fold less efficient than release induced by intact virus. Moreover, neither intact penton base protein nor the other major external proteins (hexon, fiber) caused release of liposome contents (data not shown). Thus, it is unlikely that the penton base contains a pore-forming structure. This is consistent with our observation that release of marker from individual liposomes exhibited a graded rather than an all or none response, as was the case with pore-forming cytolysin (Blumenthal et al., 1984). Marker release is more likely to be caused by a transient perturbation of the lipid bilayer structure (Blumenthal & Klausner, 1982).

Since the fiber protein is bound to the receptor, the penton base is probably in close apposition to the target membrane. Lowering the pH in the endocytic vesicle could enable the hydrophobic residues in the penton base to react with a lipid patch as the initial event in target membrane disruption. The finding that adenovirus permeabilizes liposomes provides an assay which can now be used in an attempt to reconstitute the receptor into liposomes to study the entry process under conditions closer to those occurring in the cell.

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Myosin Subfragment 1 Has Tertiary Structural Domains†

Stefan Highsmith*

Department of Biochemistry, School of Dentistry, University of the Pacific, San Francisco, California 94115

Don Eden

Department of Chemistry, San Francisco State University, San Francisco, California 94132

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ABSTRACT: Transient electrical birefringence measurements were made on skeletal muscle myosin subfragment 1 (S1) at 3.7 °C in 10 mM tris(hydroxymethyl)aminomethane–acetate and 0.10 mM $MgCl_2$, pH 7.0. The specific birefringence for 4.5 μM S1 was determined from steady-state measurements to be $(8.1 \pm 0.3) \times 10^{-7}$ (cm/statvolt)^2. For electric fields in the range of 2.47–24.7 statvolts/cm, the alignment was due to a large permanent dipole moment for S1, estimated to be 8500 ± 2000 D. The duration and the strength of the transient electric field was varied, and the temporal response of the decay of the birefringence signal was analyzed. The rate of rotational motion after the field was removed increased with increasing field strength for short (0.35-μs) pulses and decreased with increasing pulse lengths for all field strengths. The rate of decay from a steady-state birefringence signal was independent of field strength. A model of S1 structure is proposed, which is consistent with these data and most other data on S1 structure. In this model, S1 is composed of two tertiary structural domains that are connected by a flexible linkage with a substantial restoring force. The electric dipole moments on the two domains are arranged head to tail. The segmental movement of the domains is restricted to certain directions. The average conformation of the molecule is elongated, but it can be made more compact by the torque exerted by an electric field. The structural changes depend on the strength and duration of the pulse. This hypothesized structure is discussed in regard to cross-bridge mechanisms of force generation that require S1 to bend and to an elastic element in the cross-bridge.

Force generation by actomyosin and ATP in muscle occurs while myosin is bound to actin. The portion of myosin that interacts with ATP and actin is called the cross-bridge, and a variety of cross-bridge mechanisms have been proposed for the force-generating step. These mechanisms vary widely in the details of the structural changes involved, including suggestions that the attached cross-bridge rolls, bends, or shrinks in order to cause the thick and thin filaments to interdigitate.

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This literature has been reviewed (Huxley, 1969; Huxley & Simmons, 1971; Eisenberg & Hill, 1978; Tregear & Marston, 1979; Morales et al., 1982; Highsmith & Cooke, 1983; Goody & Holmes, 1983; Harrington & Rodgers, 1984). The bending mechanisms require the part of the attached cross-bridge called subfragment 1 (S1)[1] to change its shape. Put another way, in this mechanism, S1 bound to actin would display segmental motion as one of its tertiary structural domains moved with respect to another in order to generate force.

