at the tips of the filaments and thereafter gradually progress inward toward the central bare-zone.

The simulations described in this paper were prompted by the in vitro experiments of Saad and colleagues in which the rapid and random exchange of subunits along the full length of the pH 7.0 class of synthetic thick filaments was documented

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(Saad et al., 1986, 1991). Their experiments served to renew a general interest in mechanisms of subunit exchange and turnover by offering an unexpected mechanism with important implications for consideration. From a kinetic perspective, their results are of interest because a random mechanism of exchange is inconsistent with the sequential nature of the kinetics of assembly where subunits can only be lost from or added to the tips of thick filaments.

In vivo, observations of subunit exchange and the long-term stability of the subunit composition of filaments are less supportive of a random mechanism of exchange. The complete interchange of one myosin isoform with another occurs in a matter of days in cardiac muscle in response to hormone treatment. Under these conditions, a preferential exchange of subunits is observed at the tips of the thick filaments (Wenderoth & Eisenberg, 1987). The authors point out that the kinetics of length regulation of myosin filaments provide a plausible basis for these observations. The localization of neonatal, embryonic, and adult myosin isoforms in thick filaments during the development of chicken muscle provides evidence for the existence of static isoform distributions which would prove unstable were random exchange functional (Taylor & Bandman, 1989). However, it should be noted that the validity of these data has recently been questioned (Gordon & Lowey, 1992).

The simulations described in this paper quantify the process of subunit exchange using kinetic data obtained from experiments performed on the pH 8.0 class of synthetic thick filaments. Monte Carlo methods are readily applicable to the simulation of the time-dependent behavior of small groups of interacting molecules. The technique is therefore ideal for tracking the dynamics of the assembly of thick filaments in a volume similar to that of a single sarcomere. Time-dependent fluctuations in the size of individual thick filaments and the exact sites of exchange of myosin molecules can readily be modeled. In certain instances, simulation allows one to explore aspects of behavior at a level of molecular detail that is inaccessible to experimentation.

The simulated data have the statistical properties of a small system which allows direct comparison with experiments in which the positions of labeled myosin subunits in thick filaments are mapped by microscopy. These computations place an upper limit on the rate of exchange since the reaction occurs in the absence of structure-stabilizing proteins associated with native filaments that cause them to be longer and more stable than their synthetic counterparts in physiological solvents (Davis, 1988b).

I first show that the kinetics of thick filament assembly (three rate constants and two cooperativity parameters) (Davis, 1981b, 1985) can be used to compute normal filament length distributions and the critical concentration of free subunits at equilibrium with filaments. Exchange, as anticipated, first occurs at the tips of the filaments where fluctuations in size occur frequently, spreading thereafter toward the center of the filament at an ever decreasing rate. Significant exchange is seen in milliseconds while full exchange takes many years to complete. It is therefore improbable that full exchange can be attained in vitro or in vivo at equilibrium in a reasonable length of time. Observations of random exchange in pH 7.0 filaments are ascribed to a layer of myosin that coats the surface of these filaments and exchanges via a different mechanism to the hidden nativelike core of these filaments. Facilitated exchange, in which rapid partial dissociation and reassembly function together to change the subunit composition of filaments, is proposed as a viable alternative mechanism for exchange in vivo. Rates of filament assembly and disassembly are discussed in this context. A preliminary account of the work has been published in abstract form (Davis, 1989).

METHODS

The stochastic simulation was performed according to the method described by Gillespie (1976, 1977). The program was written in FORTRAN and was run on Hewlett Packard 1000/A600+ and 9000/835 computers. Truncation and other errors were checked for by changing the precision of critical variables and testing different random-number generators operating at different degrees of precision. All the computed data presented were obtained using DRAND48 as the random-number generator on a Hewlett Packard 9000/835 computer.

RESULTS

Kinetic Model for the Simulation of Subunit Exchange. The rate constants used as the source of the transition probabilities for stochastic simulation come from pressure-jump experiments on the assembly of myosin thick filaments at atmospheric pressure (Davis, 1981b) and on the disassembly of myosin at elevated hydrostatic pressure (Davis, 1985). For the purpose of the simulation, individual mechanisms that govern association and dissociation of subunits are integrated into an overall mechanism of assembly. In doing so, published kinetic parameters and stoichiometric relations are recast in a unified form. Details of the procedures used to obtain the values of the kinetic constants for simulation and how these data were employed in the simulation are outlined below.

Assembly (addition of subunits to the tips of the growing filament) is governed by a composite rate constant (k_{obs}) defined by the reaction sequence presented in eq 1–3 where

$$M + M \overset{K_1}{\rightleftharpoons} D + F_{i-1} \overset{k_2}{\rightarrow} F_i \quad (1)$$

