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SEGMENTAL FLEXIBILITY IN THE MYOSIN MOLECULE: Evidence From Binding Studies of Myosin Fragments with Actin

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From comparative studies of the association with polymeric actin of the bifunctional species heavy meromyosin and its monofunctional constituents, information about the relative freedom of these paired elements can be derived. An isotherm for the former binding process is presented which involves, as an experimentally determinable parameter, the local concentration of a second segment after the first of a pair is attached to the lattice. From combined data for these two association reactions a value of 10^{-4} M is obtained for this quantity. The large degree of segmental flexibility reported for the free heavy meromyosin is still manifested in the association with actin.

INTRODUCTION

For some years it has been known that myosin and its trypsin cleavage product, heavy meromyosin (HMM)* are duplex (1, 2). There is evidence from the binding of small molecules, e.g., substrate analogs, that the two appendages of these species behave independently and equivalently (3). However, some studies of enzymatic breakdown of adenosine triphosphate argue against these elements or heads being identical (4, 5).

Mechanisms for muscle action all entail a cycle of association, hydrolysis of adenosine triphosphate, and dissociation involving bridges between the myosin contained in thick filaments with the F-actin composing the thin filaments. While changes in attitude of the bridge during this cycle are common to these schemes, explicit provision is usually not made for the bifunctional character of the myosin (6, 7). One model, however, proposes an "arm-over-arm" progression of myosin elements along the actin filaments (8, 9) in the contractile process. Such a scheme clearly requires a measure of motile independence of these elements.

Early studies of steady state fluorescence depolarization have suggested a degree of flexibility of the two myosin appendages (10). Recent determinations of the changes in polarization anisotropy decay times of a fluorophore attached to myosin, HMM, and S1

*Abbreviations: HMM, heavy meromyosin; S1, subfragment 1 of heavy meromyosin; F-actin, fibrous actin; G-actin, globular actin component of fibrous actin.

argue for a large degree of segmental flexibility of the S1 elements probably at a pivotal junction with the myosin stem (11).

Here we report on a different approach to the question of segmental flexibility of the myosin duplex. A comparison of the binding constant for a bifunctional species, e.g., HMM, with that of its constituent monofunctional elements, i.e. S1 with a linear lattice such as F-actin should provide information germane to the degree of motional freedom possessed by these paired moieties. Moreover, the seat of the flexibility must clearly be localized in the HMM portion of the myosin molecule.

THE MODEL ISOTHERMS

We consider here a linear array of sites which can be filled by a bifunctional ligand capable of attachment by one appendage or by two. A schematic depiction of this process is presented in Fig. 1a. F-actin is a double helix of globular G-actin monomers (12). Consequently, two modes of interaction of HMM can be envisioned, one with two adjacent G-actin sites in the same chain, or, alternatively, one with G-actin sites in opposite strands. Electron micrographs appear not to permit a decision on this point (13). For the model presented here it is, of course, immaterial which type of bimodal attachment occurs. However, for this model to retain its simple linear character requires that for each site there be only two equivalent nearest neighboring sites, so that one of the above two modes of attachment must predominate.

The binding equilibrium can be treated by recourse to a partition function derived from a 3 × 3 matrix whose elements represent the nine possible statistical weights of the transitions arising from the three states for each site. Each site can be either empty (statistical weight of 1 by convention), filled by one appendage (weight of α), and lastly filled by a second appendage (weight of β). This last pair of adjacent sites would have a weight of $\alpha\beta$. Figure 1a displays these weights beside the appropriately bound species.

This is an example of a linear Ising system whose treatment is now widely described in the literature (14, 15). The partition function for an assembly of N sites can then be written as a repeated matrix product.

$$\Gamma_N = (1 \;\; 1 \;\; 1)\mathbf{M}^{N-1}(1 \;\; \alpha \;\; 0)^T . \tag{1}$$

The first unit of which must be either empty or filled by the first appendage of the bifunctional ligand is represented by the transpose (superscript T) of the row vector $(1 \;\; \alpha \;\; 0)$. The matrix **M** for this system is

$$\mathbf{M} = \begin{pmatrix} 1 & 1 & 1 \\ \alpha & \alpha & \alpha \\ 0 & \beta & 0 \end{pmatrix}. \tag{2}$$

The first and third columns contain the weighting factors for the two transitions available from an antecedent empty site and a prior site filled by a second appendage, respectively. The second column represents the three transitions from a pre-existent first appendage occupied site.