There are several reasons to suspect that S1 has tertiary structural domains that could undergo segmental motion of the type suggested: S1 is readily cleaved by trypsin into discrete primary structural domains (Yagi, 1975; Mornet et al., 1979); one of the primary structural domains, when isolated, appears to bind actin (Mulrad & Morales, 1984); X-ray diffraction measurements on crystallized S1 indicate it has a narrow middle portion with larger segments on either side (Winkelmann et al., 1985); two sulfhydryl groups on S1 can be chemically cross-linked over a substantial range of distances between the two groups (Wells & Yount, 1980); ^{1}H and ^{13}C NMR measurements indicate that there is a large amount of internal mobility for the amino acid side chains and backbone structure of S1 (Highsmith et al., 1979; Prince et al., 1981; Eads & Mandelkern, 1984); two conformations of S1 are detected in solution by ^{31}P NMR measurements of chemical shifts from bound nucleotides (Shriver & Sykes, 1981); and finally, spin-labeled S1 moieties in muscle fibers appear to generate force with at least a portion of the S1's orientation remaining unchanged (Cooke et al., 1982). It has been hypothesized that S1 bends to generate force (Highsmith & Cooke, 1983; Goody & Holmes, 1983).

Nonetheless, the S1 tertiary structural domains and their segmental motions are speculative. The data above are consistent with an S1 that has no segmental structure and undergoes only local conformational changes (Highsmith & Cooke, 1983). Studies of S1 structural dynamics in solution have been interpreted in terms of a rigid S1 (Mendelson et al., 1973; Thomas et al., 1975; Kobaysi & Totsuka, 1975; Yang & Wu, 1977; Kretzschmar et al., 1979; Garrigos et al., 1983; Morel & Garrigos, 1983).

Reported here are the rates for rotational motion of S1 in solution determined from the decay of birefringence that has been induced by transient electric fields of varying strengths and durations. An internally consistent analysis of the data requires a model of S1 that can exhibit reversible segmental motions as well as rotational alignment when subjected to a transient electric field. Our results are the first observation that S1 is capable of segmental motion in solution and support cross-bridge mechanisms involving S1 bending to generate force.

Experimental Procedures

S1 was prepared from rabbit dorsal muscle myosin by the action of α-chymotrypsin in the absence of Mg^{2+} (Weeds & Pope, 1976) and purified by size-exclusion chromatography by using Sephacryl S-400. Light chains 1 and 3 were present, light chain 2 was absent, and the 95 000-dalton heavy-chain fragment was homogeneous when analyzed by gel electrophoresis in the presence of SDS (Weeds & Pope, 1976; Highsmith & Eden, 1985). ATPase activities at 25 °C in 100 mM KCl, 5 mM $CaCl_2$, 2 mM ATP, and 10 mM MOPS (pH 7.5) were typically 3.1 s^{-1}. Samples of 5–20 μM S1 were dialyzed against 10 mM Tris–acetate (pH 7.0) and 0.1 mM $MgCl_2$ at 4 °C and centrifuged at 80000g for 1 h before birefringence measurements were made. The stock S1 solutions were diluted with the final dialyzate to obtain the desired [S1].

Steady-state electric birefringence measurements were made by using the high-power pulse generator and the instrument described previously (Eden & Elias, 1983; Highsmith & Eden, 1985). In each series of measurements, for the first two voltages used, data consisting of one-tenth the number of electric field pulses needed for good statistics were collected, stored, and used to obtain a steady-state birefringence that corresponded to a fresh S1 sample. The values for the birefringence were always equal, within experimental error, to the values obtained subsequently with 10 times as many pulses. The field strengths were varied randomly, and at least one early measurement was repeated at the end of a series to ensure further that the electric field was not altering the samples. Pulses were 5 and 7 μs long and varied between 2.47 and 24.7 statvolts/cm (1 statvolt = 299.78 V). The rise of the birefringence signal was fit to the expressions derived by Tinoco and Yamaoka (1959) to determine the contributions of permanent and induced dipoles, assuming that the induced dipole was rapid.

