Volume 278, number 1, 131-132 FEBS 09315 January 1991

ADONIS 001457939100038R

Commentary

Kinetics of the actomyosin ATPase

Four or six states?

Leonard A. Stein

Laboratory of Molecular Cardiology, Departments of Medicine and Physiology, SUNY at Stony Brook Medical Center, New York, USA

Received 19 November 1990

Tesi et al. [(1990) FEBS Lett. 260, 229-232] use a misinterpretation of the four-state controversy as a springboard to this paper. The authors give no data to characterize the proteins, making it impossible to compare the proteins with those from other laboratories. The analysis is flawed by the authors' failure to verify that the steady state rates obtained compare reasonably well with published rates. Although a four-state model may be a satisfactory approximation for certain limited purposes, it is not a sufficient basis for a complete analysis of the actomyosin system.

In a recent article, Tesi et al. [1] assert that the four state kinetic model [2] can adequately describe the interactions of Subfragment-1 with actin and ATP in solution: 'Since we find that the P_i burst at high actin is low, we conclude that the four-state model is sufficient to describe the actomyosin ATPase.' This conclusion is unwarranted on at least two grounds: (A) the data they present do not convincingly establish that the P_i burst does approach zero at infinite actin, and (B) their assertion is based solely on burst measurements, and there are other properties of the actomyosin system which are unaccountable by a four-state model.

A. The initial P_i burst

The P_i burst of myosin Subfragment-1 (S-1) was first observed in the absence of actin. After formation of the **M·ATP** complex, a rapid isomerization occurs which hydrolyzes ATP on the myosin surface to the state **M·ADP·P_i**. The fraction of the S-1 in the state **M·ADP·P_i** at steady state is, by definition, the magnitude of the burst. Because the release of products is very slow in the absence of actin, the burst is the virtual plateau of phosphate production at steady state (see Tesi et al., Fig. 1). In the presence of actin, however, measurement of the P_i burst is much more difficult due to the high ATPase rate in the steady state. In practice, one usually measures the P_i production until it becomes linear and then extrapolates back to zero time. The resulting y-intercept gives a **reduced** estimate of the burst magnitude, sometimes called the **'apparent burst'** [3]. This extrapolation always underestimates the 'true' P_i burst, or concentration of myosin-product states, because it is based on the false assumption that the steady-state rate begins at $t = 0$. Calculation of the true burst depends upon the model. With four-states [3,4][a] (footnote):

True Burst = apparent burst + (steady state rate/burst rate).

It is not clear that Tesi et al. [1] made this distinction, but we will assume that the burst they referred to is the apparent burst. Practical difficulties abound when attempting to obtain a good estimate of the apparent burst, and since the measurement involves extrapolation, any process that delays the attainment of the full steady state (e.g. low [ATP], high viscosity and poor mixing, etc.), will lead to an underestimation of the apparent burst. Furthermore, any other error in the steady state rate estimation will have an effect on the apparent burst magnitude.

Correspondence address: L.A. Stein, Health Sciences Center, T-17, Room 020, SUNY at Stony Brook, Stony Brook, NY 11794, USA

[a]This equation is based on the assumption that the binding of actin to myosin in the presence of ATP can be represented by rapid equilibria. It is also based on the approximation that during the burst the free actin concentration does not change appreciably. If $K_3 = K_{13}$, then no change in actin does occur, but even if K_3 and K_{13} differ by a factor of 2-3 the approximation is still excellent. The equation should not confuse the reader to believe that any choice of the variables is possible. The four-state model also requires that:

(True Burst) > (Apparent Burst) > (True Burst)^2

where the magnitudes are fractions of the total myosin S-1 present. Therefore a solution of the equation given in the text with the Apparent Burst = 0 and the True Burst = 1 is not possible with the four-state model.

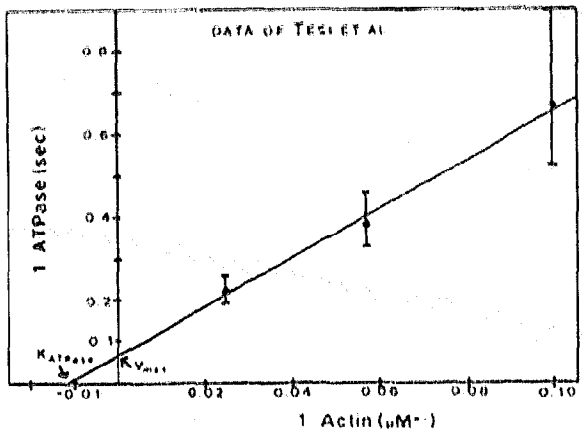

Fig. 1. Data taken from Table I of Tesi et al. [1] and error bars come from the reported standard deviations from this source as well.