The dimensionless parameter $\alpha = cK$, where K is taken to be the intrinsic association constant for one element (vide infra) and c is the concentration of attachable myosin elements. The parameter $\beta = c_L K$, where c_L is the "local" concentration of a second element after the first has been conjoined with the F-actin. β and, ultimately, c_L are the quantities of basic physical interest.

In the brief treatment outlined here we will concentrate on a version of this model for which $\beta \gg \alpha$ or $c_L \gg c$ for the accessible range of ligand concentration. That is to say, whenever one element is attached, the second must automatically follow. The system allows no single hangers as shown in Fig. 1a. Figure 1b displays this mode of binding. The matrix M can be replaced by

$$M' = \begin{pmatrix} 1 & 0 & 1 \\ \alpha & 0 & \alpha \\ 0 & \beta & 0 \end{pmatrix} \tag{2'}$$

The fractional extent of saturation (θ') follows from equation (1) with the transition matrix given by equation (2') by customary procedures of diagonalization of M' to evaluate Γ_N' and operation on $\ln\Gamma_N'$ by the sum: $\frac{\alpha}{N}\frac{\partial}{\partial\alpha} + \frac{\beta}{N}\frac{\partial}{\partial\beta}$. This yields for θ' the following simple expression

$$1 - \theta' = \frac{1}{(1 + 4\alpha\beta)^{1/2}} \, . \tag{3}$$

This is to be compared with the result for the familiar Langmuir isotherm also written as fractional extent of unsaturation

$$1 - \theta'' = \frac{1}{1 + \alpha} \tag{4}$$

The latter would be expected to describe the binding of S1 to the F-actin bihelix as displayed in Fig. 1c.

DISCUSSION

Equation (3) is identical in form to the result for the adsorption of a diatomic molecule on a linear lattice in the limit of large N (16, 17) obtained by a different argument. We note from this result that $\theta' = \frac{1}{2}$ when $\alpha_{1/2}\beta = \frac{3}{4}$, and also, the Hill coefficient $n_{1/2} = \frac{3}{4}$. This should be contrasted with $\theta'' = \frac{1}{2}$ when $\alpha_{1/2} = 1$ with $n_{1/2} = 1$ for the usual Langmuir case. Clearly we are dealing in the former case with an anticooperative binding process ($n_{1/2} < 1$). This is a consequence of the occurrence in the course of random occupation of neighboring pairs of sites of an increasing number of single isolated empty sites (Fig. 1b).

With the limiting form of the transition matrix given by equation (2′) such sites are unutilizable by the bifunctional adsorbent. Consequently, association is disfavored at higher levels of saturation. This is an effect which exists irrespective of any possible steric restriction imposed on the availability of sites by the size of the globular elements of HMM or myosin – a property of the adsorbent not well represented in Fig. 1.

Anticooperative isotherms do not exhibit the graphic departure from those for ideal noninteracting systems which their cooperative counterparts manifest in the canonical sigmoidal behavior at low levels of saturation. The most striking feature of the isotherm for a bifunctional adsorbent over that for the binding curve of the corresponding monofunctional species is a depression of the midpoint to a substantially lower value in the former case, as depicted in Fig. 2. It is the possibility of combining information from studies of these two binding species which warrants the expression of the single variable in equation (3) as a product of two parameters.

The pictorial similarity of the anticooperative and noninteracting isotherm shown in Fig. 2 may account for the success in treating data for HMM binding to F-actin by a Scatchard plot which is really suitable only to the latter.

Studies on HMM binding to F-actin generally agree on an extrapolated saturation value of two G-actin monomers per HMM (18–21). However, there is considerable variation in the magnitudes of the binding constants derived from these data and a concomitant variation in the constant for the S1 binding. A comparison of the concentrations at the midpoints of the isotherms for these two species provides a means of estimating β and c_L. A more precise determination would require fitting the entire HMM/F-actin isotherm to equation (3) or some variant thereof.