The time course of the decay of the birefringence signals was determined by analyzing the decay after a steady-state signal was obtained by using a 7-μs pulse or by using fresh samples of S1 and 0.35-μs pulses produced by a cable-discharge pulser. The maximum signal obtained with the short pulses was typically about one-tenth of that obtained with a 7-μs pulse of the same voltage. For the short pulses, many more applications of the electric field were required to obtain adequate statistics. The CaATPase activity has been determined after measurements to be within 90% of the original value (Highsmith & Eden, 1985).

It was noticed that a small slowly decaying signal of opposite sign to that of the birefringence was observed after the short pulses were terminated, when the laser beam was blocked. Before and after every determination of a rate of decay, data sets were collected with the laser beam blocked, and these were used to correct the raw data. This correction consistently gave a decay time 8–10% slower than that obtained with the raw data.

The decay of the birefringence signal (after correcting for the pick up obtained with the laser beam blocked) was fit by using a multiexponential decay analysis program titled DISCRETE (Provencher, 1976a,b). The base line (typically <1% of the maximum signal) was a floating parameter, and DISCRETE determined the minimum number of exponential decays that were required to fit the data, given its accuracy. In over 80% of the cases, the best fit was obtained with a single-exponential decay. In the remaining cases, the single exponential was the second best fit. There was no correlation between the cases where the single-exponential decay was second best to the strength or duration of the pulse or the signal to noise ratio. All the decay constants reported here are for single-exponential functions.

Results

When solutions containing 3–7 μM S1, 10 mM Tris–acetate (pH 7.0), and 0.10 mM $MgCl_2$ at 3.7 °C were subjected to a 7-μs pulse ranging from 2.47 to 24.7 stratvolts/cm, a steady-state birefringence signal was obtained. The birefringence responses showing the buildup and decay obtained with

[1] Abbreviations: S1, myosin subfragment 1; τ, rotational decay time; Φ, orientation function; μ_p, permanent electric dipole moment; Δn_{av}, steady-state birefringence; Δn_s, staturation birefringence; E, electric field; K_{sp}, specific birefringence; Tris, tris(hydroxymethyl)aminomethane.

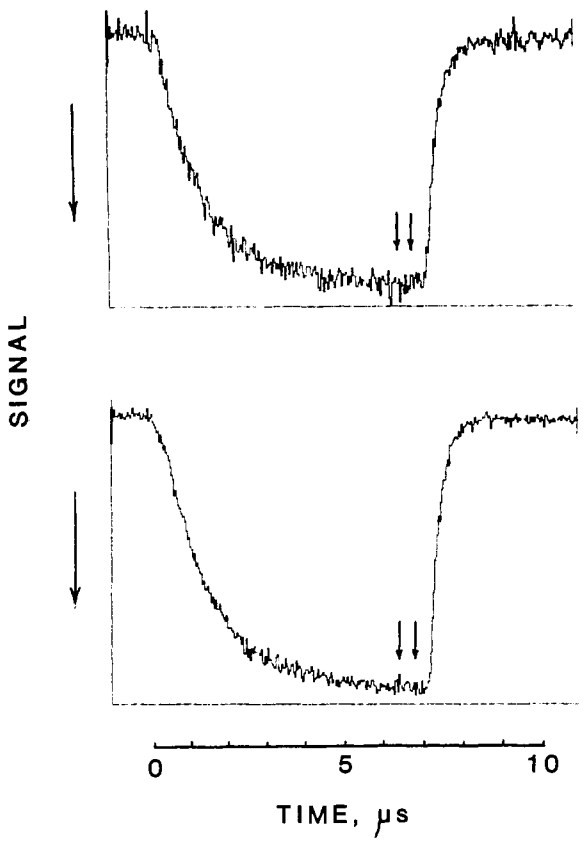

FIGURE 1: Birefringence signal. The transient electric birefringence signals for a solution of 4.5 μM S1, 10 mM Tris–acetate, and 0.10 mM $MgCl_2$, pH 7.0, at 3.7 °C when the 7-μs square-wave pulse of 2.47 (upper trace) or 19.7 (lower trace) statvolts/cm is applied are shown. The arrows indicate the region used to determine the region for the steady-state birefringence.

