Force Generation and Temperature-Jump and Length-Jump Tension Transients in Muscle Fibers

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ABSTRACT Muscle tension rises with increasing temperature. The kinetics that govern the tension rise of maximally Ca^{2+}-activated, skinned rabbit psoas fibers over a temperature range of 0–30°C was characterized in laser temperature-jump experiments. The kinetic response is simple and can be readily interpreted in terms of a basic three-step mechanism of contraction, which includes a temperature-sensitive rapid preequilibrium(a) linked to a temperature-insensitive rate-limiting step and followed by a temperature-sensitive tension-generating step. These data and mechanism are compared and contrasted with the more complex length-jump Huxley-Simmons phases in which all states that generate tension or bear tension are perturbed. The rate of the Huxley-Simmons phase 4 is temperature sensitive at low temperatures but plateaus at high temperatures, indicating a change in rate-limiting step from a temperature-sensitive (phase 4_a) to a temperature-insensitive reaction (phase 4_b); the latter appears to correlate with the slow, temperature-insensitive temperature-jump relaxation. Phase 3 is absent in the temperature-jump, which excludes it from tension generation. We confirm that de novo tension generation occurs as an order-disorder transition during phase 2_{slow} and the equivalent, temperature-sensitive temperature-jump relaxation.

INTRODUCTION

Raising the temperature of a muscle results in an increase in tension. In skinned rabbit psoas fibers, tension increases in a sigmoidal fashion from a low value near 0°C to a maximum at ~45°C (Ranatunga, 1994). The kinetics that give rise to this temperature-induced increase in tension can be readily investigated in laser temperature-jump (T-jump) experiments in which fiber temperature is raised a few degrees in <1 μs. The resultant tension transient consists of three exponential phases (Davis and Harrington, 1987a, 1993a,b). Only the slower two are a direct result of the effect of temperature on the cross-bridge. The fastest ($1/\tau_1$), arises from a mini length-jump/step (L-jump) imposed on the fiber by the step expansion of the proteins following the T-jump (Goldman et al., 1987; Davis and Harrington, 1993a). The dominant biphasic tension transient that accounts for all of the tension rise on heating consists of a slow temperature-insensitive step ($1/\tau_3$), coupled to a medium speed, temperature-sensitive transition ($1/\tau_2$), considered to be associated with de novo tension generation (Davis and Harrington, 1993a,b). A simple, base mechanism of contraction, consistent with the T-jump kinetics, is presented in the Discussion.

Mechanical transients resulting from the perturbation of muscle fibers by small, abrupt changes in length provide the reference system for cross-correlating tension transients seen with other techniques (Huxley and Simmons, 1971; Ford et al., 1977). Unlike the response to a laser T-jump described above, the response to an L-jump is complex and multiphasic in form, as all states that generate tension or bear tension (either transiently or continuously) are perturbed. In small-perturbation experiments, all of these processes occur simultaneously as a sum of a series of exponentials (Davis and Harrington, 1993a). A step change in the length of a muscle fiber contracting under isometric conditions results in an immediate change in tension called phase 1. Return to the pre-jump isometric tension after phase 1 is typically governed by the sum of a few exponential processes. It is during phase 2 that most of the initial isometric tension, lost during phase 1, is recovered. Phase 3 is evident next and characteristically has an amplitude opposite in sign to that of phase 2. Phase 4, the slowest of the relaxations, returns fiber tension to the pre-jump isometric value.

Phase 2 has long been considered to be the primary force-generating transition in muscle fibers (Huxley and Simmons, 1971). Unlike other kinetic steps, its apparent rate constant is remarkably sensitive to fiber tension, slowing at high tensions and accelerating at low. Davis and Harrington (1993a) presented evidence to show that phase 2 is heterogeneous consisting of (1) a fast, virtually temperature-insensitive step (phase 2_{fast}) with properties of a damped elastic element and (2) a slower temperature-sensitive step (phase 2_{slow}) with an amplitude that correlates with isometric tension. Comparative L- and T-jump experiments showed that phase 2_{slow} correlates with the medium speed T-jump relaxation ($1/\tau_2$), the order-disorder transition thought responsible for de novo tension generation (Davis and Harrington, 1993a).

Kawai and colleagues have proposed a different scheme with phase 3 as the step in which de novo tension is generated (Kawai and Halvorson, 1991; Kawai and Zhao, 1993). Hitherto, the consensus was that phase 3 arose from cross-bridge detachment from actin (Huxley and Simmons, 1973; Ferenczi et al., 1982; Ford et al., 1985). According to Kawai's

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mechanism, phase 2_{fast} and phase 2_{slow} (their phases d and c) are not tension generating and arise from a two-step binding of ATP to the cross-bridge at the end of the contractile cycle. To reconcile their mechanism with the T-jump data (Zhao and Kawai, 1994), they assigned phase 3 (their phase b) and not phase 2_{slow} (their phase c) to the medium speed, de novo tension-generating step seen in the laser T-jump experiments of Davis and Harrington (1993a).

The aim of this paper is to correlate the T-jump and L-jump mechanical tension transients with a view to understanding their role in the contractile cycle. First, a simple and fundamental kinetic scheme for tension generation based on the T-jump kinetics is presented. Emphasis is placed on the slower steps (L-jump Huxley-Simmons phases 3 and 4 and the slow speed T-jump relaxation ($1/\tau_3$)), as earlier comparative studies focused on faster steps in the time domain of L-jump phases 1 and 2 and T-jump relaxations $1/\tau_1$ and $1/\tau_2$ (Davis and Harrington, 1993a). Phase 3 is excluded from its proposed role in tension generation (Kawai and Halvorson, 1991; Kawai and Zhao, 1993; Zhao and Kawai, 1994) by showing that it is kinetically quite distinct from $1/\tau_2$, the de novo tension-generating, medium speed, T-jump relaxation. In addition, we reason on thermodynamic grounds that the negative amplitude of phase 3, which opposes tension recovery in L-jump experiments, effectively excludes it from a role in tension generation. The implied uncoupling of de novo tension generation from phosphate release is discussed elsewhere (Davis and Rodgers, 1995, and manuscript in preparation). Mechanistic roles for the various T- and L-jump slow steps are also considered.

