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THEORETICAL CONSIDERATIONS IN THE EQUILIBRIUM BINDING OF MYOSIN FRAGMENTS ON F-ACTIN

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In a previous paper, equilibrium constants for the binding of myosin fragments onto F-actin were assumed known and the statistical problems encountered when the actin sites are occupied to an arbitrary fractional extent were analyzed. The object of the present paper is to attempt to understand the observed order of magnitude of these equilibrium constants in terms of the statistical mechanical degrees of freedom involved. That is, we examine here the equilibrium constants themselves rather than the statistical consequences of the equilibrium constants. The treatment given amounts to a semi-quantitative sketch or outline of the problem. Structural details are much too uncertain to warrant a careful and rigorous treatment at this time. But the discussion suffices to establish the essential qualitative features of the problem. The procedure used is to examine the important equilibrium constants, one at a time, in terms of the factors (partition functions) that contribute to each constant, together with numerical estimates for these factors.

1. Introduction

In a previous theoretical paper [1], equilibrium constants for the binding of myosin fragments onto F-actin were assumed known and the statistical problems encountered when the actin sites are occupied to an arbitrary fractional extent were analyzed. This analysis was found to be useful in determining experimental values for the equilibrium constants [2]. The object of the present paper is to attempt to understand the observed orders of magnitude of these equilibrium constants in terms of the statistical mechanical degrees of freedom involved. That is, we examine here the equilibrium constants themselves rather than the statistical consequences of the equilibrium constants.

The theoretical treatment presented below amounts only to a semi-quantitative sketch or outline of the problem. Structural details are much too uncertain to warrant a careful and rigorous treatment at this time. But we feel that our discussion suffices to establish the essential qualitative ingredients of the problem: the methodology used is more important than the numerical results. Revisions and refinements of the theory can be made in the future, when warranted.

Our procedure will be to examine the important equilibrium constants, one at a time, in terms of the factors (partition functions) that contribute to each constant, together with numerical estimates for these factors.

Two earlier papers on myosin physical biochemistry [3,4] are somewhat related to the present one. Also, the superficially similar one-headed and two-headed antigen-antibody binding problem has been treated [5] and has been used [6,7] in muscle biochemistry. The analysis presented here is much more detailed in the degrees of freedom considered and is formulated explicitly for the actomyosin problem, which has several features not present in the antibody-antigen problem: assumed structure of the head; directionality of the actin filaments; 45° optimal binding angle; molecular distortion necessary to bind two heads; and non-equivalence of the two bound heads (see details below).

2. Binding constant and partition functions

We give necessary statistical thermodynamic background material here. Consider the equilibrium

free ligand in solution $\rightleftarrows$ ligand bound on sites . (1)

Our object is to derive the corresponding binding equilibrium constant K_b as simply as possible. For this purpose, we take the free ligand to be at a very low concentration so that very few sites are occupied by bound ligand. The sites are associated with F-actin monomers in the present problem. The chemical potentials of free and bound ligand molecules [ref. 8, eqs. (4-22) and (7-8)]

$$\mu_f = -kT \ln (q_f/N_f), \quad \mu_b = -kT \ln (q_b/\theta), \tag{2}$$

$$q_f = (2\pi mkT/h^2)^{3/2} V q_f', \quad \theta = N_b/M, \tag{3}$$

where N_f is the number of free molecules in a volume V, N_b is the number of bound molecules on M possible binding sites, the q's are (dimensionless) partition functions, $(\ )^{3/2}V$ is the translational partition function, q_f' refers to non-translational degrees of freedom, and q_b includes a Boltzmann factor with the binding *free energy* in the exponent (this free energy, in turn, includes any solvent effects associated with the binding process).

The two chemical potentials are equal at equilibrium. Therefore

$$q_b/(q_f/V) = \theta/(N_f/V) = \theta/c \equiv K_b . \tag{4}$$

Thus,

$$K_b = q_b/(\ )^{3/2} q_f' \quad (\text{cm}^3 \text{ molecule}^{-1}), \tag{5}$$

$$= q_b(N_0/1000)/(\ )^{3/2} q_f' \quad (\text{M}^{-1} \text{ or } \ell \text{ mole}^{-1}), \tag{6}$$

where $(\ )^{3/2}$ is in units of cm^{-3}, $c = N_f/V$ (concentration), and N_0 is Avagadro's number. The last form, eq. (6), gives K_b in the conventional units M^{-1}.

Suppose there are two different bound states, 1 and 2, at equilibrium with each other. Then eq. (2b) applies to either state. Thus for the process $1 \rightleftarrows 2$, with equilibrium constant K_b', we have

$$q_b^{(2)}/q_b^{(1)} = \theta_2/\theta_1 = K_b' . \tag{7}$$

This constant is dimensionless.

The equilibrium constants of interest to us in this paper are (using the notation of ref. [1]): K_s for the binding of S-1 (subfragment 1 of HMM, heavy meromyosin) on F-actin; K_1 for the one-headed binding of HMM on F-actin; K_2 for the two-headed binding of HMM on F-actin; K for *one-headed binding* $\rightleftarrows$ *two-headed binding* of HMM on F-actin; and K' for the *in vivo* one-headed binding of a myosin cross-bridge onto an actin site that is optimally placed longitudinally relative to the cross-bridge position. Of course K_1, K_2, and K are not all independent; they are related by $K_2 = K_1 K$. The constants K_s, K_1, and K_2 are special cases of eq. (6) whereas the constants K and K' are special cases of eq. (7). The latter is true for K' because bound state 1 in eq. (7) corresponds to a cross-bridge already attached ("bound" in the sense of not being freely diffusible) to a *myosin* filament while bound state 2 represents a cross-bridge still attached to the myosin filament but also bound by one head to an actin site on an actin filament. Thus, formally there is merely a change in the bound state of a cross-bridge when it attaches to an actin site.