$$d[F_i]/dt = K_1 k_2 [M]^2 2[F_{i-1}] \quad (2)$$

$$k_{obs} = K_1 k_2 2[F_{i-1}] \quad (3)$$

M is monomeric myosin, D is myosin dimer with a 44-nm axial stagger between parallel-packed subunits (Davis et al., 1982), F_i are filaments comprised of i myosin dimers, K_1 is the association constant for dimer formation, and k_2 is the rate constant for the addition of dimer to the filament ends. As mentioned, the value of the pseudo-second-order rate constant k_{obs} (more precisely, k_2) is independent of length—its constant value is symbolized in vector form by the two arrows in the lower half of Figure 1. A value for k_{obs} of 8.8×10^6 M^{-1} s^{-1} at a filament concentration of 2.4 mg mL^{-1} [see Figure 2 of Davis (1981b)] is obtained from an analysis of the kinetics of the regrowth of pressure-shortened thick filaments (Davis, 1981b). Revision of the molecular weight of myosin from 465 000 to 520 000 raises the value of k_{obs} to 9.84×10^6 M^{-1} s^{-1}. In all kinetic schemes and calculations in this paper, dimer is the intermediate that is added to, or lost from, the tips of the thick filament. Consequently, the value of k_{obs} (originally specified in terms of monomeric myosin added to the filament) was doubled to 1.97×10^7 M^{-1} s^{-1}. The relationship of eq 3 was used to calculate the composite third-order rate constant, $K_1 k_2$, used for the simulation, from k_{obs} and the concentration of filament ends ($2[F_{i-1}]$). Filament concentrations are calculated by assuming that native and

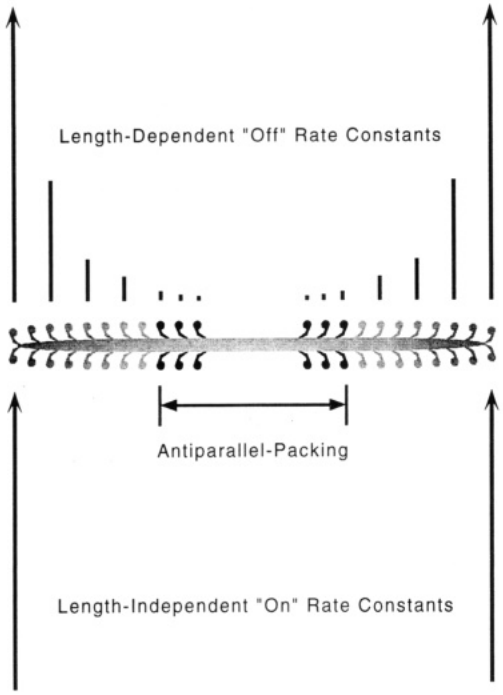

FIGURE 1: Mechanism of growth and length regulation in thick filaments of purified myosin. Arrows of identical size represent equal "on" and "off" rates for the addition and loss of dimer subunits from full-length filaments at equilibrium. The "on" rate constants are the same whatever the filament length because they are diffusion-limited and insensitive to changes in thick filament structure. Slower, length-dependent "off" rate constants for various shorter filaments are depicted as a series of different-sized vertical bars in the upper half of the diagram. The "off" rates decline exponentially in two phases: one associated with the dissociation of the parallel-packed arms of the thick filaments, the other with the dissociation of antiparallel-packed myosin located about the central bare-zone. Dimer subunits can only be added to or lost from the tips of the filaments, a sequential reaction.

pH 8.0 filaments have the same number of myosin subunits per unit length [see Davis (1988a)]. A value of 2.33×10^{14} M^{-2} s^{-1} for K_1k_2 results.

Dissociation is governed by a length-dependent "off" rate (see upper half of Figure 1). In earlier work, four parameters (k_1, k_0, α, and β) were shown to govern the kinetics of subunit dissociation from zones of parallel-packed and of antiparallel-packed myosin in the thick filament (Davis, 1985). These four kinetic parameters are recast to conform with the mechanism for the dissociation of thick filaments outlined in eq 4–8:

$$F_i \xrightarrow{k_{-i}} F_{i-1} + D \tag{4}$$

$$d[F_{i-1}]/dt = k_{-i}2[F_i] \tag{5}$$

$$k_{obs} = k_{-i}2[F_i] \tag{6}$$

For antiparallel-packed myosin:

$$\text{for } i = 0\text{–}10,\ k_{-i} = k_{apara}e^{\beta i/54} \tag{7}$$

For parallel-packed myosin:

$$\text{for } i = 11 \text{ to } \infty,\ k_{-i} = k_{para}e^{\alpha i/54} \tag{8}$$

where k_{-i} is the rate constant for the dissociation of subunit from a filament of i subunits, k_{para} and k_{apara} are the boundary/reference rate constants for the dissociation of hypothetical zero-length ($i = 0$) parallel-packed and antiparallel-packed filaments, respectively, and α and β are their associated cooperativity parameters that govern the extent of the exponential change in the dissociation rate constants as the filaments grow. The four parameters k_{para}, k_{apara}, α, and β, in their new form, were calculated as follows from pressure-jump data (Davis, 1985). The pseudo-zero-order rate constant k_1 [units of change in absorbance (turbidity) at 320 nm per second (Davis, 1981a)] for the dissociation of a full-length filament of parallel-packed myosin has a rate of 3.0 A_{320} s^{-1}, and k_0 for the dissociation of a zero-length filament of antiparallel-packed myosin has a value of 0.018 A_{320} s^{-1}. Both rates were obtained at a filament concentration of 0.4 mg^{-1} mL^{-1} and a pressure of 27.5 MPa (275 atm). First-order "off" rate constants for dimer dissociation of 1350 s^{-1} for k_1 and 10.5 s^{-1} for k_0 were calculated using a specific turbidity of 0.15 A mg^{-1} mL^{-1}, a molecular weight of 1 040 000 for the myosin dimer, and a filament length at atmospheric pressure of 0.65 μm comprising 54 dimer subunits. The "off" rate constants measured at elevated pressures in the upward pressure-jump were normalized to atmospheric pressure using the appropriate volumes of activation ($\Delta V^{\ddagger}$) (Davis, 1985). Values of 162 s^{-1} for k_1 and 0.354 s^{-1} for k_0 resulted. Similarly corrected values of –0.458 for α and 1.177 for β, each for a 10% change in filament length, were obtained for the cooperativity parameters. Conversion to the nomenclature used in the mechanism presented above (eq 7 and 8) resulted in values of 1.66 s^{-1} for k_{para}, 0.354 s^{-1} for k_{apara}, 4.58 for α, and 11.77 for β for a change in length from 0 to a full-length filament of 54 dimer subunits. These deterministic rate constants and cooperativity parameters were converted to their probabilistic form for computer simulation (Gillespie, 1976, 1977).