MATERIALS AND METHODS

Fiber preparation and solutions

Rabbits were sacrificed and fiber bundles were prepared and chemically skinned according to Davis and Harrington (1993a). Aluminum T-clips (Goldman and Simmons, 1984) fitted ~4 mm apart were used to attach the fiber to stainless steel mounting hooks in the tension transducer cell. In some instances, fiber ends attached to the clips were treated with glutaraldehyde (Chase and Kushmerick, 1988) to reduce end-compliance. Fiber lengths and diameters (at three positions along the fiber length) were measured in the transducer cell containing relaxing solution after setting the sarcomere spacing using the diffraction pattern from a He/Ne laser. Fibers were first activated at 1°C by washing with preactivating solution and then rapidly flowing activating solution into and through the cell (Davis and Harrington, 1993a). After activation, the fiber temperature was rapidly (<35 s) raised to the temperature of the experiment.

The ionic strength of the solutions was 0.2 M and were the same as those used by Davis and Harrington (1993a). Disodium glycerol 2-phosphate is used as a temperature-insensitive buffer (Goldman et al., 1987). Relaxing solution: 7.52 mM $MgCl_2$, 5.48 mM vanadium-free ATP, 20 mM EGTA, 20 mM creatine phosphate, 15 mM disodium glycerol 2-phosphate. Preactivating solution: 7.52 mM $MgCl_2$, 5.48 mM vanadium-free ATP, 0.1 mM EGTA, 19.9 mM HDTA (1,6-diaminohexane-*N,N,N',N'*-tetraacetic acid), 19.9 mM creatine phosphate, 15 mM disodium glycerol 2-phosphate. Activating solution: 7.39 mM $MgCl_2$, 5.52 mM vanadium-free ATP, 20 mM Ca-EGTA, 20 mM creatine phosphate, 15 mM disodium glycerol 2-phosphate. All solutions contained 2 mg/ml creatine phosphokinase and were adjusted to pH 7.1 at room temperature with 1 N KOH.

Tension measurements

A Model 407A (Cambridge Technology, Cambridge, MA) capacitor-based force transducer with a resonant frequency of 12 KHz, 100-μs rise time, and compliance of 0.1 μm/g was used for all tension measurements (Davis and Harrington, 1993a).

Laser T-jump

The fiber in the 2 × 2 × 15-mm cuvette is heated uniformly by the 1.315-μm infrared beam in less than 1 μs. The laser T-jump heated the fiber 5°C with a 1.7-J pulse of infrared light. Details of the use, design, and construction of the infrared laser and its associated optics are described elsewhere (Davis and Harrington, 1987b, 1993a).

L-jump

An ergometer (Model 300S, Cambridge Technology) is used for the L-jump experiments. In the −1.5-nm per half sarcomere L-jump experiments used in this paper, the 100% rise time of the step motor is 160 μs (Davis and Harrington, 1993a). In most experiments, the fiber ends attached to the clips were treated with glutaraldehyde (Chase and Kushmerick, 1988) to reduce end-compliance, and the length cycling procedure of Brenner (Brenner, 1983; Sweeney et al., 1987) was applied to reduce sarcomere length dispersion during activation. Differences in end-compliance that exist between carefully treated, freshly skinned fibers and those with ends stabilized by cross-linking appear to have little effect on the kinetics (amplitudes and rates) of tension generation in small-perturbation experiments (Davis and Harrington, 1993a). Sarcomere lengths were measured from the diffraction pattern produced by a 10-mW He/Ne laser set at the Bragg angle to the fiber. Changes in the diffraction pattern in experiments up to 16°C were followed with an SL15 super linear position sensor and model 301DIV-30 position-sensing amplifier (UDT Sensors, Hawthorne, CA) (Davis and Harrington, 1993a).

Data collection and analysis

The voltage output from the force transducer amplifier is recorded on a digital oscilloscope (model 430, Nicolet Instrument Corp., Madison, WI). Variable length records (generally 8000 data points) of 12-bit data were recorded. An in-house program with National Instruments LabView software running on a Gateway 486DX266V personal computer with an AT-GPIB interface card (National Instruments, Austin, TX) was used to control and acquire data from the digital oscilloscope. Details of the methods used for data analysis remain unchanged (Johnson and Frazier, 1985; Davis and Harrington, 1993a).

RESULTS

The data presented here extend earlier T- and L-jump studies on faster kinetic steps (Davis and Harrington, 1987a, 1993a,b) to include the slow step ($1/\tau_3$) of T-jump data and the slower phases 3 and 4 of the response to an L-jump. Changes in length are used to perturb all states that generate tension or bear tension (either transiently or continuously) in a muscle fiber. The maximal number of kinetic steps associated with various mechanical interactions between thick and thin filaments respond in these experiments. A laser T-jump results in simpler kinetics, as the additional property of temperature sensitivity is required. The T-jump response is particularly important in understanding the mechanism of

muscle contraction because it should contain the step(s) associated with de novo tension generation. This follows directly from the fact that both phases 2_{slow} and 3, candidate reactions for de novo tension generation in muscle, are themselves endothermic with equilibrium constants close to unity (Zhao and Kawai, 1994).