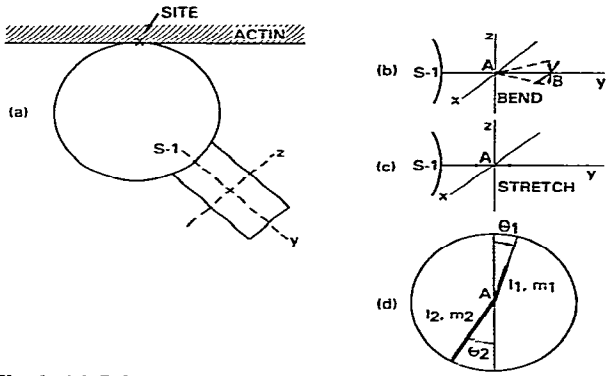

Fig. 1. (a) Schematic shape used for S-1 (bound here on an actin site). Center of cylindrical appendage is origin for axes x (out of paper), y, z. Relative dimensions chosen: cylinder, 1×2; sphere radius, 1.5. Total length of S-1, 120 Å. (b) Appendage (schematic) with bend point at A. Bending can occur in x and z directions. (c) Appendage can stretch or contract along y axis, with center point A. (d) Model used to deduce partition function for bending. See text for details.

3. Two choices for the range of binding angle

Is the cross-bridge elasticity located primarily in the actin site–S-1 angle or in S-2 (subfragment 2)? This question is discussed in detail elsewhere [9], and is not a settled matter. In the former case, there would be a

broad range possible in the actin site–S-1 angle in *in vitro* binding, whereas in the latter case the range in binding angle would be rather narrow (as is more conventional in enzymology). We have used the former choice in our recent work on muscle models [9,10]. In the present paper we consider both possibilities. Actually, the latter choice happens to be much simpler to handle theoretically because in this case (but not in the other) the same rotational coordinate system can be used for both free and bound S-1 moieties. In fact, we have already exploited this feature in earlier work [3]. Therefore, for simplicity and brevity, in each of the following sections the primary discussion refers to the latter choice (rather rigid binding angle). The other case (broad range in binding angle) is taken care of in a separate subsection merely by making an order of magnitude correction to the primary analysis.

4. Binding of S-1 on F-actin

The shape of S-1 is uncertain. But we are obliged to make some definite choice; we adopt, rather arbitrarily, the shape [11,12] shown in fig. 1a (where an S-1 is bound to a schematic actin site). This figure represents a sphere with an attached right circular cylinder. The mass of S-1 is M_S. (We are defining S-1 as that part of a myosin molecule from the tip of one head to the connection point with the other head.)

To derive an approximate expression for K_S, using eq. (6), we have first to specify the degrees of freedom of S-1 to be taken into account. We make the conventional approximation and simplification that the different degrees of freedom are independent of each other (i.e., the Hamiltonian is separable). Free S-1 in solution has three translational and three rotational (rigid body) degrees of freedom. The latter may be approximated using a spherical model. On binding, the translational degrees become three degrees of vibrational motion of the center of mass about the minimum in the binding free energy surface. This surface is determined by the interaction of S-1 with the actin site, including effects due to the release of water molecules in the binding process. The depth of this free energy well is denoted by U, relative to a zero with S-1 far from the binding site. The three rotational degrees become, on binding, three degrees of rocking (vibrational) motion within a rather restricted angular range [3] (see the preceding section).

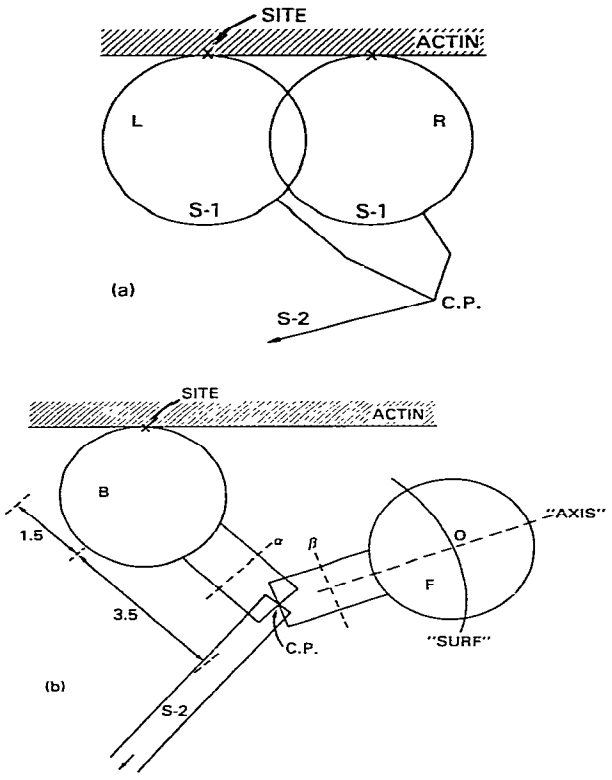

Fig. 2. (a) Schematic two-headed binding of HMM on actin, showing various distortions necessary to acoomodate the binding. Twist of sites on F-actin not shown. (b) One-headed binding of HMM (see text for details).

There are thousands of internal vibrational degrees of freedom in S-1. These we ignore; that is, we assume that these degrees are essentially unperturbed on binding and hence the corresponding partition function cancel in eq. (6). We also assume that the actin filament is unperturbed by bound ligands.