Kinetic parameters were checked against independently measured equilibrium data before the simulation was initiated. This is done by calculating the equilibrium constant (monomer or critical concentration) at atmospheric pressure for the full-length filaments for comparison with values measured directly in experiments:

$$K_1k_2[M]^2 2[F_{i-1}] = k_{-54}2[F_i] \tag{9}$$

Since $2[F_{i-1}]$ closely approximates $2[F_i]$, we may write

$$K_1k_2[M]^2 \cong k_{-54} \tag{10}$$

Substitution of a mean dissociation rate constant of 162 s^{-1} for k_{-54} (k_{-i} for a myosin filament at atmospheric pressure calculated from the relation $k_{-54} = k_{para}e^{\alpha}$) and an assembly rate constant of 2.33×10^{14} M^{-2} s^{-1} for K_1k_2 in eq 10 yields a critical concentration of 0.43 mg mL^{-1} monomer, twice the value, 0.21 mg mL^{-1}, measured experimentally for the mixture of monomer and dimer (Davis, 1985). This does not constitute a large deviation from the experimentally determined value, indicating that the kinetic data result in values close to that determined independently in equilibrium experiments. Since it is important that ends of the thick filaments "see" the same myosin concentration encountered in the experiments in which the kinetics were determined, the composite "on" rate constant (K_1k_2) was adjusted a small amount to give the experimentally measured critical concentration of 0.21 mg mL^{-1} monomer. Had this adjustment not been made, the filaments would have been marginally shorter.

Stochastic Simulation of the Length Distribution at Equilibrium. The next step in the investigation of the exchange of subunits between thick filaments is to generate an equilibrium length distribution in the computer from a monodisperse system of filaments at their mean length. A volume of 1.86×10^{-13} mL containing filament and myosin monomer concentrations of 1 and 0.21 mg mL^{-1}, respectively,

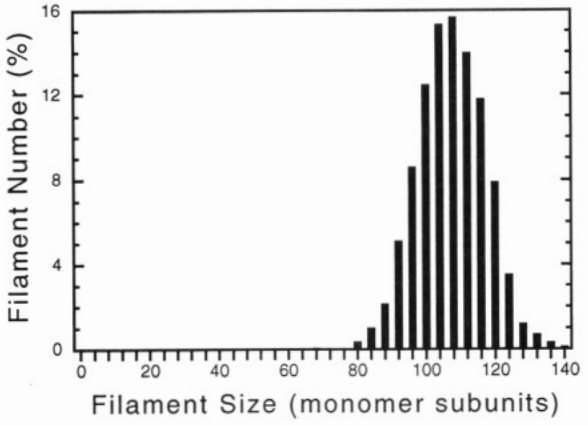

FIGURE 2: Simulated length distribution of myosin thick filaments. Note the symmetric form of the computed length distribution about a mean of 0.638 SD ± 0.052 μm, $n = 4000$. The bin size is equivalent to a pair of myosin dimers.

is used for the simulation. The system translated into a set of starting conditions for the stochastic simulation that included 2000 filaments of 54 dimer subunits each and 45 295 myosin monomers. The mechanism in deterministic form (see eq 11–16) is limited to 300 simultaneous differential equations

$$\mathrm{M} + \mathrm{M} \underset{k_{-i}}{\overset{K_1}{\rightleftharpoons}} \mathrm{D} + \mathrm{F}_{i-1} \underset{k_{-i}}{\overset{k_2}{\rightleftharpoons}} \mathrm{F}_i \quad (11)$$

$$d[F_1]/dt = K_1k_2[M]^2 2[F_0] - k_{apara}e^{\beta 1/54}2[F_1] \quad (12)$$

$$\vdots$$

$$d[F_{10}]/dt = K_1k_2[M]^2 2[F_9] - k_{apara}e^{\beta 10/54}2[F_{10}] \quad (13)$$

$$d[F_{11}]/dt = K_1k_2[M]^2 2[F_{10}] - k_{para}e^{\alpha 11/54}2[F_{11}] \quad (14)$$

$$\vdots$$

$$d[F_{54}]/dt = K_1k_2[M]^2 2[F_{53}] - k_{para}e^{\alpha 54/54}2[F_{54}] \quad (15)$$

$$\vdots$$

$$d[F_{300}]/dt = K_1k_2[M]^2 2[F_{299}] - k_{para}e^{\alpha 300/54}2[F_{300}] \quad (16)$$

for the assembly of a system with a mean filament size of 54 dimers and a maximum size of 300 dimers. In the actual simulation, an array of 4000 half-filaments is used, reducing the number of differential equations to 150.

