Biophysical Journal Volume 65 November 1993 2261-2264 2261

Letters to the Editor

Orthophosphate and the Fastest Component of the Mechanical Transient in Skinned Muscle Fibers

K. Horiuti and T. Sakoda
Department of Physiology, Oita Medical University, Oita 879-55, Japan

Dear editor:

When a small length change is applied to a contracting muscle fiber, tension recovery shows three characteristic components: Phase 2/process C and phase 4/process A are the fast and slow components of the tension recovery, respectively. Between these two processes phase 3/process B intervenes, which is a pause or reversal of the recovery (Huxley and Simmons, 1971; Kawai and Brandt, 1980). Kawai and Halvorson (1991) proposed a chemomechanical cross-bridge model which explains the effects (Kawai and Halvorson, 1989, 1991) of ATP, ADP, and orthophosphate (P_i) on processes C and B. Recently, we have closely examined their model, and found several problems with it.

The chemical steps and assumptions in the model are as follows (Kawai and Halvorson, 1991) (Scheme 1):

A. Step 6 is the slowest, rate-limiting step of the cycle of ATP hydrolysis by myosin (M) in the presence of actin (A).

B. The steps that show the second and third slowest relaxation rates are steps 4 and 2, respectively, and both are readily reversible.

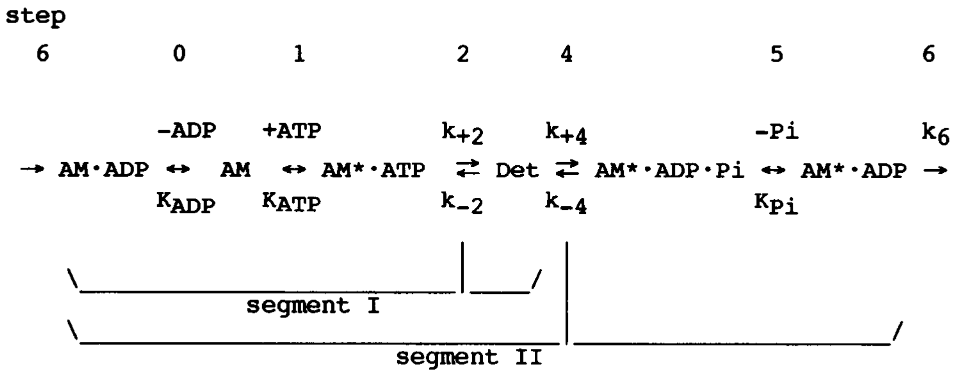
C. Association/dissociation of ADP, ATP, and P_i are very rapid compared with the other steps, and are only characterized with stability constants (K_{ADP}, K_{ATP}, and K_{Pi}).

D. State Det is the detached state of cross-bridge [(A)M · ATP plus (A)M · ADP · P_i] and thus does not bear stiffness or force, while the other attached states bear nearly equal stiffness and force.

E. The length perturbation affects the equilibrium constants of steps 2 and 4: K_2 ($= k_{+2}/k_{-2}$) and K_4 ($= k_{+4}/k_{-4}$) decrease when the muscle is released, and increase when the muscle is stretched. This assumption was not explicitly stated by Kawai and Halvorson (1989, 1991), but it is necessary to correlate the mechanical and chemical events in their model.

Qualitatively, when one releases the muscle, the reaction segment (I) around step 2 first relaxes decreasing Det, and this results in the rapid increase in tension (process C). Following this initial phase, the segment (II) around step 4 then relaxes increasing Det, which results in the decrease in tension (process B). It should be noted that no step has been assigned to the slow phase of tension recovery (process A; see Tawada and Kawai (1990)). In a recent version of the model (Zhao and Kawai, 1993), step 2 is split into two steps with insertion of an intermediate state, so that the original process C corresponds to process C plus D in the new version. However, we only discuss on the original model, because the modification does not affect our argument.

A problem with assumption *E* is that the equilibrium constant (K_4) for the force-producing step decreases when the cross-bridges are released and lose force. This effect of force on the equilibrium seems to conflict with the thermodynamic law of Le Chatelier that, when a stress is applied to a system



SCHEME 1

Received for publication 8 March 1993 and in final form 20 July 1993.

0006-3495/93/11/2261/04 $2.00

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

Herbert R. Halvorson
Henry Ford Hospital, Detroit, Michigan 48202 USA

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

---

Received for publication 9 August 1993.

0006-3495/93/11/2262/04 $2.00