Because we need to accomodate the simultaneous binding of two heads of HMM on adjacent actin sites (for K_2 and K), it is an obvious and well-known geometrical necessity that there be some flexibility in the shape of S-1. We assume that this flexibility resides in the cylindrical appendage, and involves motion relative to the axes x, y, z (fig. 1a). These three "flex" degrees of freedom are assumed, for simplicity, to take the form shown in figs. 1b and 1c, where only the axis of the cylindrical appendage is represented. The axis can

bend at point A (fig. 1b), so that point B moves essentially parallel to either the x or z axes (double arrows). This "bending at a point" is meant to simulate smoother bending over a short distance. Or the appendage can *stretch* or *contract*, along the y axis, symmetrically about point A (fig. 1c). These distortions or strain make possible the two-headed binding of HMM, depicted in fig. 2a. [The geometrical problem is to bring together the ends (point B in fig. 1b) of two S-1's, at the junction with S-2, and still maintain close to the optimal 45° binding angle for each head.] These flex capabilities are "in reserve" for two-headed binding and are presumably not very important for the other states of S-1 and HMM, free or bound.

We digress at this point to discuss the partition functions for bending and stretching. For bending, consider the idealized rotational motion in a circle, shown in fig. 1d. Two rigid arms of arbitrary shape rotate about the center A. The masses are m_i ($i = 1, 2$) and the moments of inertia about A are I_i. The rotation is free except for the potential energy $u = f_\theta(\theta_2 - \theta_1)^2/2$, which keeps the two arms colinear aside from small oscillations with $\theta_1 \neq \theta_2$. This one-dimensional vibration corresponds to the bending motion in fig. 1b, in either the x or z direction. By standard methods [8] one finds that the vibrational partition function for this system can be written

$$q_v = kT/h\nu\,, \qquad \nu = (1/2\pi)(f_\theta/\bar{I})^{1/2}\,, \tag{8}$$

where $\bar{I} \equiv I_1 I_2/(I_1 + I_2)$ is the reduced moment of inertia. We see that $q_v \sim \bar{I}^{1/2}$. Note that if $I_2 \gg I_1$, then $\bar{I} \approx I_1$ (the smaller moment of inertia). The complete "bend" partition function (x and z motion), assuming symmetry, is then

$$q_{\text{bend}} = q_v^2 \sim \bar{I}\,. \tag{9}$$

Consider, now, stretching motion along the y axis (fig. 1c), with force constant f and arbitrary masses m_1 and m_2 on either side of point A. Then we have the well-known relations [8]

$$\nu = (1/2\pi)(f/\bar{m})^{1/2}\,, \qquad q_{\text{str}} = kT/h\nu \sim \bar{m}^{1/2}\,, \tag{10}$$

where $\bar{m} \equiv m_1 m_2/(m_1 + m_2)$ is the reduced mass. If $m_2 \gg m_1$, then $\bar{m} \approx m_1$. It should be noted that f_θ and f are intrinsic properties of the appendage protein and are unaltered when additional weights (see below) are attached to either end of the appendage. The dependences $\bar{I}$ and $\bar{m}^{1/2}$ in eqs. (9) and (10) therefore suffice to give partition function ratios in such cases.

From the geometry in fig. 1a (see the caption), the ratio of masses on either side of the xz or $y = 0$ plane (midplane of the appendage) is 19 : 1 and the ratio of moments of inertia is more than 100 : 1. Therefore, for a free S-1 molecule in solution, we can use the approximations $\bar{I} \approx I_1$ and $\bar{m} \approx m_1$, where I_1 and m_1 refer here to the half-appendage $y \geqslant 0$ in fig. 1a. For a bound S-1 molecule, because of the attachment of S-1 to the actin filament at the binding site, I_2 and m_2 ($y \leqslant 0$) are essentially infinite. Thus the same approximation holds, but more accurately. As a result, $q_{\text{flex}} = q_{\text{bend}} q_{\text{str}}$ has essentially the same value in the free and bound S-1 equations below, and can be cancelled in a ratio.

In view of the above discussion, we have, for S-1,

$$q_f(\text{S-1}) = (2\pi M_s kT/h^2)^{3/2} V q_{\text{rot}} q_{\text{flex}}\,, \tag{11}$$

$$q_b(\text{S-1}) = q_{\text{CMv}} q_{\text{rock}} q_{\text{flex}} e^{-U/kT}\,, \tag{12}$$

and

$$K_s(\text{M}^{-1}) = \frac{q_{\text{CMv}} q_{\text{rock}} e^{-U/kT}(N_0/1000)}{(2\pi M_s kT/h^2)^{3/2} q_{\text{rot}}}\,, \tag{13}$$

where CMv refers to vibration of the center of mass and U is defined above.

The experimental value of K_s is about $10^7\,\text{M}^{-1}$ at 25°C and 0.2M ionic strength [6,13–16]. Our procedure here will be to estimate $q_{\text{CMv}}/(\ )^{3/2}$ and $q_{\text{rock}}/q_{\text{rot}}$ in eq. (13), and then calculate the value of U required to give $K_s = 10^7\,\text{M}^{-1}$.

In connection with q_{CMv}, consider a classical one-dimensional harmonic oscillator, on the x-axis, with mass m and $u = fx^2/2$. We want to replace this potential with an equivalent effective square-well potential, $u = 0$ in $-L < x < +L$, and $u = \infty$ otherwise. To find the effective L, we equate the two corresponding partition functions, $kT/h\nu$ and $(\ )^{1/2} 2L$, and find for L, $fL^2/2 = \pi kT/4$. L is very close to the turn-around point $x = x_0$ for an oscillator with the mean thermal energy kT: $fx_0^2/2 = kT$. We *estimate* the range of the motion of S-1 in the actin site as $2L = 4$ Å. Then we can write for the effective three-dimensional partition function,

$$q_{\text{CMv}} = (2\pi M_s kT/h^2)^{3/2} v\,, \tag{14}$$

where $v = (2L)^3 = 64\,\text{Å}^3$. Thus $q_{\text{CMv}}/(\ )^{3/2}$ in eq. (13) is just v (in cm^3).