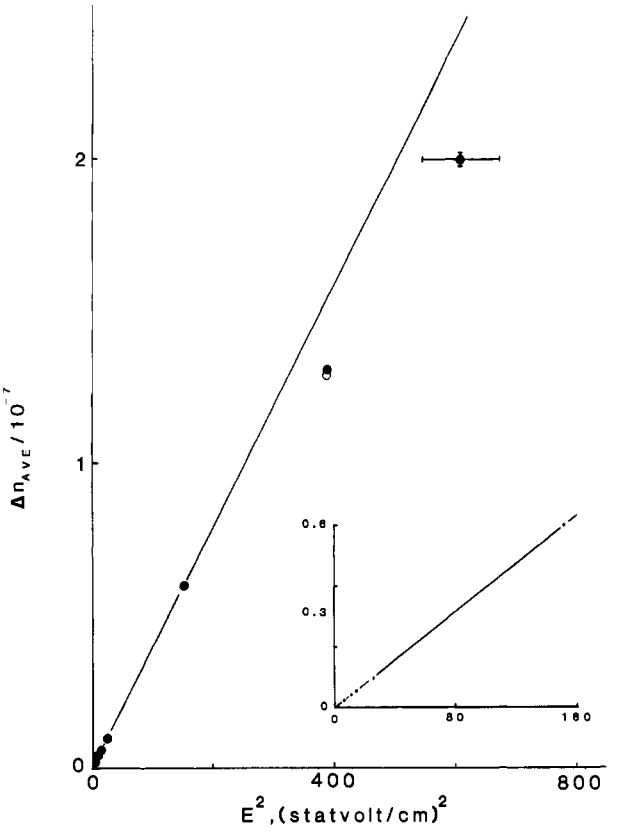

FIGURE 2: Kerr plot. The values for the steady-state birefringence Δn_{av} plotted as a function of the square of the applied electric field. The inset shows the lower field results in greater detail. Data with 7-μs (●) and 5-μs (○) pulses are shown.

2.47 and 19.7 statvolts/cm pulses are shown in Figure 1. Note that the rise time is much slower than the decay. The rise time was analyzed by using the theory of Tinoco and Yamaoka (1959) for the case of a permanent dipole and a rapidly induced dipole with their orientations along the symmetry axis of the molecule. The ratio of the permanent to induced dipole contributions to the birefringence signal was greater than 20 in both cases. Thus, for the solutions and field conditions used, the alignment of S1 is due primarily to its permanent dipole moment.

The steady-state birefringence Δn_{av} determined from the amplitude of a 400-ns region near the end of the pulse (see arrows in Figure 1) for each of seven voltages is plotted as a function of the square of the electric field in Figure 2. Pulses that were 5 μs long gave slightly smaller numerical values for the birefringence but were statistically within experimental error of the 7-μs results. At lower voltages (Figure 2, inset), the Kerr law is obeyed, and the specific birefringence K_{sp} for 4.5 μM S1 in 10 mM Tris–acetate and 0.1 mM $MgCl_2$, pH 7 at 3.7 °C, is $(8.1 \pm 0.3) \times 10^{-7}$ (cm/statvolt)^2. Above 12 statvolts/cm, the data deviate from the behavior predicted by the Kerr law. At the highest voltage used, the birefringence is 17% lower than predicted. A value for the saturation birefringence Δn_s was estimated from a plot (not shown) of Δn_{av} vs. $1/E^2$ extrapolated to $1/E^2 = 0$ (Fredericq & Houssier, 1973). This value for Δn_s was used to calculate values for the orientation function Φ. By use of the method of O'Konski et al. (1959) and if a negligible induced dipole moment is assumed, the permanent dipole moment μ_P for S1 was estimated to be 8500 ± 2000 D. Although the precision for this estimate of μ_P for S1 is low, the information is useful. It is clear that

Table I: Rotational Relaxation Times Obtained at 3.7 °C at Zero Field after Transient Electric Field Pulses of Different Strengths and Durations

E (statvolt/cm)	τ_s (μs)[a]	τ_L (μs)[b]
3.3	0.236 ± 0.003	0.321 ± 0.004
19.7	0.174 ± 0.004	0.311 ± 0.005

[a] Obtained after a 0.35-μs pulse. [b] Obtained after a 7.0-μs pulse.