T-jump experiments and the kinetics of the slow relaxation $1/\tau_3$

A laser T-jump of a muscle fiber contracting isometrically results in an increase in fiber tension governed by three apparent rate constants (Davis and Harrington, 1993a). The fastest ($1/\tau_1$) arises from a mini L-jump imposed on the fiber by the step expansion of the proteins after the T-jump (Goldman et al., 1987; Davis and Harrington, 1993a). The medium speed ($1/\tau_2$) and slow ($1/\tau_3$) steps are of particular interest, because they arise from the direct effects of temperature on the cross-bridge cycle. In earlier experiments, attention was focused on correlating the medium speed relaxation ($1/\tau_2$) with phase 2_{slow} and de novo tension generation (Davis and Harrington, 1993a,b). A correlation of the slow T-jump relaxation ($1/\tau_3$) with equivalent slow L-jump relaxations (possibly phases 3 or 4) was not included, as they were considered peripheral to step(s) associated with de novo tension generation. Since then, the debate about the endothermic step(s) responsible for de novo tension generation has extended beyond a consideration of phase 2 to include these slower kinetic steps. These data are also essential in the assignment of the kinetics of interconversion of the various intermediate states populated during the contractile cycle. We therefore present data in sufficient detail to allow a cross-correlation of all L-jump and T-jump small-perturbation relaxations.

Fig. 1 illustrates a series of six tension transients recorded at different post-jump temperatures. The three relaxations that comprise the tension transient are illustrated in the 6°C panel. The quality of the residuals obtained by subtracting the fit from the raw data and the confidence limits on the estimated parameters show that the kinetic parameters are generally well determined. Fiber tensions, normalized to cross-sectional area, are low at post-jump temperatures of 26°C and above, indicating fiber deterioration. The question is whether the determination of the relaxation times at these two temperatures is compromised. Recent experiments (in the presence of 5 mM phosphate) in which the fiber sarcomere pattern is preserved on rapid heating (<1 s) from 2 to 30°C show a 4-fold increase in fiber tension (Pate et al., 1994). On average, we obtain a 3.3-fold increase in tension on heating fibers over a smaller temperature range from 1 to 26°C in the presence of 15 mM phosphate, solvent conditions that result in lower fiber tensions and consequently less fiber damage. Phosphate resulted in small and fixed increases in the rates of the two relaxations ($1/\tau_2$ and $1/\tau_3$) over the full temperature range; in other words, we see an offset but identical response of the kinetic parameters to temperature under conditions in which fiber deterioration is minimal (Davis and Rodgers, 1995, and manuscript in preparation). These observations, together with the good fiber to fiber reproducibility (SEM) of the rate parameters point to the kinetics being well determined at all temperatures. Temperature dependence of the

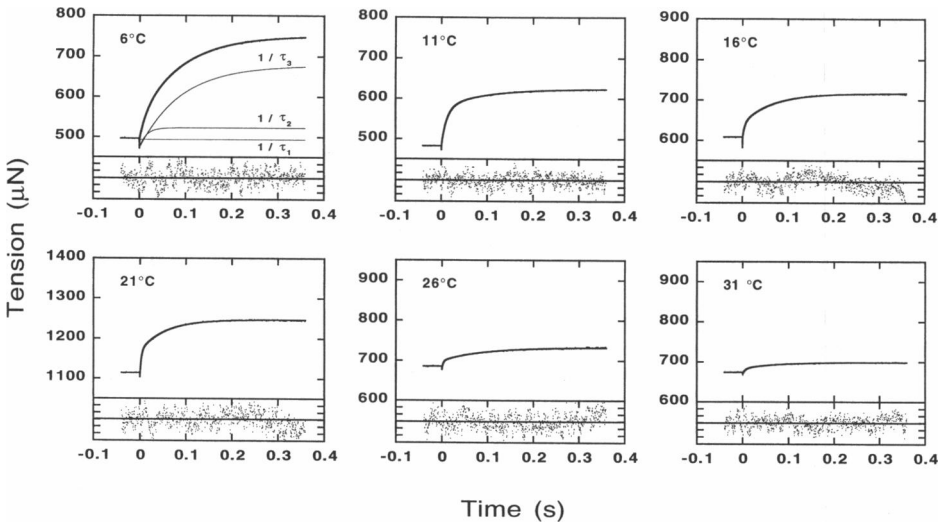

FIGURE 1 T-jump transients of muscle fibers at various temperatures. The response of fibers to a 5°C T-jump is presented. The post-jump temperature is indicated on each panel. Each fiber was activated at 1°C and the temperature was raised to the selected pre-jump temperature in ~35 s immediately before the T-jump. For more details, see Davis and Harrington (1993a). Data were analyzed by nonlinear least-squares fitting to the sum of three exponentials as described in detail in Davis and Harrington (1993a). The best fit line is superimposed on each data set, and residuals of the fit are presented below each data set. The scale of the residuals is ±3 μN in each case. Typical resolved exponential components that sum to form the total signal are shown in the 6°C panel. Fiber dimensions were as follows: length = 3.8, 3.9, 4.0, 4.2, 4.0, and 4.2 mm, and cross-sectional area = 8659, 6244, 6128, 10237, 5346, and 5026 μm^2 for 6, 11, 16, 21, 26, and 31°C, respectively. Initial sarcomere spacing was 2.6 μm in each case. Relevant fitted rate constants for $1/\tau_3$ are 11.2 (10.9–11.5), 11.7 (11.0–12.5), 15.4 (15.2–15.7), 18.3 (17.8–18.7), 11.2 (10.6–11.9), and 13.6 (11.8–15.6) s^{-1} for 6, 11, 16, 21, 26, and 31°C, respectively. The values in parentheses represent the 67% joint confidence interval for each parameter.

slow T-jump relaxation ($1/\tau_3$) is presented in Arrhenius form in Fig. 2. It is evident from Fig. 2 that the apparent rate constant for the slow relaxation is invariant with temperature, having an average value of 13.2 SEM ± 0.7 s^{-1} (n = 6). The constancy of $1/\tau_3$ has been commented on in earlier publications (Davis and Harrington, 1991, 1993a).