We disgress to mention that if we write eq. (13) as

Table 1
Estimated factors contributing to equilibrium constants in broad-range binding angle case

Equil. const.	Symm. no.	S-2	Trans.	Rot.	Flex.	$e^{-U/kT}$	$e^{-W/kT}$	Product
K_s	1	1	$3.9\times10^{-2}\,M^{-1}$	2.9×10^{-3} (2.6×10^{-4}) a)	1	8.9×10^{10} (1.0×10^{12})	1	$1\times10^{7}\,M^{-1}$
K_1	2	0.6	$3.9\times10^{-2}\,M^{-1}$	2.8×10^{-4} (2.5×10^{-5})	8	8.9×10^{10} (1.0×10^{12})	1	$0.9\times10^{7}\,M^{-1}$ b)
K	1	1	0.5	6.8×10^{-6} (9.7×10^{-6})	0.25	8.9×10^{10} (1.0×10^{12})	1.3×10^{-2} (8.3×10^{-4})	1×10^{3}
K'	2	0.05	1	9.9×10^{-5}	9.6	8.9×10^{10} (1.0×10^{12})	1	8.4×10^{6} (9.5×10^{7})

a) Values in parentheses are changes required for the narrow-range binding angle case.
b) The experimental value we use for K_1 is $1.9\times10^{7}\,M^{-1}$ [7].

$$K_s(M^{-1}) = \left(\frac{v}{1000/N_0}\right)\left(\frac{q_{rock}}{q_{rot}}\right)e^{-U/kT}\,, \qquad (15)$$

the quantity $1000/N_0$ is the volume (in cm^3) per molecule at a concentration of one mole per liter. Units other than cm^3 can also be used in $v/(1000/N_0)$ provided only that both v and $1000/N_0$ have the same units, e.g., $64\,Å^3/1660\,Å^3$ (above).

We make a similar "square-well" estimate for q_{rock}/q_{rot}. This has been discussed in detail elsewhere for a spherical macromolecule [ref. 3, eq. (25)]. The completely free motion in q_{rot} is restricted to a quite limited range in the eulerian angles θ, ϕ, ψ in q_{rock}:

$$q_{rock} \approx (R_b^2/12a^2)q_{rot}\,, \qquad (16)$$

where the allowed *linear* range of motion (from θ and ϕ) is $2R_b$ and a is the radius of the spherical molecule. We take $2R_b = 4\,Å$ again and $a = 36\,Å$ (fig. 1a). This gives $R_b^2/12a^2 = 2.57\times10^{-4}$. [It should be noted that, in the notation of ref. [3], $R_b^2/12a^2 = f_\theta f_\psi$ (bound ligand), not $f_\theta^{\ddagger} f_\psi^{\ddagger}$ (activated complex).]

We now have numerical estimates or values for all the ingredients in eq. (13) except U. On substituting these numbers in eq. (13) we can calculate (at 25°C) $e^{-U/kT} = 1.01\times10^{12}$, or $U = -16.4$ kcal mole^{-1}.

Broad range in binding angle. Above [eq. (16)], the tip-of-the-head area of motion associated with minimal freedom in the eulerian angles θ and ϕ is $(R_b^2/4a^2)\times(4\pi a^2) = \pi R_b^2$ [see eq. (25), ref. 3 for details]. Here, as an estimate, we replace this by the area $2R_b \cdot 2d$ of a rectangular strip, where $2d$ is the effective linear range allowed by a broad angular free energy function. [We assume, rather arbitrarily, that an increased range exists in the d (axial) direction but not in the R direction.] To estimate d, we use $fd^2/2 = \pi kT/4$ (as above for L), where f is obtained from $f(80\,Å)^2/2 = 16kT$, a property of the free energy function used in ref. [10]. This gives $d = 17.7\,Å$, compared to the more restricted $R_b = 2\,Å$. Thus R_b^2 in eq. (16) is to be replaced by $4R_b d/\pi$: $4\,Å^2 \to 45.1\,Å^2$, an increase by a factor of 11.3. Consequently, in this "broad range" case, q_{rock}/q_{rot} is increased to 2.90×10^{-3} while $e^{-U/kT}$ is decreased correspondingly to 8.94×10^{10} (or $U = -14.9$ kcal mole^{-1}). That is, when the S-1 can rock more on the actin site after binding, the loss of rotational freedom is less than when the S-1 is bound rigidly to the actin. Thus a lower binding free energy U suffices to compensate for the loss of rotational freedom.

The values above are included in table 1, which exhibits the separate contributions to the several equilibrium constants. The "Trans" column includes the factor $N_0/1000$ [see eq. (15)] for K_s and K_1. Values in parentheses in table 1 are changes required for the narrow-range binding angle case (see above).

Our estimates of the loss of translational and rotational freedom on the binding of S-1 to actin probably are accurate only to an order of magnitude. Nevertheless, it is clear that, when S-1 binds to actin, there must be a large loss of translational and rotational freedom which is more than offset by a large free energy of binding (to give a binding constant of $10^7\,M^{-1}$). Presumably this free energy of binding includes the usual entropy increase of water molecules [17].

5. One-headed binding of HMM on F-actin

We consider K_1 here. Fig. 2b shows an HMM molecule with one head bound (B, bound; F, free). We introduce the label H (where necessary) for either head in a *free* HMM molecule. C.P. refers to the "center point", which we use as a pseudo center of mass below. Motion about the three-way joint (at C.P.), between the two heads and S-2, is assumed to be completely free. The mass of HMM is taken as $3M_s$.