S1 has a large permanent dipole moment that will interact with nearby charges and electric fields. For the measurements here, the interaction energy $\hat{\mu}_P \cdot \hat{E}$ for the alignment of S1 is much smaller than the thermal energy, $^3/_2kT$, for the smaller electric fields used and is larger than $^3/_2kT$ for the larger fields. Thus, it is likely that the values for Δn_{av} (Figure 2) that are less than those predicted by the Kerr law reflect the approach of the saturation of the birefringence.

The decay of the birefringence was analyzed after long (7-μs) and short (0.35-μs) pulses to obtain the rotational relaxation times τ_L and τ_S, respectively. Values obtained for τ_S are plotted as a function of field strength in Figure 3. As the field strength was increased from 2.47 to 20.4 statvolts/cm, the rotational relaxation time decreased by 21% from 0.236 ± 0.003 to 0.174 ± 0.004 μs. This substantial increase in the apparent rate of rotational relaxation was reproducible and independent of the order in which the varying field strengths were applied to the sample. A limited number of measurements on solutions at higher ionic strength (not shown) suggested the increase in the rate of rotation with increasing field strength was enhanced by increasing [KCl] and/or [$MgCl_2$].

If the pulse duration was increased so that the birefringence signal reached steady state, the relaxation time after the field was removed, τ_L, increased and became independent of the field strength (Table I). For 7-μs pulses, the observed rota-

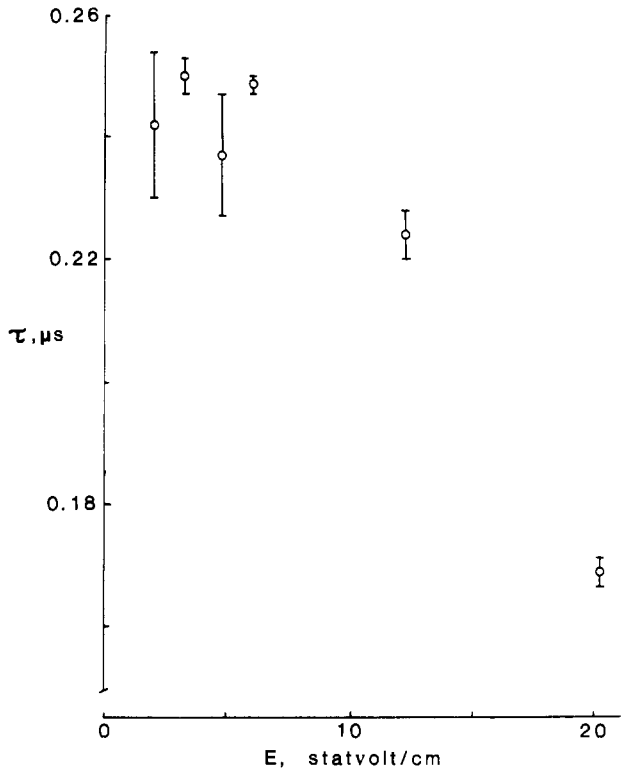

FIGURE 3: Electric field strength dependence of relaxation rates. The values for the rotational relaxation time τ_S, when the decay after the electric field was fit to the expression $\Delta n/\Delta n_{max} = A \exp(-t/\tau_S) + B$, are plotted as a function of the strength of the 0.35-μs electric field pulse. Error bars are 1 SD.

tional relaxation times for 3.3 and 19.7 statvolt/cm fields differed by less than 3%.