The main question is whether a monodisperse system of 4000 half-filaments of 27 dimers each and 45 295 monomers will generate a population of filaments with the width and form of a length distribution measured independently in the electron microscope. A typical computed length distribution, after 100 s of equilibration time has elapsed, is illustrated in Figure 2. The symmetric histogram gives a clear representation of the form of the distribution. In order to compare measured and computed length distributions, a larger bin size of 0.05 μm (equivalent to 5.25 dimers) is used to partition the computed data. Figure 3 illustrates the computed length distribution (0.638 SD ± 0.052 μm, $n = 4000$) together with an experimentally determined length distribution (mean length of 0.664 SD ± 0.083 μm, $n = 100$) obtained from electron micrographs of positively stained synthetic thick filaments

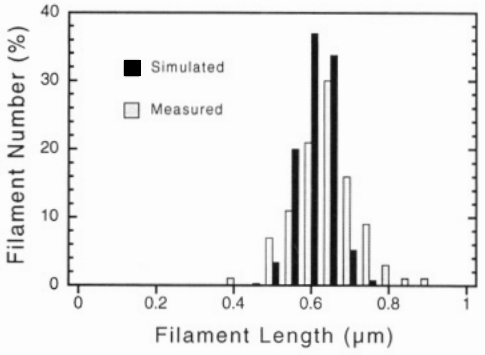

FIGURE 3: Simulated and measured length distributions of synthetic thick filaments. Length distributions computed from the kinetics of myosin thick filament length regulation and those measured from a sample of positively stained thick filament in the electron microscope are similar in form. Means of 0.638 SD ± 0.052 μm, $n = 4000$ (simulated), and 0.664 SD ± 0.083 μm, $n = 100$ (measured), were determined for the length distributions. The bin size is 0.05 μm.

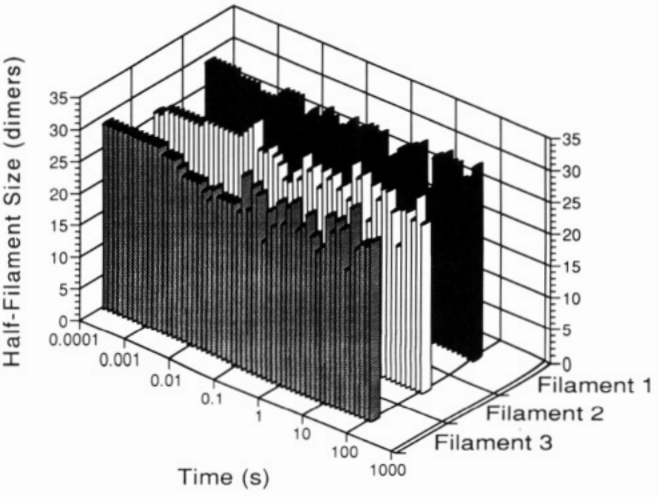

FIGURE 4: Fluctuations at equilibrium in the sizes of three half-filaments. Filament lengths change little at short time intervals of 100 μs; as the sampling interval increases, the correlation between the sizes of adjacent filaments declines steadily. Fluctuations in size appear constrained about the mean length of the filaments, reflecting the length distributions illustrated in Figures 2 and 3. The potential for subunit exchange exists whenever subunits are added or released by fluctuations in filament size.

(Davis, 1988b). It is evident from these data that both the simulated and measured length distributions have a common form. The kinetic mechanism and the method of simulation generate equilibrium properties close to that observed in independent experiments, thereby validating the approach.

Time-Dependent Fluctuations in Thick Filament Size. Fluctuations in the size of individual thick filaments that are otherwise at equilibrium mediate exchange. An impression of the extent and time scale of these fluctuations over 6 decades of time can be gained from an inspection of Figure 4. The lengths of the three half-filaments appear to change little when sampled serially at 100-μs intervals; as the sample interval is increased, the correlation between lengths appears increasingly random. Overall, it is evident that the fluctuations in size are relatively small in amplitude and cluster about the mean length of the filaments. The time-average length distribution of a single filament recorded over an extended time period should, according to theory, be equivalent to the measured length distributions of Figures 2 and 3. That is, the smaller the fluctuations in size, the narrower the resultant length distribution. Quantification of exchange under these conditions is addressed next.

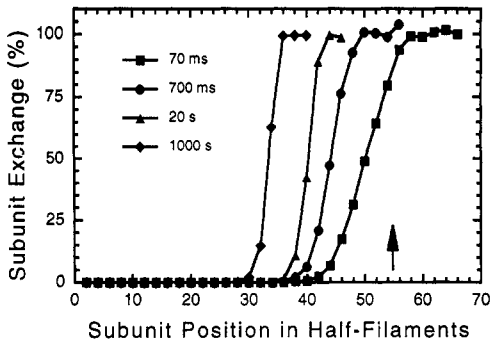

FIGURE 5: Time dependence of the physical location of subunit exchange along the lengths of a population of synthetic thick filaments. Exchange was initiated in the computer by "mixing" labeled and unlabeled populations of myosin half-filaments at equilibrium (see Figure 2 for the length distribution of these filaments). Note that the boundary between sites along the lengths of the half-filaments that have undergone exchange and those still to exchange sharpens progressively with time. Overall extents of exchange at 70 ms, 700 ms, 20 s, and 1000 s are 10, 20, 30, and 40%, respectively. The stochastic nature of the computation is apparent in the fluctuations of the 100% exchange data. The arrow indicates the mean size of the half-filaments at equilibrium.