We write the partition function for a free HMM molecule as [compare eq. (11)]

$$q_f(\mathrm{HMM}) = \tfrac{1}{2}(6\pi M_s kT/h^2)^{3/2} V q_{\mathrm{surf}}^2 q_{\mathrm{axis}}^2 q_{\mathrm{flex}}^{(\mathrm{H})2} q_{\mathrm{S2}} \cdot \tag{17}$$

We now discuss the factors on the right-hand side. (i) The factor of two in the denominator is the symmetry number [8] of free HMM (two heads). (ii) $q_{\mathrm{surf}} q_{\mathrm{axis}}$ is the rotational partition function of a single head in free HMM. q_{surf} refers to the free motion (θ, ϕ) of the head on a spherical surface with center at C.P. As an approximation, the full mass M_s can be thought of as moving on the surface ("Surf") shown in fig. 2b. However only about 75% of this surface is available to the center of the head because of the presence of the other head and of S-2; q_{surf} includes this correction. q_{axis} refers to rotation (ψ) about the "axis" in fig. 2b. (iii) $q_{\mathrm{flex}}^{(\mathrm{H})}$ relates to flex motion in the neighborhood, say, of β (fig. 2b) when head B is also free. In this case the mass M_s (approximately) is on the right side of β while the mass $2M_s$ (from B and S-2) is on the left side of β. However, B and S-2 "flop" about C.P. so that their masses and moments of inertia have a much smaller effect on the flex motions than they would have if they were a *rigid* part of an arm, as in fig. 1d. The *full* mass of B or of S-2 is effective only when the axis of B or of S-2 happens essentially to coincide with the direction of a given flex motion. This will generally not be the case. For $q_{\mathrm{flex}}^{(\mathrm{H})}$ we therefore *approximate* B and S-2, together, on the left as having the same effect that F has by itself on the right. Thus, in eq. (10), $q_{\mathrm{str}}^{(\mathrm{H})} \sim (M_s/2)^{1/2}$. Similarly, in eq. (9), we write $q_{\mathrm{bend}}^{(\mathrm{H})} \sim I_s/2$, where I_s is the moment of inertia of F about β (this can be approximated by putting the full mass M_s at 0 in fig. 2b). (iv) Finally, q_{S2} represents the rotational and possible flex motions for S2, as in (ii) and (iii) above (but details are not needed).

For an HMM molecule with one head bound, as in fig. 2b, we have

$$q_b^{(1)}(\mathrm{HMM}) = q_{\mathrm{CPv}}^{(1)} q_{\mathrm{rock}}^{(\mathrm{B})} q'_{\mathrm{surf}} q_{\mathrm{axis}} q_{\mathrm{flex}}^{(\mathrm{B})} q_{\mathrm{flex}}^{(\mathrm{F})} q'_{\mathrm{S2}} e^{-U/kT} \tag{18}$$

where U has the same value as in eq. (12) for S-1. That is, we assume that the HMM head binds to actin in the same way that S-1 does.

We now discuss the factors in eq. (18) in turn. (i) The binding free energy well (for the interaction of B with the actin site) has the same shape here as in the S-1 case above (CMv). C.P. undergoes three degrees of vibrational motion which we represent (effectively) by

$$q_{\mathrm{CPv}}^{(1)} = (6\pi M_s kT/h^2)^{3/2} v \,, \tag{19}$$

as in eq. (14), where v has the same value (64 Å^3). (ii) The bound head B has rocking rather than rotational (surf, axis) motion. As in eq. (16),

$$q_{\mathrm{rock}}^{(\mathrm{B})} \approx (R_b^2/12a^2)(q_{\mathrm{surf}}/0.75) q_{\mathrm{axis}} \,, \tag{20}$$

where $2R_b = (3.5/5.0)4$ Å $= 2.8$ Å and $a = (3.5/1.5)36$ Å $= 84$ Å, in which correction has been made for the different geometry (see the double-headed arrows in fig. 2b). This leads to $R_b^2/9a^2 = 3.09 \times 10^{-5}$. (iii) q'_{surf} (for the free head) and q'_{S2} are reduced fractionally from q_{surf} and q_{S2}, respectively, to take into account another geometrical effect: with head B bound, the free head F and S-2 have surfaces of rotation ("Surf") that are further restricted because the F-actin structure is in the way. This effect will be larger for S-2 because it is very long. (iv) For $q_{\mathrm{flex}}^{(\mathrm{B})}$, the effective mass above α (fig. 2b) is infinite because of the attachment to actin. Below α, the two flopping masses are again approximated as a single mass M_s. Thus $q_{\mathrm{str}}^{(\mathrm{B})} \sim M_s^{1/2}$ and $q_{\mathrm{bend}}^{(\mathrm{B})} \sim I_s$. For $q_{\mathrm{flex}}^{(\mathrm{F})}$, the mass to the left of β is effectively infinite whereas to the right it is M_s. Thus $q_{\mathrm{str}}^{(\mathrm{F})} \sim M_s^{1/2}$ and $q_{\mathrm{bend}}^{(\mathrm{F})} \sim I_s$, the same as for $q_{\mathrm{flex}}^{(\mathrm{B})}$.

From eqs. (6), (17), and (18) we have then

$$K_1(\mathrm{M}^{-1}) = 2v \left(\frac{q_{\mathrm{rock}}^{(\mathrm{B})}}{q_{\mathrm{surf}} q_{\mathrm{axis}}}\right)\left(\frac{q'_{\mathrm{surf}} q'_{\mathrm{S2}}}{q_{\mathrm{surf}} q_{\mathrm{S2}}}\right) \times \left(\frac{q_{\mathrm{flex}}^{(\mathrm{B})} q_{\mathrm{flex}}^{(\mathrm{F})}}{q_{\mathrm{flex}}^{(\mathrm{H})2}}\right)\left(\frac{N_0}{1000}\right) e^{-U/kT} \,, \tag{21}$$

where v is in units of cm^3.

The experimental value we use for K_1 is 1.9×10^7 M^{-1}. This value is based on the experimental evidence showing that the rate of attachment of HMM to F-actin is about twice that of S-1 [7] (the rate of detachment of S-1 and a single HMM head from actin are likely to be similar).