L-jump experiments and the kinetics of phases 3 and 4

As mentioned in the Introduction, the kinetic response of muscle fibers to step changes in length serves as a benchmark against which data from the perturbation of fibers by other means are correlated. The full response of a muscle fiber subjected to a −1.5-nm per half sarcomere length step on two different time bases is illustrated in Fig. 3, *a* and *d*. In these small-perturbation relaxation experiments, all four kinetic phases (phases 2_{fast}, 2_{slow}, 3, and 4) are exponential in form, allowing their associated apparent rate constants and reaction amplitudes to be obtained from a nonlinear least squares fit to the data (Davis and Harrington, 1993a). The contribution of each of these phases to the tension transients are depicted in Fig. 3, *b* and *e*. Fig. 4 illustrates four tension transients recorded at different temperatures. Isometric tensions achieved at the various temperatures in Figs. 3 and 4 are typical of the levels found for skinned rabbit psoas fibers by other workers (Pate et al., 1994; Ranatunga, 1994). These L-jump rate data, together with the laser T-jump data, provide unique insights into the physical nature of component kinetic phases associated with tension generation. Results described next on the temperature dependence of the slower kinetics of phases 3 and 4 are presented for comparison with the T-jump data of Fig. 2.

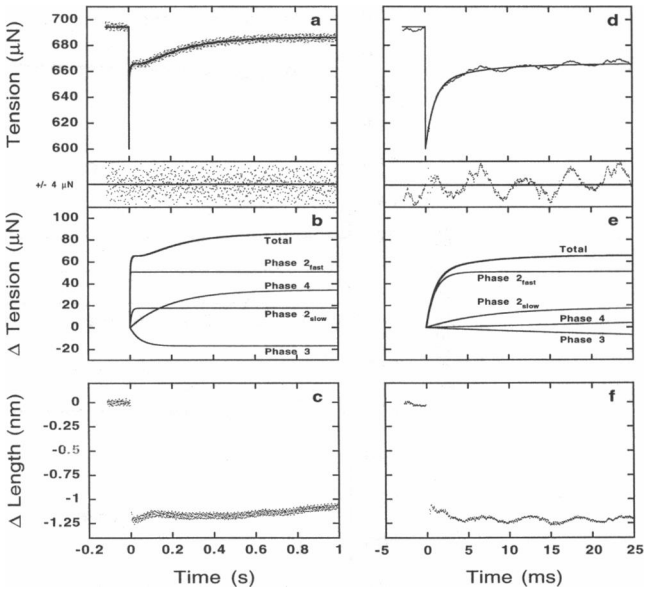

FIGURE 3 Kinetics of tension generation after the L-jump of a rabbit psoas fiber undergoing isometric contraction at 11°C. The response of a fiber to an L-jump of −1.5 nm per half sarcomere applied in 160 μs is shown on slow (*a*) and fast time bases (*d*). Eight transients were averaged in each case. The best fit line obtained by fitting to phases 2_{fast}, 2_{slow}, 3, and 4 is drawn through the data, and residuals are plotted below the transients. The resolved fits to phases 2_{fast}, 2_{slow}, 3, and 4 along with the total are illustrated in *b* and *e* at different time bases. The following parameters define the fitted data: isometric tension = 694.5 μN, phase 1 = 600 μN, and the amplitudes and rate constants for phases 2_{fast}, 2_{slow}, 3, and 4, respectively, are 50.9 μN, 925 s^{-1}; 17.7 μN, 138.1 s^{-1}; −16.8 μN, 20.5 s^{-1}; and 34.4 μN, 5.1 s^{-1}. The length change applied to the fiber was followed by using the first order diffraction maximum as described in Materials and Methods. Results are presented for each time base in panels *c* and *f* in units of nanometers per half-sarcomere. Fiber dimensions were as follows: length = 4.0 mm, relaxed fiber sarcomere spacing = 2.6 μm, and cross-sectional area = 4320 μm^2.

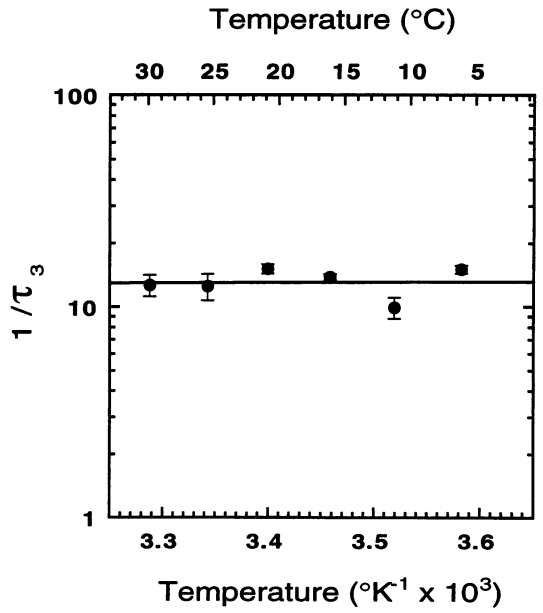

FIGURE 2 Arrhenius plot of temperature dependence of the slow T-jump relaxation. The apparent rate constant remains virtually constant with temperature with an average value of 13.2 SEM ± 0.7 s^{-1}. The mechanistic implication here is that, to be seen, the step has to be coupled to a temperature-sensitive rapid preequilibrium(a). Kinetic parameters are plotted at the post-jump temperature. These data were obtained from T-jump tension transients similar to those of Fig. 1 of this paper and Figs. 1 and 2 of Davis and Harrington (1993a). Error bars represent ±SEM for n = 37, 13, 11, 9, 4, and 8 determinations at 6, 11, 16, 21, 26, and 31°C, respectively.

