complicating factor. We consider any minor effects of phosphate on process C to be a problem for the level of approximation used in fitting the model to the data, rather than a flaw in the model itself.

Alternative arrangements of the states of this scheme either violate the sense of the enzyme mechanism or are inconsistent with the data. For example, if process C were placed at the right of the scheme, it would become isolated from the binding steps for ADP and ATP and the scheme would no longer explain the data for process C. If those binding steps were moved along with process C, then the predicted effects of ADP and ATP on process B would become inconsistent with the data.

Assumption E is not of our doing. It is inconsistent with the data, the analysis of the data, the model derived from the analysis of the data, and the ability of muscle to function. We assume that each state X_i has a definable physical length l_i. Each step of the scheme corresponds to some change in this length: $\Delta l_i = l_{i+1} - l_i$ (binding reactions have $\Delta l_i = 0$). The effect of a change in overall tension $\delta\tau$ on the equilibrium constant $K_i = ([X_{i+1}]/[X_i])$ for a step is then given by Hill (1960):

$$\delta \ln K_i = \Delta l_i \, \delta\tau / k_B T, \quad (1)$$

where k_B is the Boltzmann constant and T is temperature. Inspecting the data shows that Δl_A and Δl_C are positive (lengthening) and that Δl_B is negative (shortening). Accordingly, increasing the tension increases K_A and K_C, and decreases K_B.

The basic flaw in the argument of Horiuti and Sakoda is their assumption that equilibrium considerations can be applied to the system *overall*, ignoring the appreciable departure from equilibrium in the chemical potentials of ATP and the products P and ADP. In fact, the system is in a stationary state very far from equilibrium. If it were at equilibrium (essentially total consumption of ATP), there would be no contractile phase, just as they suggest, and le Chatelier's principle would apply.

The chemical relaxation experiment is characterized by perturbations too small to appreciably change the preexisting stationary state, thus the steady-state cycling continues. The nonequilibrium distribution between substrate and product drives the system through the contractile step, despite the unfavorable length-tension work. Since there is only one shortening step and at least one lengthening step, $|\Delta l_4| \geq \Delta l_3$. The amplitudes of the observed processes are additionally influenced by the chemical compliance (Eigen and de Maeyer, 1974; Jovin, 1975), a quantity that reflects the sensitivity of the advancement of the respective chemical steps to perturbation of the equilibrium constant. The compliance is greater for process B than process C essentially because K_4 (1/2.5) is closer to unity than is K_2 (4.9). As a consequence, the tension change for process B will exceed in absolute value that for process C.

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Kawai's Response to Horiuti and Sakoda

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Although Drs. Horiuti and Sakoda's suggestion to place process (C) after the phosphate (P_i) release step and before the rate-limiting step is interesting, this mechanism fails to explain the observed dependence of the rate constant $2\pi c$ on the MgATP or MgADP concentration (Kawai, 1982; Kawai and Halvorson, 1989). Their suggested model predicts that $2\pi c$ is insensitive to MgATP and MgADP concentrations, owing to slow process (B) (step 4) that intervenes between the MgATP/MgADP binding step and the suggested location of process (C); step 4 then kinetically uncouples the MgATP/MgADP binding step from process (C). We showed earlier that $2\pi c$ increases and saturates as the MgATP concentration is increased (Kawai, 1982; Kawai and Halvorson, 1989), and that $2\pi c$ decreases as the MgADP concentration is increased (Kawai and Halvorson, 1989). Furthermore, Horiuti and Sakoda's suggested model does not predict "delayed tension" or "oscillatory work" (Pringle, 1967); instead their model predicts two exponentials of the same sign (exponential advances) represented by $\{B \exp(-2\pi bt) + C \exp$

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$(-2\pi ct)\}\delta l$ ($b < c$, t = time) on step length increase (δl), hence the polarity of process B is clearly wrong. Our second order binding constant K_1k_2 ($0.57–2.8 \times 10^6$ M^{-1} s^{-1}) (Kawai, 1982; Kawai and Halvorson, 1989; actual value depended on the method of analysis) based on process (C) and that measured from caged ATP experiments (0.5×10^6 M^{-1} s^{-1})(Goldman et al., 1984) are in close agreement, and they are in the vicinity of those observed on solution studies of purified acto-HMM (heavy meromyosin) or acto-S1 (myosin subfragment one) ($1–4 \times 10^6$ M^{-1} s^{-1}) (White and Taylor, 1976). These are additional lines of evidence that process (C) relates to the cross-bridge detachment step.