On the right-hand side of eq. (21), v and U have the same values as in the K_s calculation above. The first ( ) on the right of eq. (21) is 3.09×10^{-5} (see above). The third ( ) has the value

$$(M_s^{1/2} I_s)^2 / [(M_s/2)^{1/2} (I_s/2)]^2 = 8 .$$

The second ( ) (referring to F and S-2), the only unknown in the equation, we then calculate as 0.97. This is too large by a factor of about two. For example, one might have guessed ( ) = 0.8 (F) × 0.6 (S-2) = 0.48. The factor 0.8 is included under "Rot." in table 1. The factor 0.6 is also included in table 1, under S-2; K_1 in the table is not the experimental value. Thus, although eq. (21) for K_1 is more complicated than eq. (15) for K_s, it turns out, on examining the factors involved, that the experimental relation $K_1 \approx 2K_s$ is quite understandable. As can be seen in table 1, as in the binding of S-1 to actin, there is a large loss of translational and rotational freedom which is more than offset by a large free energy of binding.

Broad range in binding angle. The correction here is the same as for K_s, above. The first ( ) on the right of eq. (21) is increased by a factor 11.3 to 3.49×10^{-4} and $e^{-U/kT} = 8.94 \times 10^{10}$ (see table 1).

6. Two-headed binding of HMM on F-actin

It is more convenient to examine K than K_2 (recall that $K_2 = K_1 K$). In addition to eq. (18), we need (for K) the partition function for HMM bound by two heads (as shown in fig. 2a):

$$q_b^{(2)}(\mathrm{HMM}) = q_{\mathrm{CPv}}^{(2)} q_{\mathrm{rock}}^{(\mathrm{L})} q_{\mathrm{rock}}^{(\mathrm{R})} q_{\mathrm{flex}}^{(\mathrm{L})} q_{\mathrm{flex}}^{(\mathrm{R})} q'_{\mathrm{S2}}\, e^{-(2U+W)/kT} . \tag{22}$$

The two heads are denoted L (left) and R (right); they are in principle *not* equivalent in this state because of the directionality of the F-actin and of the binding (see below). S-2 is assumed to behave in practically the same way as in one-headed binding. Hence we use q'_{S2}, as in eq. (18).

The new feature to be contended with here is that two heads can bind on adjacent sites, with both angles near 45°, only with the aid of a certain amount of distortion or strain in various degrees of freedom: the flex, rock, and CPv motions will all contribute (see below). In the equilibrium configuration of the complete HMM molecule (bound to the two sites), all of these degrees of freedom will be "off-balance" (i.e., not at their minimum free energies). Also, the two heads may well be in contact, adding a new "contact" free energy term. This might be positive, negative, or zero; there might be positive contributions owing to van der Waals or electrostatic repulsions and negative contributions from van der Waals or other attractive forces (electrostatic; hydrogen bonds), or from an entropy increase in those water molecules freed from the contact surfaces of the two heads. The contact between the two heads shown in fig. 2a should not be taken too seriously: the shape is uncertain and the actin sites twist around the F-actin axis.

The overall equilibrium configuration will correspond to the minimum in the overall free energy function; the minimum has free energy $2U + W$. Thus W is defined as the difference in the binding free energy between the two HMM heads and two S-1 molecules. W will probably be positive owing to the sum of the effects referred to above. We emphasize that W is *not* simply the contact free energy; it includes positive strain contributions because the two heads are attached to each other.

A simple analogy to the distortion effect above is the binding of a diatomic molecule on a pair of sites on a surface, one site for each atom, when the distance between sites is note quite the same as the equilibrium interatomic distance in the free diatomic molecule [18]. To accomplish the binding, a configurational compromise has to be reached such that the total energy is minimized (though no individual degree of freedom is at *its* minimum energy). Incidentally, it will usually be necessary to introduce new normal coordinates in order to treat the small vibrations around the total energy minimum.

From eqs. (7), (18) and (22),

$$K = \left(\frac{q_{\mathrm{CPv}}^{(2)}}{q_{\mathrm{CPv}}^{(1)}}\right)\left(\frac{q_{\mathrm{rock}}^{(\mathrm{L})} q_{\mathrm{rock}}^{(\mathrm{R})}}{q_{\mathrm{rock}}^{(\mathrm{B})} q'_{\mathrm{surf}} q_{\mathrm{axis}}}\right) \times \left(\frac{q_{\mathrm{flex}}^{(\mathrm{L})} q_{\mathrm{flex}}^{(\mathrm{R})}}{q_{\mathrm{flex}}^{(\mathrm{B})} q_{\mathrm{flex}}^{(\mathrm{F})}}\right) e^{-U/kT} e^{-W/kT} . \tag{23}$$

This equilibrium constant is dimensionless; there is not general agreement on its value. Experimental values for K_2 (in M^{-1}) have ranged from 10^8 to 10^{10} [6,16,19]. Since $K_2 = K_1 K$, values for K range from 10 to 10^3. The choice we make is $K = 10^3$ [16].

The middle pair of parentheses on the right of eq. (23) can be rearranged in the form

$$\left(\frac{q_{\text{rock}}^{(\text{L})} q_{\text{rock}}^{(\text{R})}}{q_{\text{rock}}^{(\text{B})2}}\right)\left(\frac{q_{\text{rock}}^{(\text{B})}}{q_{\text{surf}} q_{\text{axis}}}\right)\left(\frac{q_{\text{surf}}}{q'_{\text{surf}}}\right). \qquad (24)$$

If this is reinserted in eq. (23), there are now five pairs of parentheses on the right. The third and fourth of these have the respective values 3.09×10^{-5} and 1/0.8 (see above). In the first, second, and fifth ( ), there are a total of five ratios of the type $q^{(2)}/q^{(1)}$, each with three vibrational degrees of freedom, where (2) refers to doubly bound and (1) to singly-bound HMM (recall that $q_{\text{flex}}^{(\text{F})} = q_{\text{flex}}^{(\text{B})}$). The doubly-bound molecule is distorted and under strain, as explained above. One might expect its vibrational frequencies to be a little higher because of this. We therefore *guess* that a factor 1/2 is reasonable, as an *average* for each of the five $q^{(2)}/q^{(1)}$ quotients. (Of the total factor 1/32: 1/2 appears as "Trans." in table 1; 1/4 appears as "Flex."; and 1/4 is included in "Rot.".)