Subunit Exchange between Thick Filaments at Equilibrium. Exchange is initiated by "mixing" a labeled and an unlabeled population of filaments at equilibrium. First-order kinetics result because the concentrations of the reacting species remain unchanged during the reaction. Rates of exchange are independent both of the total myosin concentration and of the ratio in which labeled and unlabeled filaments are mixed.

Spatial and temporal aspects of subunit exchange are investigated by "mixing" a 1:1 ratio of labeled and unlabeled thick filaments and subunits at equilibrium. This ratio optimized the number of subunits tracked into and out of filaments. A typical length distribution for the filament populations used in these computations is illustrated in Figure 2. Figure 5 illustrates the extent of exchange at each monomer position in a population of half-filaments at four moments in time. It is clear that the initial and very rapid rate of subunit exchange at the tips of the thick filaments slows dramatically as time progresses and the exchange boundary moves in toward the center of the filaments. Subunits proximal to the bare-zone are therefore much less likely to undergo exchange than their distally situated counterparts. The observations relate to the exchange of parallel-packed myosin, since the boundary with antiparallel-packed myosin at the 20-subunit level is not reached in the time period of the simulation. As time progresses, the boundary between fully exchanged and yet to be exchanged subunits sharpens significantly. Fluctuations in the extent of exchange, arising from the stochastic nature of the system, are evident in regions of the plots where exchange is complete. Positional data, in which individual myosin molecules are labeled and visualized in the electron microscope, can be directly compared with these computed data.

The overall rate of subunit exchange of subunits between thick filaments is considered next. The computation is equivalent to an experiment in which the quench of donor fluorescence is followed after mixing two filament populations labeled with fluorescent donor and acceptor molecules. The rates of exchange seen on 0.1-, 1-, 10-, and 1000-s time bases are illustrated in Figure 6. Very fast rates of exchange are recorded immediately after mixing; rates decline precipitously thereafter as time progresses: 12% exchange occurs in the first 100 ms while it takes an extended 16 min and 40 s to reach 40% exchange. The form and the extended nature of

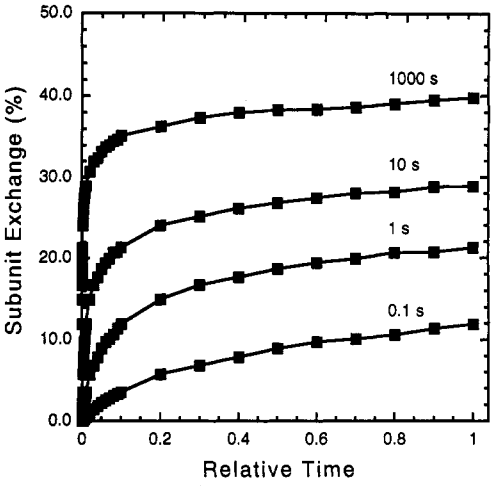

FIGURE 6: Time dependence of subunit exchange between two populations of synthetic thick filaments. Note the precipitous decline in the rate of exchange, from an initially very fast rate, as time progresses. These data are plotted with full-scale time bases ranging from 0.1 to 1000 s.

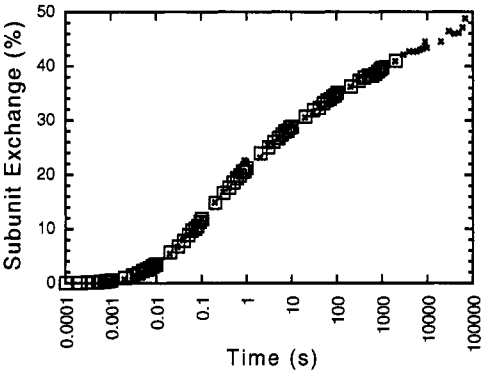

FIGURE 7: Time dependence, on a logarithmic scale, of subunit exchange between two populations of synthetic thick filaments. These data are similar to those of Figure 6. A linear extrapolation (not shown) of the latter part of the plot to the point of 100% exchange shows that full exchange would only occur after many years. Simulation times were extended by replacing the routinely used 4000 (□) half-filament array with smaller set of 400 (×) half-filaments.

the reaction are best illustrated when the data are plotted on the logarithmic time base of Figure 7. The square symbols in this figure are computed for exchange between 2 groups of 2000 half-filaments. Time constraints limit the extent of exchange that can be calculated in the computer since 3 months were required to reach the final 2000-s point on the graph. Care was taken to test whether the observed tailing-off in the reaction was a computational artifact or not. Exhaustive changes in the precision of key variables, the use of different random-number generators and running the simulations on two computers with quite different internal architectures, all failed to alter the outcome of the simulations. The time range is extended beyond the 2000-s point by reducing the size of the filament array by a factor of 10. As can be seen in Figure 7, the only consequence, apart from the required reduction in computing time, is to increase the amplitude of the fluctuations in the data. In these extended computations, it was found that 50% exchange was reached in 27 h 45 min. An indication of the time required to complete the exchange reaction can be obtained by an approximate, linear extrapolation of the 10 000–100 000-s segment of Figure 7 to an intercept at 100% exchange. The extrapolation procedure clearly does not take into account the downward trend in the data which presumably extends beyond the last data point at 50% exchange. One additional factor makes the estimate yet more conservative.