Phase 3 is distinctive because it is associated with an amplitude of opposite sign to phases 2 and 4. The temperature dependence of the apparent rate constant for phase 3 is illustrated in Arrhenius form in Fig. 5. It is evident that its apparent rate constant shows a marked sensitivity to temperature with a Q_{10} of 4.16. The 1°C datum of Fig. 5 is not well determined because of interference between the rates of phases 3 and 4. These data are similar to the rates obtained by Kawai and colleagues for phase 3 (their phase b) in the absence of phosphate (Zhao and Kawai, 1994). It is clear that the apparent rate constants for phase 3 are factors of ~15 at 6°C to ~3 at 26°C, slower than $1/\tau_2$, illustrated in Fig. 9 of Davis and Harrington (1993a) It is evident that there is no correlation between the temperature dependence of phase 3 and the slow T-jump relaxation ($1/\tau_3$) illustrated in Fig. 2. These new data provide strong support for our original proposal (Davis and Harrington, 1993a,b) that medium speed relaxation seen in T-jump experiments corresponds to phase

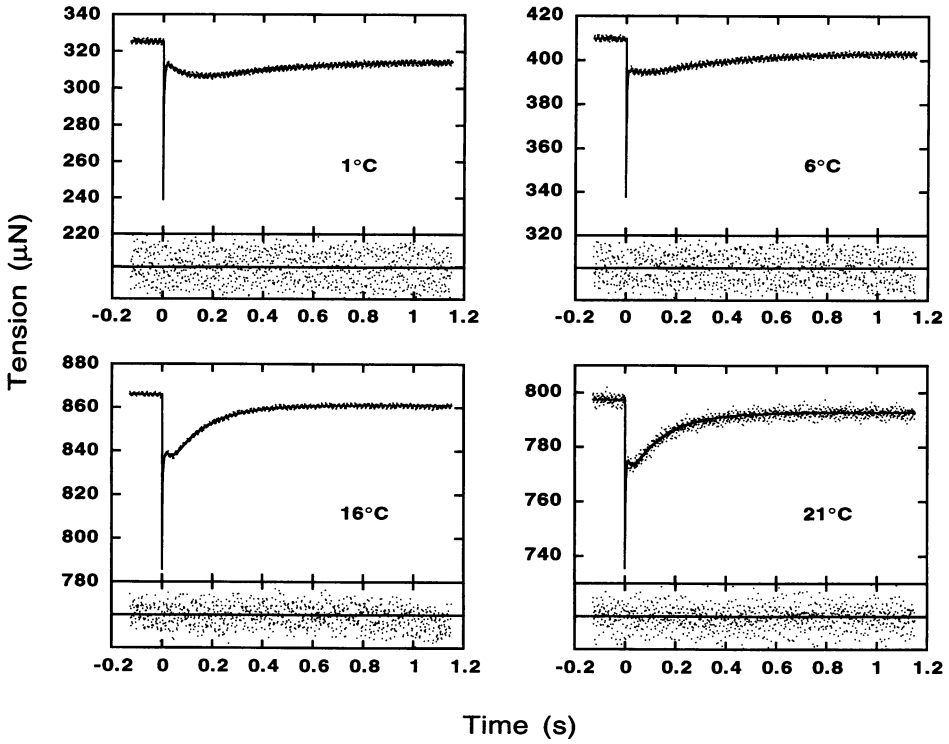

FIGURE 4 L-jump tension transients of muscle fibers at various temperatures. The response of fibers to a −1.5-nm per half sarcomere L-jump applied at four different temperatures is presented. The temperature is indicated on each panel. As in Fig. 3, eight transients were averaged in each case. The data were fitted to phases 2_{fast}, 2_{slow}, 3, and 4, and the best fit is drawn through each data set. Residuals of the fit are presented below each data set. The scale of the residuals is ±2 μN at 1, 6, and 16°C and ±5 μN at 21°C. Fiber dimensions were as follows: length = 4.2, 3.3, 4.0, and 4.0 mm; relaxed fiber sarcomere spacing = 2.6, 2.5, 2.5, and 2.6 μm; and cross-sectional area = 4320, 4418, 5026, and 4320 μm^2 for 1, 6, 16, and 21°C, respectively. Relevant rate constants are as follows: phase 3 = 12.2 (10.7–13.7), 20.8 (14.7–27.8), 76.1 (72.6–79.6), and 90.7 (78.3–103.6) s^{-1} and phase 4 = 3.03 (2.44–3.60), 2.97 (2.35–3.51), 7.09 (6.84–7.34), and 7.12 (6.60–7.65) s^{-1} for 1, 6, 16, and 21°C, respectively. The values in parentheses represent the 67% joint confidence interval for each parameter.

2_{slow} and certainly not to phase 3 as Zhao and Kawai (1994) suggest. Phase 3 appears absent from laser T-jump tension transients.

Phase 4 is the slowest kinetic step and serves to return tension to its pre-jump isometric value. This can be readily seen in the L-jump tension transients illustrated in Figs. 3 and 4. Fig. 6 shows some interesting features of its temperature dependence in the form of an Arrhenius plot. At low temperatures, the apparent rate constant is slow, rising to a plateau value at higher temperatures. The 1°C datum of Fig. 6 is not well determined because of interference between the rates of phases 3 and 4. Above 15°C, the rate remains constant at approximately 9 s^{-1}. It is interesting to note that the closest rate process to the plateau value of phase 4 is the apparent rate constant of $1/\tau_3$, the temperature-independent slow relaxation seen in the T-jump and illustrated in Fig. 2 These data imply a possible switch in the rate-limiting step from a temperature-dependent to a temperature-independent step at approximately 15°C.

DISCUSSION

Relationship to other T-jump studies

All of the kinetic data presented are obtained from small-perturbation relaxation experiments of maximally Ca^{2+}-activated skinned rabbit psoas fibers (Goldman et al., 1987; Davis and Harrington, 1987a, 1993a,b). We earlier showed (Davis and Harrington, 1987a, 1993a) that the slow component of the biexponential tension rise observed by Goldman et al. (1987) is subdivisible into two exponential components (our medium speed ($1/\tau_2$) and slow ($1/\tau_3$) relaxations).

