at equilibrium, the system readjusts to reduce the stress (Sienko and Plane, 1961).

Another problem is that the effect of P_i on process C in the model is different from that observed in experiments. The process C observed by Kawai and his colleagues (Kawai et al., 1986; Kawai and Halvorson, 1991) was independent of P_i, although in an early study of Kawai (1986) the rate of process C seemed somewhat dependent on P_i. On the other hand, in the model, the equations by Kawai and Halvorson (1991) imply that the magnitude of process C is a monotonic, increasing function of P_i, although the rate of process C is independent of P_i. This P_i dependence of C is fundamental to the model and can be intuitively understood as follows: Step 2 for process C is placed between the rate-limiting step 6 and the P_i-releasing step 5. All the connecting steps 0–4 are quite reversible. Therefore, elevation of P_i in the model inevitably shifts the cross-bridge population toward the reaction step for process C.

The present model is simple and successful in characterizing processes B and C (Kawai and Halvorson, 1989, 1991). However, there are three problems in the model: (i) the minimal effect of P_i on process C is not explained, (ii) the direction of the force sensitivity of the force-producing step is peculiar, and (iii) process A has not yet been incorporated.

REFERENCES

Huxley, A. F., and R. M. Simmons. 1971. Proposed mechanism of force generation in striated muscle. *Nature (Lond.).* 233:533–538.

Kawai, M. 1986. The role of orthophosphate in crossbridge kinetics in chemically skinned rabbit psoas fibres as detected with sinusoidal and step length alterations. *J. Muscle Res. Cell Motil.* 7:421–434.

Kawai, M., and P. W. Brandt. 1980. Sinusoidal analysis: a high resolution method for correlating biochemical reactions with physiological processes in activated skeletal muscles of rabbit, frog and crayfish. *J. Muscle Res. Cell Motil.* 1:279–303.

Kawai, K., K. Güth, K. Winnikes, C. Haist, and J. Rüegg. 1987. The effect of inorganic phosphate on the ATP hydrolysis rate and the tension transients in chemically skinned rabbit psoas fibers. *Pflügers Arch. Eur J. Physiol.* 408:1–9.

Kawai, M., and H. R. Halvorson. 1989. Role of MgATP and MgADP in the cross-bridge kinetics in chemically skinned rabbit psoas fibers. Study of a fast exponential process (C). *Biophys. J.* 55:595–603.

Kawai, M., and H. R. Halvorson. 1991. Two step mechanism of phosphate release and the mechanism of force generation in chemically skinned fibers of rabbit psoas muscle. *Biophys. J.* 59:329–342.

Sienko, M. J., and Plane, R. A. 1961. *Chemistry.* McGraw-Hill, New York. p 187.

Tawada, K., and M. Kawai. 1990. Covalent cross-linking of single fibers from rabbit psoas increases oscillatory power. *Biophys. J.* 57:643–647.

Zhao, Y., and M. Kawai. 1993. The effect of the lattice spacing change on cross-bridge kinetics in chemically skinned rabbit psoas muscle fibers. II. Elementary steps affected by the spacing change. *Biophys. J.* 64:197–210.

Halvorson's Response to Horiuti and Sakoda

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The assumption fundamental to our analysis of the dynamic stiffness (Kawai and Halvorson, 1989, 1991) is that the observations must correspond to transitions between states of the actomyosin complex that have differing physical lengths (Hill, 1960); elementary binding per se cannot be responsible. The data show that none of the transitions occur at the rate of binding or dissociation. A secondary assumption or approximation is that the binding steps are sufficiently faster than the transitions that local equilibrium pertains. This interpretation is consistent with rates of substrate/product binding for most enzymes.

The development of a minimal mechanochemical reaction scheme also requires the assumption of an ATP hydrolase operating at steady-state. The small perturbations applied to the muscle fiber legitimize the use of a chemical relaxation analysis (Hammes, 1968). In essence, if the process being studied equilibrates rapidly compared to the steady-state rate of turnover, then established analyses for conventional chemical relaxation data (Castellan, 1963; Thusius, 1977; Jovin, 1975) can be used merely by substituting steady-state concentrations for equilibrium concentrations. In general, the slowest observable process is the most difficult to analyze, not only because it is coupled to all the faster processes (as in the conventional relaxation experiment), but also because it is farthest from equilibrium (relative inequality of forward and reverse rates). This was a major reason for not attempting to incorporate process A into the scheme.

The simplest scheme relating the events of the ATP hydrolase cycle to the effect of concentrations of ATP (S), ADP (D), and orthophosphate (P) on the observed rates is given by Scheme 3 (Kawai and Halvorson, 1991). In this scheme the slowest step takes X_6 (AM*D) to X_0 (AMD), closing the cycle. This abstract scheme was not assumed, but rather derived from the behavior of the relaxation processes with respect to the solution variables. State X_{34}, also labeled "Det," is an approximation, representing a collection of states that are not resolvable by experiment.

Under the approximation that process C is significantly faster than process B ("kinetic uncoupling"), the expression for the relaxation rate is given by Eq. 3 of Kawai and Halvorson (1989). This certainly provides an adequate first-order description of the data. If indeed there is a second-order effect of phosphate on process C, the level of approximation can be improved at the expense of feasibility of analysis by solving a quadratic equation (see Eqs. 13–17, Kawai and Halvorson (1991)). The existence of process D (Zhao and Kawai, 1993), faster than process C, introduces an additional

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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.

REFERENCES

Castellan, G. W. 1963. Calculation of the spectrum of chemical relaxation times for a general reaction mechanism. *Ber. Bunsen-Ges. Phys. Chem.* 67:898–905.

Eigen, M., and L. de Maeyer, 1974. Theoretical basis of relaxation spectrometry. *In* Investigation of Rates and Mechanisms of Reactions. G. G. Hammes, editor. John Wiley & Sons, New York.

Hammes, G. G. 1968. Relaxation spectrometry of biological systems. *Adv. Protein Chem.* 23:1–57.

Hill, T. L. 1960. Introduction to Statistical Thermodynamics. Addison-Wesley Press, Reading, Massachusetts. 140–143.

Jovin, T. M. 1975. Fluorometric kinetic techniques: chemical relaxation and stopped flow. *In* Biochemical Fluorescence: Concepts. R. F. Chen and H. Edelhoch, editors, Vol. 1. Marcel Dekker, New York. pp. 307–323.

Kawai, M., and H. R. Halvorson. 1989. Role of MgATP and MgADP in the cross-bridge kinetics in chemically skinned rabbit psoas fibers. Study of a fast exponential process (C). *Biophys. J.* 55:595–603.

Kawai, M., and H. R. Halvorson. 1991. Two step mechanism of phosphate release and the mechanism of force generation in chemically skinned fibers of rabbit psoas muscle. *Biophys. J.* 59:329–342.

Thusius, D. 1977. On the analysis of relaxation amplitudes. *Biophys. Chem.* 7:87–93.

Zhao, Y., and M. Kawai. 1993. The effect of lattice spacing change on cross-bridge kinetics in chemically skinned rabbit psoas muscle fibers. II. Elementary steps affected by the spacing change. *Biophys. J.* 64:197–210.

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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