THE HILL COEFFICIENT FOR THE Ca^{2+}-ACTIVATION OF STRIATED MUSCLE CONTRACTION

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ABSTRACT The following arguments are presented for the observation that curves relating free Ca^{2+} and force development of thin filament regulated myofilaments of skinned muscle fibers have Hill coefficient (n) >4, which is the number of Ca^{2+} binding sites on troponin: (a) Activation of the myofilaments is a process relaxing to a nonequilibrium steady state or stationary state. Systems operating at nonequilibrium stationary states are known to display Hill coefficients greater than the number of interacting sites and similar results have been obtained for Ca^{2+} activation of myofilament isometric force. (b) The size of the basic subunit of thin filament regulated muscle may be the entire thin filament rather than seven actins, one tropomyosin, and one troponin. In this case the number of interacting sites may be on the order of hundreds. (c) Hysteresis in the Ca^{2+} activation of isometric force might result from multiple stationary states and also might give rise to Hill coefficients >4.

INTRODUCTION

Force development of fast skeletal myofilaments is regulated by Ca^{2+} binding to the four sites of troponin (Potter and Gergely, 1975). Yet in several studies (Brandt et al., 1980; Godt and Lindley, 1982; Brandt et al., 1982) of the relation between free Ca^{2+} concentration and the activation of stationary state (steady state) isometric force, fits of the data gave Hill coefficients >4. These reports have led to some consternation and to the proposal that "other factors must be sought" (Brandt et al., 1982), because dogma states that the Hill coefficient may not be greater than the number of interacting sites, and there are only four Ca^{2+}-binding sites on troponin. In fact, it has been argued that only two of the four sites, the so-called Ca-specific or regulatory sites, are responsible for the activation of fast skeletal muscle contraction (Potter and Gergely, 1975). We have argued elsewhere (Shiner and Solaro, 1982) that the assumptions that led to the conclusion that only two of the Ca^{2+}-binding sites on troponin are responsible for the activation may be unjustified, however, the conclusion itself is not necessarily incorrect. In the case of cardiac muscle myofilaments, pCa-activation relations have Hill coefficients similar to those observed with skeletal myofilaments (Solaro and Shiner, 1976; Fabiato and Fabiato, 1978), yet there are data (Robertson et al., 1982) consistent with the idea that there is one regulatory site per cardiac troponin. If this is the case, the situation regarding Hill coefficients >4 becomes even more puzzling. Our purpose here is to point out that having such large Hill coefficients for the Ca^{2+}-activation of isometric force does not contradict principle because one is concerned with the activation of force under nonequilibrium, stationary state conditions. In addition, we mention two other points that may contribute to the large values of the observed Hill coefficients.

DISCUSSION

The statement that the Hill coefficient may not be greater than the number of interacting sites is valid for the equilibrium binding of a single ligand to, say, a protein, as can be easily demonstrated. We know of no published proof that the corresponding statement holds for the equilibrium activation (or inhibition) of the binding of a first ligand species by a second species, but it appears to be valid as long as the effect of the second species on the binding of the first is monotonic (i.e., only activating or only inhibiting). Whatever the case may be, these statements do not apply to the Ca^{2+}-activation of stationary state isometric force. The word equilibrium, which appears in the first two sentences of this paragraph, refers to the thermodynamic equilibrium where, by definition, the net rate of all processes must vanish. The force under consideration here, however, is actively produced; i.e., its production is driven by free energy obtained from the splitting of ATP. There-

fore, the net ATPase rate is nonzero, and the appropriate stationary state is not an equilibrium state, but a nonequilibrium stationary state.

It has been shown several times, mostly in terms of enzyme kinetics, that the Hill coefficient may be greater than the number of interacting sites for a system operating at a nonequilibrium stationary state (see, e.g., Bardsley et al., 1980). For example, when the concerted model (Monod et al., 1965), one of the classical models for cooperativity, is generalized to take into account the nonequilibrium aspects of catalysis (Shiner, 1982), it is found that the Hill coefficient for catalytic binding as well as that for conformational processes may be greater than the number of interacting (catalytic) sites. Because conformational processes in this model may be generally reinterpreted as the binding of a ligand that influences the rate of catalysis (Whitehead, 1979), it has thus been shown that the number of interacting sites is also not an upper limit for the Hill coefficient for the activation (or inhibition) of a nonequilibrium process, such as enzyme catalysis or force production in the isometric stationary state.