Table I of Tesi et al. gives steady state rates estimated from the quench flow measurements. Note that Tesi et al. give neither a steady state ATPase activation plot nor a binding plot, nor do they give the kinetic parameters (i.e. K_{ATPase}, $K_{binding}$ and V_{max}) usually derived from such plots to allow comparison with prior reports. Note further that the data that are responsible for these estimates are only carried out to 250 ms, where the transient burst is not entirely over, and the full steady state rate has not been achieved. In Fig. 1, I have plotted a double reciprocal plot of the steady state rate vs actin concentration using data given by Tesi et al. The extrapolation gives a V_{max} of 14/s and a K_{ATPase} of 85 μM, and these values may be compared to the values of 6.7/s and 6 μM obtained by Rosenfeld and Taylor [3] at 20°C, or 4/s and 2-4 μM obtained by Stein et al. at 15°C (a 2-fold change in V_{max} for a 5° change in temperature is expected). A V_{max} of 14/s is 3-fold higher than previously reported at 15°C, and the K_{ATPase} is more than an order of magnitude higher. These data imply that the ATPase activity has been underestimated at low actin and overestimated at high actin, and this will raise the apparent burst at low actin and reduce the burst at high actin. The burst at 10 μM actin reported by Tesi et al. is about 88% of the magnitude in the absence of actin, which is too high even for the six-state model, and the burst at 40 μM actin is only about 27% that in the absence of actin. Review of Fig. 2c of Tesi et al. shows that if a steady state ATPase rate of 3.6/s were assumed (a 20% reduction), the extrapolated magnitude would be about 50% of the magnitude in the absence of actin. The data of Stein et al. [5] showed a magnitude at 40 μM actin that was about 55% of that in the absence of actin. Note that the burst magnitudes reported by Stein et al. [5] were corrected for inactive S-1 (using the irreversible binding magnitude), and for this reason alone appear higher than those of Tesi et al. However, at zero actin the **uncorrected** magnitude was 0.56 and identical to theirs.

Tesi et al. cite Rosenfeld and Taylor [3] as also having observed a low burst magnitude at high actin. However, examination of the data summary in Fig. 3 of reference 3 shows that overall, except for the burst at 40 μM actin, the data appear to plateau at about 0.20 or 40% of the magnitude measured in the absence of actin. An example of the data at 40 μM actin is shown in Fig. 2a. Here Rosenfeld and Taylor obtained a steady state rate of 10.7/s, nearly twice the V_{max} given in their Table I, which casts doubt on their burst measurement at 40 μM actin. The magnitude reported by Rosenfeld and Taylor for crosslinked actoS-1, considered by many to be equivalent to infinite actin, is almost identical to that of Stein et al., and was 29% at 20°C [3] vs 32% at 15°C [6].

B. The controversy: four states or six states?

The controversy over the adequacy of the four-state model really involves a **philosophical** question: how poorly does a model have to fit the data before the model is rejected? Stein et al. [2] initially rejected the four-state model when it could not adequately account for the observed 3-6-fold difference between K_{ATPase} and $K_{binding}$. However, Rosenfeld and Taylor [3] state that: .. 'a four-state model provides a good approximation to the kinetic behavior of actoS-1 . . . We do not conclude that additional states are absent or unimportant but rather that a four-state model provides a sufficiently good description.. A more direct method for detecting additional intermediates is required..'. Tesi et al. assert that their data show that the four-state model is sufficient to account for all of the available data, implying that the only difficulty for the four-state model had been the presence of a P_i burst at high actin. However the necessity of a six-state model arose from the combination of several kinds of data [4]: (1) the linear double reciprocal activation plot at low to moderate actin, (2) the 3-6-fold difference in K_{ATPase} and $K_{binding}$, (3) the rise in the tryptophan fluorescence rate at low actin and (4) the oxygen exchange kinetics [7], as well as (5) the P_i burst data.

Acknowledgements: This work was supported by The American Heart Association, New York State Affiliate. Dr Stein was a recipient of a Research Career Development Award from the N.I.H.

REFERENCES

[1] Tesi, C., Barman, T. and Travers, F. (1990) FEBS Lett. 260, 229-232.
[2] Stein, L.A., Schwartz, R.P., Chock, P.B. and Eisenberg, E. (1979) Biochem. 18, 3895.
[3] Rosenfeld, S.S. and Taylor, E.W. (1984) J. Biol. Chem. 259, 11908-11919.
[4] Stein, L.A. (1988) Cell Biophys. 12, 29-58.
[5] Stein, L.A., Chock, P.B. and Eisenberg, E. (1984) Biochem. 23, 1555-1563.
[6] Stein, L.A., Green, L., Chock, P.B. and Eisenberg, E. (1985) Biochemistry 24, 1357-1363.
[7] Stein, L.A., Evans, J.A. and Eisenberg, E. (1989) Biochemistry 28, 7747-7752.