Large-perturbation T-jump experiments have been performed on rabbit psoas fibers at different temperatures (Bershitsky and Tsaturyan, 1992). The kinetic response appears monophasic for small T-jumps and biphasic for T-jumps >15°C. It is, however, unclear how these data relate to our experiments. In part, this is due to the fact that large perturbations result in large increases in tension and in the steady-state flux through the ATPase cycle, generating a quite different kinetic response to that observed in small-perturbation relaxation experiments (see Discussion in Davis and Harrington, 1993a). In addition, there are technical reasons why the rate constants obtained from these experiments might not be a full reflection of the underlying kinetic events. First, the time domain in which the drop in tension caused by fiber expansion and the subsequent recovery during the fast relaxation ($1/\tau_1$) is obscured by electrical noise associated with heating (Bershitsky and Tsaturyan, 1992). Second, the method of heating the fiber in air with a pulse of alternating current results in the fiber cooling continuously after

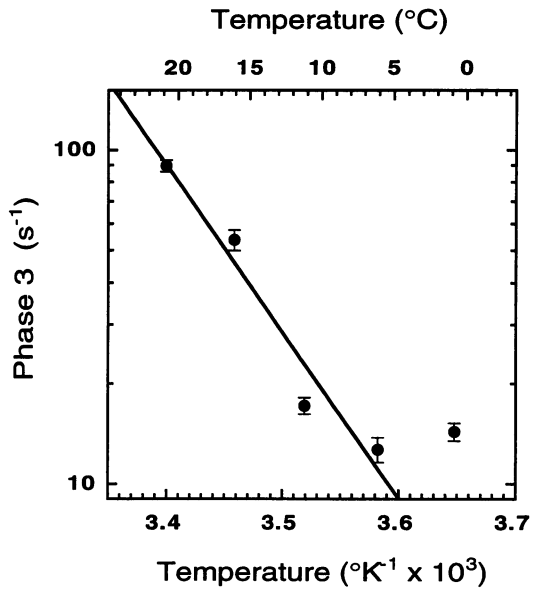

FIGURE 5 Arrhenius plot of the temperature dependence of the Huxley-Simmons phase 3. The datum point at 1°C is not well determined. Error bars indicate the standard error of the mean at each point. The number of determinations was n = 13, 18, 13, 16, and 3 for 1, 6, 11, 16, and 21°C, respectively. The line is the regression fit to the data between 6 and 21°C and corresponds to a Q_{10} of 4.16.

the T-jump with a $t_{1/2}$ of between 70 and 140 ms, thus preventing kinetic analysis about the critical 10-s^{-1} time domain of our slow speed relaxation ($1/\tau_3$). The large drop in temperature (e.g., 14°C over the 150-ms time course after a 20°C T-jump) will also cause the rate constants of temperature-sensitive steps to change significantly during the time course of the reaction. As a result, a large fraction of the 150-ms tension transients cannot be used for data analysis. In our T-jump experiments, for example, the change in fiber temperature is less than 3% of the total jump temperature (5°C) over 400 ms (Davis and Harrington, 1993a).

A base mechanism for muscle contraction

The thesis here is that the kinetics of force generation after a T-jump offers key insights into tension generation in muscle fibers. The kinetic response is simple and can be readily interpreted in terms of a fundamental, or base, mechanism that provides a well defined starting point for assigning function to the various kinetic responses recorded with other perturbation techniques. A comparison of like with like requires that all data be obtained from fibers contracting under isometric conditions subjected to either no perturbation at all (exchange experiments) or small perturbations from the steady state. Included are changes in temperature, tension, and length and in the concentrations of products and reactants of the actomyosin ATPase.

As mentioned in the Introduction, heating muscle fibers from ~0°C to 45°C results in a sigmoidal rise in isometric tension (Ranatunga, 1994). This, together with the strong likelihood that de novo tension generation results from an endothermic transition in the cross-bridge (Ford et al., 1977; Goldman et al., 1987; Davis and Harrington, 1987a, 1993a,b; Gilbert and Ford, 1988; Zhao and Kawai, 1994) makes it virtually certain that a laser T-jump of a muscle fiber will elicit a response from the primary force-generating step(s). Thus, laser T-jump experiments offer a powerful means of probing the primary step(s) associated with tension generation.

Laser T-jump tension transients consist of a temperature-insensitive slow relaxation ($1/\tau_3$) and a faster temperature-sensitive relaxation ($1/\tau_2$) (Davis and Harrington, 1987a, 1993a,b). This type of response can be readily interpreted in terms of a three-step mechanism:

$$\underset{\text{rapid preequilibrium (a)}}{\mathbf{A} \longleftrightarrow \mathbf{B}} \quad \underset{\text{rate-limiting step } (1/\tau_3)}{\longrightarrow} \quad \underset{\text{tension generation } (1/\tau_2)}{\mathbf{C} \longleftrightarrow \mathbf{D}} \qquad (1)$$

After a T-jump, the medium speed, temperature-sensitive relaxation $1/\tau_2$ governs the interconversion of intermediate C to the force-generating state D. The slow temperature-insensitive relaxation $1/\tau_3$ results from a rapid rise in the concentration of intermediate B as a result of the perturbation of temperature-sensitive equilibrium between A and B. Intermediate B is slowly interconverted to the pre-tension-generating intermediate C, which is, in turn, rapidly converted to the tension-generating intermediate D with a concomitant rise in tension that tracks the speed of the rate-limiting step. The interconversion of B and C is reversible, but we use a single arrow to indicate the direction of the flux

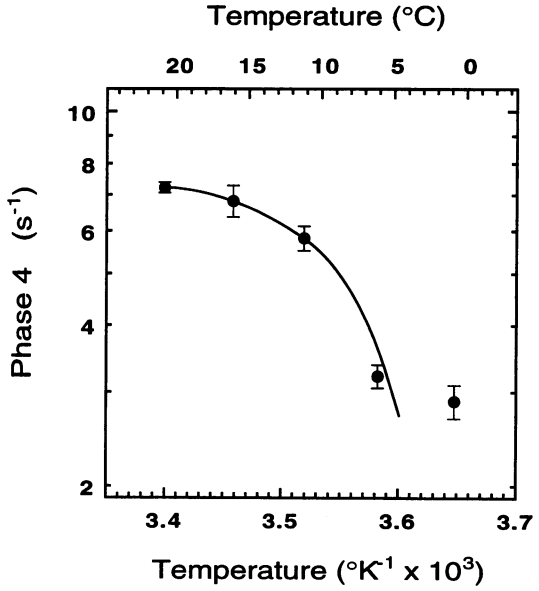

FIGURE 6 Arrhenius plot of the temperature dependence of the Huxley-Simmons phase 4. Note the rise in rate with a plateau at higher temperatures; the datum point at 1°C is not well determined. We subdivide phase 4 into a temperature-sensitive component evident at low temperatures termed phase 4_a and a temperature-insensitive component evident in the plateau region termed phase 4_b. Error bars indicate the standard error of the mean at each point. The number of determinations was n = 13, 18, 13, 16, and 3 for 1, 6, 11, 16, and 21°C, respectively. The line is a smooth curve drawn through the data.