DISCUSSION

The value for the specific birefringence of S1 is compatible with the somewhat smaller value for S1 in solutions of higher ionic strength obtained by using weak electric fields (Highsmith & Eden, 1985). Kerr law behavior at lower electric fields (Figure 2), independent of the order in which the fields were applied, indicates that no irreversible change in the structure of S1 is caused by the measurements. The deviation from the Kerr law that occurs above 12 statvolts/cm appears to be due to partial saturation of the birefringence. The observation that S1 has a large permanent dipole, on the order of 8500 D, is new structural information about myosin that may be related to cross-bridge orientation in relaxed muscle and to the interaction of S1 with actin during activation and/or contraction. Actin subunits have dipole moments that are perpendicular to the thin filament axis (Kobayasi et al., 1964). We observed changes in S1 orientation by using fields as low as 750 V/cm.

The data in Figure 3 and Table I are not compatible with a rigid monomeric S1 structure of any shape. Both localized flexibility and elasticity need to be added to the properties of S1. First consider flexibility, which is made necessary by the observed decrease in τ that occurs when the duration of a weak pulse is decreased. This observation is not compatible with an S1 structure of a uniform semiflexible nature. The elastic modulus of protein structures is of the order of 10^{11} dyn/cm^2 (Suezaki & Gō, 1976), and the persistence length of an elongated uniform semiflexible protein with a cross-sectional area equal to that of S1 would be more than an order of magnitude larger than the length of S1. Hvidt et al. (1983) have calculated that for LMM the persistence length is about

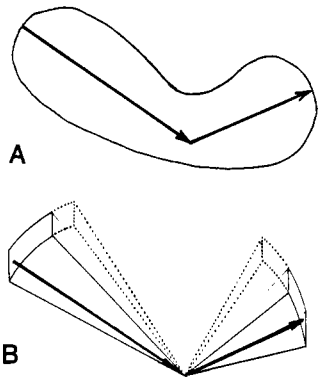

FIGURE 4: S1 structural model. In this model, S1 is segmental and flexible. Its average conformation is nearly fully elongated, and its motion is restricted. (A) Average conformation of S1 in the absence of a perturbation. Electric dipole moments are imposed on tertiary structural domains. It is flexible in the region where the dipoles join. (B) Only the electric dipoles in their average conformation are shown along with the volumes in which they can move. The solid lines indicate the volumes available as the segments move with only thermal perturbation. The dotted lines indicate the volumes available when the segmented structure is distorted by an electric field.

twice its contour length, and one can estimate that for a coiled coil of two α-helices the length of S1 the ratio of the observed τ value to that for a rigid molecule is greater than 0.95. S1 is thicker than LMM and would be much stiffer; yet the ratio is 0.74, using τ values obtained with 0.35- and 7-μs pulses and an electric field for which the interaction energy is 1/100 of the thermal energy. This is a strong indication that S1 is segmentally flexible.

Second consider elasticity. All solid materials are elastic under the appropriate conditions, and recent transient electrical birefringence measurements on DNA have also shown that strong electric fields can selectively populate internal modes of motions in that molecule (R. J. Lewis, R. Pecora, and D. Eden, personal communication; Lewis et al., 1986). The observed rotational dynamics of S1 when the electric field strength is increased (Figure 3) could be due to several causes. The less likely ones are discussed below. The most likely explanation is that S1 bending is also elastic. It is assumed that the segments that can move flexibly are also moved elastically when stressed by stronger electric fields. It seems worth pointing out that the interaction energy that induces elastic bending under these conditions is in the range above 1×10^{-13} erg per S1, assuming a perpendicular orientation of the S1 dipole moment in the field. This energy compares favorably to the energy available from ATP hydrolysis for mechanical work which is 2×10^{-13} erg per S1, assuming 5.1×10^{-13} erg/ATP (7.3 kcal/mol) for ATP hydrolysis and 40% efficiency for conversion to work. If elasticity is the best interpretation of the observed changes in S1 rotational dynamics caused by strong electric fields, the energetics of S1 bending are suitable for being quantitatively coupled to cross-bridge mechanical work. This would make S1 bending a plausible mechanism for the transient storage of mechanical energy during asynchronous cross-bridge activity in muscle.