Similar results have been obtained for an extension of the concerted model, which has more direct application to the Ca^{2+}-activation of isometric force (Shiner, J. S., unpublished observations). In accordance with the current picture of the regulation of contractile activity (Weber and Murray, 1973; Adelstein and Eisenberg, 1980), it is assumed that seven actins and one troponin (with its Ca^{2+}-binding sites) interact with one tropomyosin. As simplifications, it is additionally assumed that the actin-myosin-ATP-ADP-P_i reaction can be treated as a two-state cycle and that force is proportional to the number of myosins reacting with actins (or, more conventionally, to the number of attached crossbridges). When it is now taken into account that the stationary state for this system is a nonequilibrium one (Hill, 1974; Shiner and Solaro, 1982), the Hill coefficient for the Ca^{2+}-activation of stationary state isometric force may be as great as $8n_{Ca^{2+}}$, where $n_{Ca^{2+}}$ is the number of Ca^{2+}-binding sites on troponin that are responsible for the activation. If only the two Ca^{2+}-specific sites are the activating ones, the Hill coefficient may be as large as 16; if all four Ca^{2+}-binding sites are involved in the activation, the Hill coefficient may be as large as 32. On the other hand, the largest reported value of the Hill coefficient is 6.9 (Brandt et al., 1982), which does not even approach the smaller of these two limits.

The considerations above, which follow from the idea that the muscle contraction is a nonequilibrium phenomenon even at the stationary state, are probably the most important here. Two other points deserve mention, however. The first concerns the size of the basic subunit of the thin filament that can be considered independent. It is often assumed, as in the above paragraph, that the basic subunit consists of seven actins, one tropomyosin, and one troponin, and that these subunits are independent. There is much evidence, however, of end-to-end interactions between adjacent (overlapping) tropomyosins along the thin filament (Tawada et al., 1975; Wegner, 1979). These interactions would induce apparent interactions between the Ca^{2+} binding sites on adjacent troponins and between groups of seven actins, which in turn would imply that the basic independent subunit that must be considered is an entire thin filament, which has on the order of hundreds of Ca^{2+}-binding sites. This number of Ca^{2+}-binding sites is much larger than the four usually considered. Furthermore, Thompson (1968) has shown for the one-dimensional Ising model (of which the Koshland square model is a special case [Koshland et al., 1966]) that cooperative properties are effectively independent of the number of interacting sites when this number is greater than four. Instead of the number of sites it is the strength of the interactions that is decisive.

The second additional point concerns the hysteresis recently reported in the Ca^{2+}-activation of isometric force in barnacle fibers (Ridgway et al., 1983). The hysteresis has also been shown in the case of Ca^{2+} activation of vertebrate striated muscle (Gordon et al., 1983; Solaro, R. J., unpublished observations). We have previously predicted (Shiner and Solaro, 1982) that if this hysteresis is the result of multiple stationary states, it may also contribute to the large values of the observed Hill coefficients. Hysteresis due to multiple stationary states implies that, within some range of Ca^{2+}-concentrations along the Ca^{2+}-activation curve, the muscle has two stable states available to it. The state in which the muscle finds itself at a given Ca^{2+}-concentration is path dependent; i.e., it is determined by whether the given concentration is reached along a path of increasing or decreasing concentration. At the end points of the region of bistability, the activation curve jumps from one of the stable states to the other with a slope which is infinite in principle. The actual slope may be finite because of the finite size of muscle as a thermodynamic system, but it would still be extremely big.

We have mentioned three other factors here that all probably contribute to make the values of Hill coefficients >4 for the Ca^{2+}-activation of stationary state isometric force in vertebrate skeletal muscle. (Actually, the same considerations probably apply to other muscle types, but with appropriate changes in detail of course.) In closing, we would like to emphasize that we do not feel that the Hill equation represents an appropriate formalism for the analysis of the mechanism of the Ca^{2+}-activation. The three points mentioned here all argue against its use; other points have been mentioned previously (Shiner and Solaro, 1982). A more appropriate approach uses the concept of energies of interaction between the various subunits of the thin filament (Hill, 1983; Shiner and Solaro, 1982). The Hill coefficient does remain, however, a useful tool for the parameterization of activation curves, so that they can be easily compared.

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