through the step. The temperature-induced interconversion of C to D will also contribute to biasing the reaction to the right. The reaction step associated with the slow relaxation is passive, as it cannot generate tension because its rate does not change with temperature. Only the reaction step associated with the medium speed relaxation has a rate that changes with temperature and is thus the only plausible source of de novo tension generation; candidate faster processes coupled to this step appear absent (Davis and Harrington, 1993a,b).

The relationship between this purely kinetic scheme and various states of the cross-bridge cycle is considered next. It is highly likely that the interconversion of the pre-tension-generating state (C) to the tension-generating state (D) occurs between strongly bound actomyosin states. This is because the medium speed relaxation ($1/\tau_2$) is likely to have the same properties as phase 2_{slow}, its L-jump equivalent (Davis and Harrington, 1993a), and it is known that fiber stiffness and thus the attachment state of the cross-bridge does not change significantly during the overall time course of phase 2 (Ford et al., 1974). The coupled processes of the rapid preequilibrium(a) and the temperature-insensitive slow transition (A to C) precede tension generation. We later discuss (see below) a probable correlation between these two steps and the rise in tension seen when caged ADP is photolyzed in a fiber contracting under isometric conditions (Lu et al., 1993). The interpretation of this apparently related ADP-jump response is that the kinetics are dominated by a change in the flux of cross-bridges through various steps, including phosphate release, but not by a reversal of tension generation (D to C in our scheme). The slow T-jump relaxation could thus arise from the perturbation of rapid preequilibrium(a) that generates a flux through the $\sim$10-s^{-1} rate-limiting step with both component reactions located between ATP binding to the actomyosin cross-bridge after the power stroke and tension generation after phosphate release. It is unclear which transition is associated with the temperature-insensitive interconversion of B to C; its speed and temperature-insensitive character indicate that actomyosin complex formation is an unlikely candidate. The temperature-dependent rapid preequilibrium(a) between A and B could comprise contributions from the binding of ATP to the cross-bridge (after ADP release after step D), cross-bridge detachment, ATP hydrolysis, and possibly the formation of the actomyosin complex. The rate-limiting transition we describe is slower than the kinetics of the tension rise that occurs after the release of caged ATP (Goldman et al., 1984) and caged Ca^{2+} (Ashley et al., 1987) and the rapid restretching of fibers (Brenner and Eisenberg, 1986). Pre-steady-state kinetics governs the rise from zero tension to the isometric value in these last mentioned experiments. Our kinetic experiments are quite different in that they result from the perturbation of the isometric steady state with a quite different distribution of cross-bridges in both tension-bearing, tension-generating, and unloaded states. It is thus unsurprising that the kinetics leading to force generation appear different under these two quite different experimental conditions. Their integration will require a deeper understanding of the effects of tension and the geometry of thick and thin filaments on the kinetics of the cross-bridge cycle. Interestingly, the lack of temperature sensitivity of $1/\tau_3$ is indicative of a reaction in which there is virtually no change in the solvation state of the proteins. Protein-based changes with these properties are rare. These kinetics are considered again in the discussion of the relationship between phase 4 and $1/\tau_3$.

Experiments suggest that two kinetic steps that occur after a T-jump are manifestations of structural changes in the proteins of the cross-bridge. The first evidence for this is that a remarkably similar biexponential kinetic response to a T-jump can be obtained from muscle fibers in rigor. Rigor contraction is observed when muscle fibers devoid of chemical energy are heated a few degrees above the working temperature of the muscle (Davis and Harrington, 1987a, 1993b). Increasing evidence is accumulating to show that rigor contraction and normal contraction under physiological conditions generate tension by a common mechanism. The working hypothesis that resulted from these experimental observations is that ATP hydrolysis by fibers, or the heating of rigor fibers, supply the energy to bias intrinsic (preexisting) structural equilibria in the cross-bridge proteins toward tension generation (Davis and Harrington, 1993a,b). These experiments further emphasize the fundamental, protein structure-related nature of the biphasic tension transient seen in laser T-jumps of muscle fibers contracting in the presence of ATP and Ca^{2+}.

Correlation of T-jump relaxations with L-jump kinetic phases

We extend the approach used before in which the temperature dependence of the apparent rate constants of a particular step are used as a signature of that step/phase (Davis and Harrington, 1993a,b). These signatures, characteristic of each phase, are used to cross-correlate the L-jump and T-jump kinetics. Only small-perturbation kinetic data, obtained from fibers contracting under isometric conditions, are used. This simplifies the interpretation of the kinetics and allows rate data to be compared at similar tensions. Only one apparent rate constant changes markedly with tension and is thus faster in L-jump step-release experiments in which tension falls than in T-jump experiments in which tension rises (Davis and Harrington, 1993a,b). This sensitivity to tension is manifest in the classical asymmetry of the tension transients induced in similar sized L-jump step-release and step-stretch experiments (Huxley and Simmons, 1971).

Phase 2_{fast} and phase 2_{slow} were assigned to the fast T-jump relaxation, $1/\tau_1$ and to the medium speed relaxation, $1/\tau_2$ in earlier work (Davis and Harrington, 1993a,b). Despite criticism (Zhao and Kawai, 1994), there appears to be no reason to change this assignment on the basis of these new data.