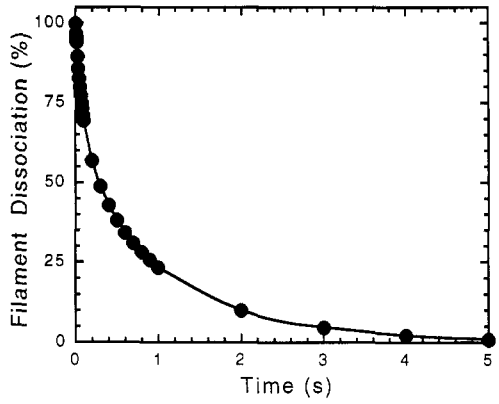

FIGURE 8: Simulation of the rate of dissociation of synthetic thick filaments, initially at equilibrium, on reducing the concentration of nonfilamentous myosin to zero. The rate profile shows that myosin thick filaments can disappear very rapidly once dissociated subunits, normally present at equilibrium, are removed from the system. The observed rapid rate of filament dissocation and final disappearance is in marked contrast to the extremely long period of time required for subunit exchange illustrated in Figures 5–7.

Exchange up to the 50% point is governed by the faster kinetics typical of parallel-packed myosin: the rate will slow even further when the boundary between parallel-packed and antiparallel-packed myosin is crossed. It would clearly take many years for full exchange to occur.

Thick Filament Dissociation on Reducing the Concentration of Nonfilamentous Myosin to Zero. Evidently subunit exchange at equilibrium as described above is far too slow to form the basis of many of the experimental observations of rapid and extensive exchange made in vitro and in vivo. This makes it worthwhile to explore the consequences of accelerating subunit exchange by inducing a dissociation–reassembly cycle to facilitate the interconversion. Figure 8 shows that thick filaments dissociate fully in 5 s when the concentration of free myosin is reduced to 0. A quantitative measure of speed of the similarly fast reverse reaction can be judged from the rate at which pressure-shortened filaments regrow (Davis, 1981b). These data imply that subunit exchange mediated by myosin filament dissociation and reassembly can be completed in a minimum time of a few seconds.

DISCUSSION

Relationship between the Computed and Independently Measured Equilibrium Properties. The kinetic mechanism of thick filament assembly used for simulation was verified by demonstrating that the independently measured equilibrium properties of the system such as the bell-shaped thick filament length distributions seen in the electron microscope and the critical concentrations recorded at equilibrium can be arrived at by calculation. The outcome is gratifying since it provides the first independent test of the various assumptions used to interpret and analyze the kinetics of the assembly and disassembly of thick filaments (Davis, 1981b, 1985). Exclusion of the nucleation reaction from the simulation is unlikely to affect the calculations because the cooperativity of assembly ensures that filaments at equilibrium are stable and unlikely to dissociate fully and re-form afresh by nucleation and growth. Stochastic simulation can therefore be reliably used to probe the spatial and temporal properties of myosin assembly in a small system at a level of detail that in some instances exceeds that currently accessible to experiment.

New information about myosin assembly is provided by the similar values obtained for the critical concentration by calculation and direct measurement. Experimental values (monomer and dimer measured as total myosin subunits) are determined by the entire mechanism which includes both nucleation and growth. Calculated values (measured as monomer) are obtained from the kinetics of growth alone (eq 11–16). At first glance, it would appear that the two values cannot be compared because different molecular species are measured. However, cross-linking experiments show that myosin monomer predominates at atmospheric pressure in measured samples (Davis et al., 1982). Reasonable estimates of monomer concentration are therefore provided by the calculated concentration of 0.43 mg mL^{-1} (nucleation excluded) and the experimentally measured value of 0.21 mg mL^{-1} (nucleation included). It can therefore be concluded that the nucleation step in myosin filament assembly has low cooperativity since the two critical concentrations differ only by a factor of 2–3. This sets myosin assembly apart from virtually all other protein polymerization reactions in which an energetically unfavorable nucleation step is followed by a series of like, favorable reaction steps.

Subunit Exchange between pH 8.0 Filaments. Myosin thick filaments formed at pH 8.0 provide the simplest experimental system of direct relevance to thick filament assembly and subunit exchange in vivo. Freedom from the stabilizing influence of certain copolymerizing proteins ensures that dynamic processes such as fluctuations in size and rates of exchange occur at their maximum rates. Their merit lies in the fact that, of all the distinct classes of synthetic thick filaments, they most closely resemble native filaments in structure [see Davis (1988a)]. A lack of copolymerizing proteins and a lack of a precisely determined length are the principal characteristics that distinguish them from their native counterparts.

The potential for exchange between thick filaments (fully equivalent to exchange between thick filaments and dissociated subunits) can be judged from the time dependence and magnitude of the spontaneous fluctuations in filament size. Computer simulation provides a vivid impression (Figure 4) of the rapidity with which the apparently static lengths of filaments seen in micrographs change in reality. Size fluctuations in individual filaments are first evident at millisecond time intervals. As the time interval between observations increases, filament sizes before and after the interval appear less correlated and increasingly random in appearance. The observation that the fluctuations appear constrained about the mean lengths of the filaments gives a clear qualitative picture of the basis for the preferential exchange of subunits at the tips of the thick filaments. It should be noted that length distributions (number average of filament size) are equivalent to a distribution of filament sizes obtained by measuring the size of a single filament at a series of equal time intervals (time average of filament size). The determination of a length distribution thus provides information about the fluctuations in filament size, but not their frequency.