Another comment is in order about the first ( ). When two heads are bound, the unperturbed (by strain) binding free energy and associated force constant, for motion of C.P., are double that for single-headed binding. But the mass is also doubled so the unperturbed vibrational frequency is unchanged.

If we use the above values and estimates together with $e^{-U/kT} = 1.01 \times 10^{12}$, we can calculate the remaining unknown quantity as $W/kT = 7.10$, or $W = 4.2$ kcal mole^{-1}.

Because of new uncertainties introduced by distortion of doubly-bound HMM, the above calculation of K is more nebulous than is the calculation of K_s or K_1. But it is still possible to understand the value $K = 10^3$ in a general way.

If we use $K = 10^3$, the value of $K_2 = K_1 K$ is 10^{10} M^{-1}. An explicit theoretical expression for K_2 follows from eqs. (6), (17), and (22), but we omit this.

Incidentally, because of two heads bound *versus* one, the suggestion has been made that the experimental values of K_1 and K_2 might simply satisfy $K_2 = K_1^2$. It suffices to point out that even the dimensions of this equation are wrong.

Broad range in binding angle. Referring to eq. (23), two corrections to be made in this case (see the discussion of K_1, above) are: $e^{-U/kT} = 8.94 \times 10^{10}$; and $q_{\text{rock}}^{(\text{B})}$ is to be increased by a factor of 11.3. A third correction in eq. (23) is that $q_{\text{rock}}^{(\text{L})} q_{\text{rock}}^{(\text{R})}$, together, should be multiplied by $11.3/2^{1/2}$, or 8.0. This follows because the two heads in doubly-bound HMM must be bent as a unit. Consequently, $fd^2/2$ (in the K_s discussion) becomes fd^2 here. Thus $d = 12.5$ Å; two heads have a smaller effective range than one head. As a consequence of these three corrections, the "broad range" value for W/kT is found to be 4.33, or $W = 2.6$ kcal mole^{-1} (see table 1). Of course if we had chosen $K < 10^3$, then $W > 2.6$ kcal mole^{-1}.

The two heads are not bound independently. Both bound heads of HMM would "like" to bind at the S-1 binding angle (say 45°), without strain. This could be called "independent binding" of the two heads, if it occurred, and if the contact free energy were zero. But because the two heads are attached to each other (at C.P., fig. 2a), this could be the equilibrium state of doubly-bound HMM only if the cylindrical appendages of the two S-1's behaved like strings. However, this is not credible because such an S-1 could not sustain a tension in muscle contraction. Thus we conclude that there is distortion in the doubly-bound HMM, as discussed above and as shown schematically in fig. 2a: the two heads do not bind independently. This lack of independence appears in table 1 as two contributions to K: $e^{-W/kT}$ and 1/32. As mentioned above, the latter contribution appears as decreases in the values of the translational, rotational and flex contributions to K, owing to a general stiffening of the HMM molecule when it binds to actin with both heads. Note that if both contributions $e^{-W/kT}$ and 1/32, were omitted, we would estimate K to be (broad-range case) 2.4×10^6. This, in turn, would mean that K_2 would have the value 5×10^{13} M^{-1}, much larger than is observed experimentally.

As mentioned above, the contact free energy between the two bound HMM heads could be positive, negative or zero. However, it appears from a comparison of theoretical [1] and experimental [2,20,21] S-1 and (two-headed) HMM binding curves that the contact free energy between neighboring S-1 and HMM molecules is close to zero. Therefore, the contact free energy between the two bound HMM heads may well be approximately zero. If so, W should definitely be positive (as in table 1) because of the distortion effects already discussed.

If the other contributions to K in table 1 are held constant, numerator and denominator in $e^{-W/kT}/32$ can be altered so long as the quotient retains the value (broad range case) 4.1×10^{-4}; recall that $32 = 2^5$ is just a guess. For example, if 2^5 is changed to 3^5, then $e^{-W/kT} = 1.0 \times 10^{-1}$ ($W/kT = 2.30$). That is, since more stiffening of the HMM is occurring on binding, less of the distortion is expressed in W.

7. One-headed *in vivo* binding of a cross-bridge

We consider here the dimensionless constant K' (introduced in the first section) for the one-headed attachment of an *in vivo* cross-bridge to a fixed but optimally placed actin site [10], as illustrated in fig. 3. As already explained, eq. (7) is applicable to this case, with state 2 the attached state and state 1 the unattached state. The geometry is especially uncertain in this example, but the essential qualitative features can be outlined. An experimental value for K' is not available.

In order to avoid new notation for this special case, we write [eq. (7)]

$$K' = 2(\text{S-2})(\text{Rot})(\text{Flex})\, e^{-U/kT}\,, \qquad (25)$$

and consider these factors one at a time. The S-2 factor includes translation (see below). In each quotient ( ), the state 2 (attached) partition function is in the numerator.

The initial factor of 2 arises because the unattached state (B is free in fig. 3) has a symmetry number of 2 [as in eqs. (17) and (21)].