Phase 3 has a distinctive temperature-dependent signature in L-jump experiments with a markedly temperature-dependent apparent rate constant (Q_{10} of $\sim$4.16). Our data are similar to values obtained by Kawai and colleagues in ex-

periments performed in the absence of phosphate (Zhao and Kawai, 1994). Inspection of the T-jump data shows that the equivalent of phase 3 is absent. There is clearly no case for correlating phase 3 with any of the relaxations seen in T-jump experiments. We conclude that phase 3 is absent from laser T-jump tension transients in the absence of phosphate.

Phase 4 has an interesting biphasic temperature dependence of its apparent rate constant. At low temperatures, its rate is slow, increasing moderately up to ~15°C, above which its value plateaus. There appears to be a correspondence between the apparent rate constants for phase 4 in the plateau region and the slow temperature-independent T-jump relaxation, $1/\tau_3$. These data imply that different rate-limiting steps limit tension recovery during phase 4 at different temperatures. This is discussed later in the final section.

De novo tension generation and phases 2_{slow} and 3

The task here is to determine which phase in L-jump experiments correlates with the primary tension-generating step in the T-jump. Candidate reactions include phase 2_{slow} (Davis and Harrington, 1993a,b) and phase 3 (Kawai and Halvorson, 1991; Kawai and Zhao, 1993; Zhao and Kawai, 1994). We develop two arguments against the mechanism of Kawai and colleagues. First, it is unlikely that phase 3, a step with a negative amplitude that opposes the tension return to the isometric value after a step-release L-jump, would generate tension. Phases 2_{fast}, 2_{slow}, and 4 with positive amplitudes in step-release are significantly more plausible candidates. Second, the reassignment of the medium speed T-jump relaxation from phase 2_{slow} to phase 3 is wrong on the basis both of the original data (Davis and Harrington, 1993a) and the additional, supporting data presented in this paper. In fact, it appears that phase 3 is totally absent from laser T-jump tension transients.

Phase 3 is observed in L-jump but not in T-jump experiments. This observation has important mechanistic consequences, because there is little doubt that a laser T-jump will perturb the concentrations of the two intermediate states that isomerize during phase 3. This follows from the fact that the reaction is well populated, with an equilibrium constant close to one, and is markedly temperature sensitive (Zhao and Kawai, 1994). As we point out in Results, its rate ranges from 12.7 s^{-1} at 11°C to 89.5 s^{-1} at 21°C. Direct comparison with the temperature dependencies of phase 2_{slow}, and its T-jump correlate $1/\tau_2$ (Davis and Harrington, 1993a,b), leaves little doubt that they arise from quite different transitions in the cross-bridge. We are thus left in no doubt that phase 3 is not associated with de novo tension generation.

There is another straightforward reason why phase 3 is an implausible candidate as the primary force-generating step in muscle fiber contraction. The step causes a reversal of tension recovery after the step change in length of an isometric, activated fiber. The response is counter to the expected on the basis of Le Chatelier's principle that states that, if a system at equilibrium is subjected to a stress, the system tends to react in such a way as to oppose the effect of the stress. Application of the principle to phase 3, a single step in a series of steps, is somewhat more complex. It requires that the response of the step in question be uncoupled from adjacent steps so that the impact of the applied stress be observed on it alone. This is because kinetic coupling to a faster, large amplitude adjacent step could, under exceptional but imaginable circumstances, result in a reversal of the reaction from that expected were the step perturbed in isolation. In the case of phase 3, this is implausible because phase 2_{fast} and phase 2_{slow}, the candidate faster kinetic steps to which it could be coupled, are, according to Kawai's mechanism, effectively isolated from phase 3 by cross-bridge detachment and reattachment to actin and ATP hydrolysis. We know these steps are relatively slow. For example, the phosphate burst resulting from the on enzyme hydrolysis of ATP in skinned rabbit psoas fibers occurs at a slow 60 s^{-1} at 12°C (Ferenczi, 1986). This, together with other step(s) in the ATPase reaction, effectively isolate phase 3 kinetically. It therefore seems reasonable to conclude that the association of phase 3 with cross-bridge detachment, and not de novo force generation, offers a far more plausible explanation of its role in tension generation (Huxley and Simmons, 1973; Ford et al., 1985). This leaves phase 2_{slow} as the endothermic step associated with de novo tension generation and not phase 3 as supposed by Kawai and colleagues. We further consider the role of phase 3 in a paper in which we investigate the interrelationship between the chemistry of the ATPase cycle and mechanical tension transients (Davis and Rodgers, 1995, and manuscript in preparation).

Mechanistic role of phase 4 and the slow T-jump relaxation, $1/\tau_3$

Phase 4 probably correlates with the slowest processes of the cross-bridge cycle and has generally been attributed to cross-bridge reattachment that occurs after an L-jump (Ferenczi et al., 1982). However, the response is clearly more complex than this because it appears to be coupled to different steps at different temperatures. At low temperatures, the overall rate of phase 4 is governed by a temperature-sensitive step in the cross-bridge cycle that is not observed in laser T-jump tension transients. At higher temperatures in the plateau region, the rate-limiting step switches over to a temperature-insensitive step that appears to correlate with the slow T-jump relaxation. We term the temperature-sensitive component phase 4_a and the temperature-insensitive component phase 4_b. An experimental observation that may be relevant here is that the release of ADP from caged precursor (an ADP-jump) causes a rise in tension at a rate of ~10 s^{-1} at 15°C under the conditions similar to our experiments (Lu et al., 1993). The interpretation provided of the ADP-jump data is that the kinetics are dominated by a flux of cross-bridges through the various steps, which includes phosphate release, and not from a reversal of tension generation. Similarly, the slow T-jump relaxation could arise from the perturbation of rapid preequilibria that generate a flux through the ~10-s^{-1}

rate-limiting step, which would include steps like ATP binding, recognition, and hydrolysis. A switch in rate-limiting step was proposed as the basis for the abrupt change in the activation energy of tetanic tension development in rat muscle at approximately 20°C (Ranatunga and Wiley, 1983). There is a distinct possibility that the change in the rate-limiting step that we observe in phase 4 underlies this observation.

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