A quantitative measure of the way in which fluctuations in size mediate exchange is obtained by examining the time-dependent movement of the interface between exchanged, and yet to be exchanged, subunits. It is broad when first observed at the tips of the thick filaments, sharpening progressively with time as it moves toward the center of the filament. This observation will be useful when interpreting experimental data in the future, since the steep boundary between exchanged and yet to be exchanged subunits provides a characteristic marker or "signature" for exchange (Figure 5).

Typically, the rate of exchange declines by an approximate factor of 10 for every decade of time. The parallel nature of a series of exchange versus time records (Figure 6) or the quasi-linear nature of the rate of exchange versus logarithm of time (Figure 7) is thus a unique reflection of myosin exchange. Like the boundary between exchanged and yet to be exchanged subunits mentioned in the previous paragraph, overall exchange versus time profiles provide a second characteristic "signature" whereby exchange influenced by the myosin length regulation kinetics can be identified. Simulation shows that it takes 27 h 45 min to reach 50% exchange. Crude extrapolation of the exchange data beyond the 50% exchange point revealed that it would take hundreds of years for complete exchange to occur.

Exchange between Synthetic Myosin Filaments. Rapid exchange of subunits at the tips of synthetic thick filaments is a common feature of both pH 8.0 (Figure 5) and pH 7.0 (Saad et al., 1991) synthetic filaments. Beyond this stage, the exchange properties of the two systems diverge markedly. Rates of exchange among pH 8.0 filaments decline precipitously with time as the reaction progresses inward toward the centers of the filaments. By contrast, exchange appears evenly distributed along the entire length of the pH 7.0 filament and is complete in under 1 h (Saad et al., 1991). The issue is important because the two mechanisms have entirely different and opposing implications for exchange in myosin filament systems. The question is: How can these apparently contradictory sets of data be reconciled? One can only speculate. If the experimental data are accepted as correct, then the most plausible explanation is that differences in exchange arise from known differences in structure. Origins for the similar and the divergent behaviors of pH 7.0 and 8.0 synthetic filaments are considered next.

Common structural features probably give rise to the shared feature of rapid exchange at the tips of the filaments. Both classes of filaments are bipolar and have bell-shaped length distributions (Josephs & Harrington, 1966; Kaminer & Bell, 1966). Length distributions normalized to a mean filament length are wider in absolute terms for pH 7.0 filaments, indicating reduced cooperativity (the stronger the cooperativity, the larger α and β, and the narrower the length distribution). Similarities outweigh differences at the level of structure we are considering, and it seems reasonable to assume that fluctuations in length similar to that described for pH 8.0 filaments occur in pH 7.0 filaments. Like pH 8.0 filaments then, the form of the length distributions measured in the electron microscope probably correlates with rapid fluctuations about the mean of the length distribution. That fluctuations in filament length provide a common pathway for the rapid exchange of subunits at the tips of both classes of myosin filaments seems a reasonable conclusion. Rapid exchange has also been observed among filaments prepared from smooth muscle (Trybus & Lowey, 1987), but these filaments have a quite different structure.

Structural differences provide clues as to the basis of the random and length-independent component of pH 7.0 exchange. Filaments formed at pH 7.0 are spindle-shaped and lack a central bare-zone. Native filaments and pH 8.0 filaments, on the other hand, have parallel sides, tapered tips, and a central bare-zone (Josephs & Harrington, 1966; Kaminer & Bell, 1966; Davis, 1988b). It has been proposed that the distinctive structure of pH 7.0 filaments is the result of variable amounts of adventitious myosin that coats the surface of thinner, normal sized synthetic thick filaments of pH 8.0 type (Kaminer & Bell, 1966). The excess of myosin subunits per 14.3-nm repeat is estimated to range from one to three (Emes & Rowe, 1978). Furthermore, the spindle shape indicates a preferential accumulation of myosin about the centers of pH 7.0 filaments.

There are thus two quite distinct molecular environments for myosin molecules in pH 7.0 filaments which provide a plausible basis for the observed differences in the mechanisms of exchange of pH 8.0 and 7.0 filaments. The variable amount of surface myosin is likely to associate and dissociate from the filament surface by a random mechanism as opposed to the strictly sequential assembly mechanism of pH 8.0 filaments. This variability excludes a sequential mechanism of assembly in which subunits are added in sequence and in a fixed ratio to length in the outer layer of pH 7.0 filaments. By contrast, pH 8.0 filaments, which have a sequential mechanism of assembly, exhibit a fixed ratio of subunits per unit length (Davis, 1981a). However, the core of pH 7.0 filaments is likely to exhibit the exchange properties typical of pH 8.0 filaments. Dual, parallel pathways thus appear to operate in the assembly of pH 7.0 filaments. A sequential pathway gives rise to the bipolar structure and narrow length distribution while a second pathway results in the random addition of a surface layer of subunits. Accordingly, the adventitious myosin is free to associate and dissociate randomly from the surface of the thick filament, leading to the experimental observations of Saad et al. (1991). The possibility of random exchange functioning in vivo now seems more remote in light of the above interpretation of the pH 7.0 exchange data.