One recent investigation of these systems reports dissociation constants for HMM and S1 to F-actin of 2.8×10^{-8} M and 3.24×10^{-6} M, respectively (20). Utilizing these results as measures of the ligand concentrations at half saturation, we calculate $\beta = 42$ and $c_L = 1.35 \times 10^{-4}$ M from the midpoint formulae quoted in the previous section. The smaller the magnitude of c_L, the greater the degree of structural flexibility displayed by the S1 elements of HMM. Other data available on these systems (18, 21) suggest smaller values of β and c_L. In fact the requirement of $\beta \gg 1$ from which equation (3) is obtained is inapplicable under these conditions and a treatment based on the complete matrix of equation (2) must be employed.

CONCLUSION

The interpretation of an enhanced association constant for a bifunctional ligand over that for its monofunctional components as arising from an increased local concentration of a second appendage after the first is bound does not rest on any specific form for either isotherm. The actual magnitude of this quantity (c_L) is dependent on the nature of the isotherms. Moreover, the structural origin in HMM of this degree of freedom must be largely conjectural. For example, if the S1 element is taken to be a rod of approximately 150Å in length (11, 13, 22), a local concentration of about 10^{-4} M is equivalent to the unhindered motion of one appendage in a spherical shell of this radius and of 30-Å thickness. Such a measure of motile freedom is qualitatively in accord with the decay times of the fluorescence polarization anisotropy of HMM and S1 previously reported (11).

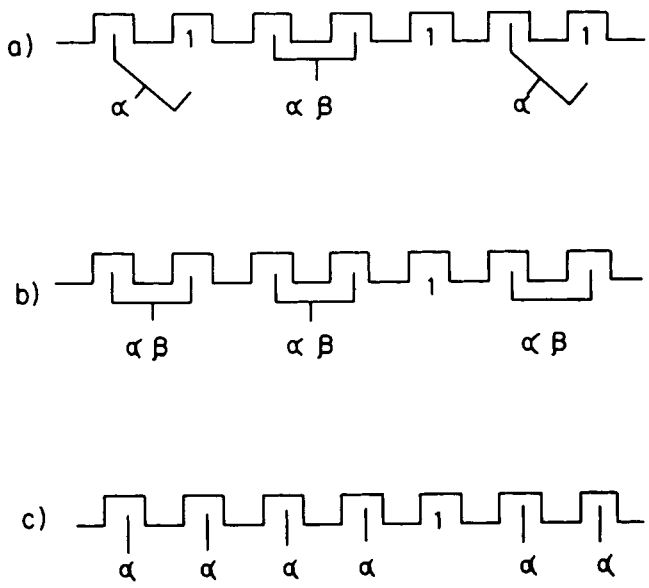

Fig. 1. Binding of a bifunctional ligand to a linear lattice with attachment by one or both elements (a) or exclusively by both elements (b). Association of a monofunctional moiety of this ligand with the lattice (c). The statistical weights are indicated by the binding sites.

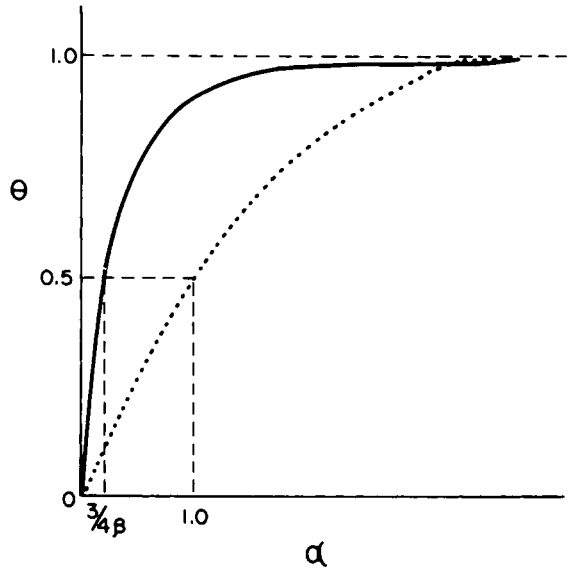

Fig. 2. Sketches of the two isotherms appropriate to the binding processes of Fig. 1b (–) and Fig. 1c (. . .).

Most significantly, this large degree of flexibility must still be manifested by the second S1 element of a pair after the first such element is attached to an F-actin filament.

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