On the basis of the above discussion, a S1 structure is suggested, which has two segments, each with a substantial permanent diple moment, connected head to tail by an elastic flexible linkage. The average conformation has the segments nearly fully extended. A schematic picture is shown in Figure 4A, which is meant to represent a structure of approximately the proportions obtained from X-ray crystallography (Winklemann et al., 1985) with permanent dipole moments imposed on the hypothesized segments. Each dipole moment moves

with its segment. The linkage is flexible, but to conform to the observation that stronger fields only cause faster rotation, there are restricted asymmetric volumes available for the segmental motion (Figure 4B). The structure is akin to an extended arm (from wrist to shoulder) with an elbow that allows it to bend only in restricted directions.

With this model of S1 structure, the increase in the rate of rotational relaxation with decreasing pulse length (Table I) is a result of a segment being moved more rapidly by the field than is the entire molecule. After a short pulse, the distribution of conformational orientations of both the segments and the entire molecule is redistributed to favor dipole orientations aligned with the field, but the segments align more quickly, and their relaxtion dominates the signal decay. This ability to selectively populate the orientational conformations of a smaller segment by appropriate electric field pulse characteristics has been demonstrated for heavy meromyosin (HMM) (Highsmith & Eden, 1985), a molecule that is known to be segmental and flexible (Mendelson et al., 1973). In the HMM case, the ratio of the lengths of the segments is about 4, and the alignment of the smaller segment and its relaxtion were resolved (Highsmith & Eden, 1985). In the present case, the relative sizes of the hypothesized segments are unknown. The apparent segments in low-resolution structures of crystallized S1 obtained by X-ray diffraction (Winklemann et al., 1985) and the relative sizes of the primary structural domains obtained with trypsin (Mornet et al., 1979) both suggest the ratio would be smaller for S1 than it is for HMM. If true, resolution of independent segmental motion, as observed for HMM, would be more difficult, and the observed relaxation times would reflect coupled modes of rotational motion. Transient electrical birefringence measurements on myosin rod, which has segments more nearly equal in length, did not permit resolution of individual segmental motion (Highsmith et al., 1977), although a faster mode compatible with the segmental motion has been detected (Highsmith et al., 1982).

The second observation, the decrease in τ_s with increasing field strength (Figure 3), is also consistent with a flexibly segmental S1. For the higher field strengths, the interaction energy is large (see above), and the greatest torque is exerted on dipoles that are perpendicular to the field. This allows a transient distortion of some of the S1's into conformations more compact than those occurring due to the thermally induced distribution of conformations. The observed decrease in τ's could be due to more rapid end-over-end rotation of the more compact S1 or to faster segmental motion driven by a restoring force.

For high or low fields, after sufficient time, the head-to-tail dipole arrangment makes the elongated conformation the most preferred. The independence of τ_L on E (Table I) suggests that S1 cannot be distorted into a conformation significantly more elongated that its average conformation, by the field strengths used here. Figure 4A shows the diple moments and the segments. The segments move with the dipoles. In Figure 4B, the dipoles are shown (without the segments, for clarity) along with the suggested volumes to which their movement would be confined during thermal and strong electric field perturbations.