Relevance of the Exchange and Facilitated Exchange to Exchange in Vivo. Computations on subunit exchange among pH 8.0 filaments suggest that exchange at equilibrium is unlikely to be directly relevant to situations where complete exchange occurs in vivo and that a different mechanism has to be invoked. Exchange in labile synthetic filaments is simply too slow after approximately 50% of subunits are randomized. Overall, native thick filaments are more stable than synthetic thick filaments. This property is seen at its most fundamental when the length of native filaments is compared with synthetic thick filaments generated under similar conditions: native thick filaments are much longer than their synthetic counterparts. Copolymerizing proteins that form part of, and stabilize, native filaments would serve to depress the rate of exchange even further. For example, C-protein, a protein that binds to pH 8.0 filaments in physiological ratios, can increase their length up to and beyond that of the native filaments through stabilization (Davis, 1988b). Using the argument presented earlier, the fixed length of native filaments attests to an absence of fluctuations in size associated with exchange in synthetic filaments.

Experimental observations that cast doubt on the universal functioning of a random exchange mechanism in vivo are considered first. Isoforms of myosin expressed in a muscle fiber are incorporated locally and do not diffuse and exchange with subunits in adjacent myofibrils (Gauthier, 1990). In developing chicken pectoral muscle, isoforms are segregated into neonatal myosin at the center of the filament with the embryonic form in the filament arms; a uniform mixing of the distribution of subunits through random exchange does not occur (Taylor & Bandman, 1989). These observations are somewhat tempered by a recent paper in which isoform segregation was not observed in pectoral muscle (Gordon & Lowey, 1992), a neutral observation in the context of our discussion. Myosin injected into cells is rapidly localized in the region of the A-band; however once bound, these molecules appear to remain in place and do not readily exchange into

adjacent thick filaments (Johnson et al., 1988). These data leave the distinct impression that circumstances exist where the subunit composition of filaments remains stable in vivo despite a proximity to a potential source of subunits that would exchange were random exchange at equilibrium operational.

The static picture presented above is in marked contrast with the high rates of exchange seen in striated muscle during isoform switching and myosin turnover. It is evident that the stability of thick filaments in muscle cells can be modulated over a wide range. For example, it takes a matter of days to switch a uniform distribution of the β-myosin heavy-chain isoform over to the α form in cardiac muscle. The complete replacement of one isoform by another is induced by hormone treatment. Preferential exchange was observed at the ends of the thick filaments where a 50% excess of label is recorded compared to a 40% below average level about the centers of the filaments (Wenderoth & Eisenberg, 1987). The labeling pattern is consistent with the kinetics of myosin self-assembly in which preferential exchange is expected at the tips of the thick filaments. The observed pattern is unlikely to arise from exchange at equilibrium because it is simply too slow even among kinetically labile synthetic thick filaments. Similar conclusions apply to myosin turnover in vivo where rates of turnover of myosin and associated proteins in muscle are high ($t_{1/2}$ of 5.4 days for rat cardiac myosin) and occur in the absence of loss of function (Zak et al., 1977, 1979). The kinetics of a mechanism for the acceleration of subunit exchange above that encountered at equilibrium are outlined next.

An attractive way of accelerating exchange in vivo is by facilitated exchange in which partial or complete dissociation of filaments is followed by the introduction of new subunits on reassembly. It is a potentially important mechanism because subunit exchange at equilibrium is clearly far too slow to form the basis of many of the experimental observations alluded to above. The rate of facilitated exchange depends not on fluctuations in filament size but on the speed at which filaments dissociate and reassemble.

Rates of thick filament dissociation relevant to facilitated exchange have been recorded at elevated pressures (Davis, 1981b, 1985). These data are, however, not directly relevant to filament dissociation at atmospheric pressure because the reaction kinetics (eq 4–8) exhibit a marked and complex dependence on hydrostatic pressure. Stochastic simulation of dissociation profiles (Figure 8) at atmospheric pressure overcomes the problem and shows that pH 8.0 filaments can dissociate fully in 5 s odd. Estimates of the rate of the reverse reaction can be judged directly from pressure-jump data. Typical reaction profiles [see Figure 1 of Davis (1981b)] recorded at atmospheric pressure show that shortened filaments can grow to their equilibrium length in seconds or less. A few seconds thus appears to be the minimum amount of time required for a full cycle of filament disassembly and reassembly among synthetic thick filaments, in marked contrast to the extremely slow limiting rates recorded for exchange at equilibrium. While it is improbable that these rates can be attained in vivo, the results do point to a modulation of the concentration of nonfilamentous myosin (and copolymerizing proteins etc. in more complex systems) as an effective and potentially rapid means for first removing old subunits and later replacing them with new. In vivo, the rate-limiting step will depend on the speed at which old subunits are removed and new subunits are introduced by the degradative and synthetic processes of the cell. A boundary between exchanged and yet to be exchanged subunits, resembling that observed for exchange at equilibrium, will also form in cases of facilitated exchange when the filaments are partly dissociated.

In conclusion, it appears that despite the intuitive appeal of exchange at equilibrium as a universal mechanism for replacing myosin subunits in thick filaments, it seems likely that its functioning is limited both in vivo and in vitro to the exchange of subunits away from the center of the filament. Excluding the discovery of an as yet undescribed mechanism of exchange, it appears that the exchange of subunit in and out of thick filaments by a random mechanism of exchange is most unlikely. Facilitated exchange offers an alternative mechanism in which old subunits are released by dissociating filaments by lowering the concentrations of subunits or their affinity for thick filaments followed by reassembly during which newly synthesized subunits are incorporated. A precedent exists in muscle hypertrophy and atrophy which are mediated by the addition and loss of thick filaments to sarcomeres without compromising muscle function. The mechanism thus provides a plausible and potentially very fast means of removing and replacing subunits in myosin thick filaments.

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