The assumption "E" is not of ours, and it is either an inference or conclusion incorrectly drawn from the analysis of our model by Horiuti and Sakoda. In particular, the strain sensitivity of K_4 is wrong. All that is required in our modeling is the strain sensitivity of K_1 or K_2 (Kawai and Zhao, 1993; Kuhn, 1981). Although there may be strain sensitivities in K_4 in accordance with Le Chatelier's principle, this is not essential to our model. If we assume that K_1 or K_2 increases with stretch (and decreases with release), then we can explain the tension transients: a length increase will facilitate the cross-bridge detachment (step 2) which is observed by phase 2 (process (C)), and this in turn will increase the number of cross-bridges in the detached state (Det). Since step 4 is slower than step 2, an increase in the number of cross-bridges in the Det state will result in a slow cross-bridge attachment, resulting in a delayed rise in tension (phase 3). The delayed rise in tension is equivalent to process (B) in sinusoidal analysis, and our model is capable of predicting both of these phenomena. Our result (Kawai and Halvorson, 1991) as well as others (Fortune et al., 1991; Dantzig et al., 1992) indicate that force generation occurs on step 4 and before P_i is released. The results of four laboratories (Kawai and Halvorson, 1991; Fortune et al., 1991; Dantzig et al., 1992; Walker et al., 1992) with three different techniques (sinusoidal analysis, pressure-release experiment, caged P_i study) agree well in terms of steps 4 and 5, hence we believe that our mapping of process (B) to step 4 is correct (see Kawai and Zhao (1993) for review).

Process (A) may not represent an elementary chemical step of the cross-bridge cycle, hence it was not included in our model. The reason is that process (A) is very small (or absent) in insect flight muscles (Pringle, 1967), cardiac muscles (Saeki et al., 1991), and partially cross-linked skeletal muscles (Tawada and Kawai, 1990). These preparations have more rigid sarcomere structure than fast twitch skeletal muscles, they hydrolyze ATP, and generate oscillatory work. Therefore, we believe that process (A) represents sliding of thick and thin filaments that involves multiple cross-bridge cycles. Process (A) may also include signals from a slow approach to the steady-state in the cross-bridge cycle.

Our model is an approximate description of the complex cross-bridge cycle, hence there may be areas that do not fit the data precisely. In addition, experimental results are not absolute, and may be subject to error. For these reasons, we aimed at explaining the large effects in our modeling. The effect of P_i on $2\pi b$ is much larger than the effect of P_i on $2\pi c$, hence this fact was the major consideration in our modeling. Furthermore, it is more desirable to explain the rate constants ($2\pi b$ and $2\pi c$) than magnitudes (amplitudes of exponential processes), because the former is the subject of less experimental error than the latter, and the analytical form is simpler in rate constants than magnitudes. While we agree in our model that magnitude C must decrease to a small number as the P_i concentration is reduced to 0, this is impossible to achieve experimentally, because of (i) P_i contamination in the activating solution, (ii) continuous liberation of P_i by muscle fibers owing to ATP hydrolysis, and (iii) k_6 is not 0 and it is finite (the cross-bridge cycle is at the steady state instead of equilibrium) so that there will be a repopulation of the states AM through AM*DP in the absence of P_i. All of these effects will increase the rate of AM*S $\leftrightarrow$ Det interconversion, hence increase the magnitude C. Since the major effects of MgATP, MgADP, and P_i are consistent with the model, we believe that our model is an appropriate (if not perfect) description of the cross-bridge cycle. Finally, I am pleased to note that Horiuti and Sakoda state that "the present model is simple and successful in explaining processes (B) and (C)."

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