A possible alternative explanation of the increase in τ with increasing pulse length is size heterogeneity. The mass variation due to light chain 1 or 3 being present is less than 4% of the mass of the heavy chain–light chain complex. Rates of rotation have been measured by fluorescence anisotropy decay, and the ratio of the decay times for S1A1 and S1A2 is 1.1 (Wadzinski et al., 1979), while in Table I, τ_L/τ_S is 1.4. It seems unlikely that size heterogeneity due to light-chain composition is the source of the dependency of τ on pulse length. Size heterogeneity could also be due to S1 having two conformations in solution with different shapes. Kinetic heterogeneity for acto–S1 has been reported (Tonomura & Inoue, 1977), and Shriver and Sykes (1981) have resolved two free S1 conformations in solution by making ^{31}P NMR measurements on bound nucleotide. Two interconvertible bound S1 conformations have been detected from multiphasic Stern–Volmer quenching curves for a bound fluorescent ATP analogue (Rosenfeld & Taylor, 1984). The two interconvertible states of S1 detected when it is free and bound could have significantly different rotational correlation times and could be the cause of the dependence of τ on pulse length for *weak* fields (Table I; 3.3 statvolts/cm). This would be a very interesting special case of S1 segmental motion. However, there is no information of the sizes or shapes of the two S1 structures that exist for free (Shriver & Sykes, 1981) or bound (Rosenfeld & Taylor, 1984) S1, and no definite connection between those results and the results in Figure 2 and Table I can be made here. It must be added that, although two S1 structures might explain the increase in τ with pulse length, they cannot explain the decrease in τ_S, but not in τ_L, with increasing field strength.

It is worth noting that the rotational relaxation times obtained after the steady-state signal are larger than the values obtained with probes to measure rotational Brownian motion (Mendelson et al., 1973; Thomas et al., 1975). This is consistent with a flexible S1 structure. It is also interesting that the relaxation times observed after a 7-μs pulse (Table I) give a length for S1, assuming 0.3 g of water/g of protein and using the Perrin shape factor with an axial ratio of 3.5 for a prolate ellipsoid, of 19–20 nm, a length that is consistent with that derived from electron micrographs (Elliott & Offer, 1978).

Serious consideration was given to the possibility that the dependence of on the field strength and pulse duration was due to the presence of a mixture of S1 monomer and dimer. It has been reported that S1 dimerizes under some conditions (Morel & Garrigos, 1982). Some of the data here are compatible with a monomer–dimer equilibrium that is driven in the monomer direction by an electric field. The increase in τ with pulse length at low-field strength would then be due to increasing the signal from the dimer, which takes longer to align, and the decrease in τ_S with increasing field strength would be due to field-induced dissociation. However, at high field strength, the same field that induced hypothetical dimer dissociation during short pulses would also have to induce association during a longer pulse. This inconsistency makes it unlikely that S1 dimerization is the cause of the behavior observed here. It should be noted that the volume ratio of S1 to solvent is 1:3000 and that the volume searched by diffusion during a 7-μs pulse, according to Berg and von Hippel (1985), is less than 4% of the solvent volume per S1 for the conditions used, making it unlikely that monomers would collide.

Our model of S1 as a molecule with tertiary structural domains connected by a flexible elastic linkage that restricts the motion of the segments to particular directions is a very resonable one for a cross-bridge that would bind to actin in an elongated form and then bend in a particular direction. Undirected flexibility would be less reasonable. If S1 does bend to produce force, it is likely that its relaxed conformation would be elongated and necessary that the power stroke be less than the length of S1. The prediction of a shortened power stroke is consistent with the recent suggestion by Huxley and Kress (1985) that the cross-bridge moves 4 nm instead of 12

nm during the force-producing part of the cycle.

The flexibility observed when the interaction energy for the S1 dipole moment in the electric field is less than $^3/_2$ kT and the distortion to a more compact conformation observed when the interaction energy is larger than $^3/_2$ kT may both occur under the conditions of actin binding and ATP hydrolysis product dissociation. In such a case, the bending would contribute to the orientational changes in fibers that are presumably correlated with the observed changes in acto–S1 affinity, as the cross-bridge nucleotide binding site is occupied progressively by ADP·P_i, ADP, and nothing (Highsmith, 1976; Green & Eisenberg, 1978; Hibberd et al., 1985). It is also possible that the distortion of S1 structure that can be induced by an electric field has a role as an elastic element for the cross bridge, to allow it to store energy during the asynchronous cycling of cross-bridges during contraction.

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