We assume that S-2 is capable of swinging freely about the joint J between S-2 and the myosin filament, i.e., about the S-2, LMM (light meromyosin) junction. We also assume that S-2 can bend and stretch in the sense already described for S-1. Rotation of S-2 around its own long axis is probably unimportant. As a result of S-2 swinging and stretching, C.P. in state 1 (B in fig. 3 is free) can move within a portion of spherical shell whose extent is quite limited by the presence of the surrounding actin and myosin filaments [22]. When B is attached (state 2), C.P. is even more confined: it can oscillate as in $q^{(1)}_{\text{CPv}}$ in eq. (18) (perturbed, however, by the S-2 attachment at J). Also, the possible bend motions of S-2 are more restricted in state 2 than in state 1. For an overall *estimate* of these losses of S-2

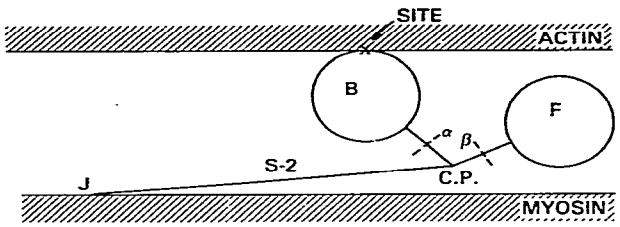

Fig. 3. Schematic one-headed binding of a cross-bridge *in vivo*. S-2 is longer than shown. See text for details.

freedom on attachment of B, we take (S-2) = 1/20 in eq. (25).

The factor (Rot) refers to "surf", "axis", and "rock" motions for the two heads. In state 1, B and F in fig. 3 are free and equivalent; this partition function is $q^2_{\text{axis}} q''^2_{\text{surf}}$, where q''_{surf} is roughly $0.3\, q_{\text{surf}}$ because of collisions of the heads with actin and myosin filaments (this is averaged over the positions of S-2 as it swings in its small solid angle about J). In state 2, the F head has partition functions q_{axis} and approximately $1.2\, q''_{\text{surf}}$ (because C.P. is now always near the center of the interfilament space, which provides maximum space for F). For the B head, we estimate $0.8\, q^{(\text{B})}_{\text{rock}}$ [see eq. (20)] because of restraints introduced by the joint J. Thus

$$(\text{Rot}) \approx \left(\frac{1.2 q''_{\text{surf}}}{q''_{\text{surf}}}\right)\left(\frac{0.8 q^{(\text{B})}_{\text{rock}}}{q_{\text{axis}} q_{\text{surf}}}\right)\left(\frac{q_{\text{surf}}}{q''_{\text{surf}}}\right)$$

$$\approx 1.2 \times 0.8 \times 3.09 \times 10^{-5}/0.3 = 9.88 \times 10^{-5}\,. \qquad (26)$$

For the flex motions of the heads, in state 1, we have M_{s} to the right of β and we estimate effectively $2M_{\text{s}}$ to the left (free flopping of B; restrained flopping of S-2) – compare $q^{(\text{H})}_{\text{flex}}$ in eq. (17). Thus, from eqs. (9) and (10), for each head in state 1, $q_{\text{bend}} \sim 2I_{\text{s}}/3$ and $q_{\text{str}} \sim (2M_{\text{s}}/3)^{1/2}$. For F in state 2, we have M_{s} to the right of β and an infinite mass to the left: I_{s} and $M_{\text{s}}^{1/2}$. For B in state 2, we have an infinite mass above α and effectively $2M_{\text{s}}$ below (see above): $2I_{\text{s}}$ and $(2M_{\text{s}})^{1/2}$. Thus

$$(\text{Flex}) \approx 2^{3/2}/(2/3)^2 = 9.55\,. \qquad (27)$$

Using $e^{-U/kT} = 1.01 \times 10^{12}$ (because we are considering attachment with the actin site at the optimal position relative to the cross-bridge) and the other factors just discussed, eq. (25) leads to $K' = 9.51 \times 10^7$. This is essentially the same value (10^8) assumed for

this constant, for modeling purposes, by Eisenberg et al. [10]. Although the close agreement is fortuitous (e.g., see below), the order of magnitude is comprehensible on the basis of the present crude analysis. Just as with K_s and K_1, a considerable loss of rotational freedom is more than offset by the large binding free energy.

Broad range in binding angle. In the above case, when the head is attached, the binding angle is quite inflexible but S-2 is (potentially) stretchable. Here the angle is (potentially) flexible but S-2 is relatively stiff. In both cases, the one stiff element, the fixed actin site, and the restraint at J (not present with K_s, K_1, or K) combine to severely limit rocking motion. Thus there is no "rocking" correction here. However, for consistency, we must take $e^{-U/kT} = 8.94 \times 10^{10}$. This leads to $K' = 8.43 \times 10^6$ (see table 1).

8. Discussion

The values in table 1 present a reliable over-all semi-quantitative picture of the present problem, based on current structural and binding observations. However, as we have taken care to point out, many individual contributions are based on intuitive estimates or guesses. Generally speaking, the calculation becomes somewhat more uncertain as we move down the rows of table 1. The dominant columns are "Rot." and $e^{-U/kT}$.

The calculation of K_s and K_1 are relatively straightforward. However, to explain the relatively low experimental value for the binding constant of the second HMM head to actin (K), we suggest, in agreement with other workers [6,16,19], that considerable distortion occurs when both HMM heads bind to actin. Also, we calculate a quite large value for the binding constant of the first myosin head to actin *in vivo* (K'), about equal in magnitude to the pseudo first-order binding constant of ATP to myosin *in vivo* [10].

It has been observed experimentally [20,21] that AMP·PNP, ADP, and increasing KCl concentration all weaken the binding constants of S-1 and HMM to actin. For example, the binding of ADP or AMP·PNP to the active site of S-1 decreases K_s 30-fold and 300-fold, respectively. However, the ratio K_s^2/K_2 seems to maintain a constant value of about $10^4\,M^{-1}$. The fact that this ratio remains constant suggests (but does not prove) that AMP·PNP, ADP, and increasing KCl affect the binding of each of the myosin heads to actin nearly identically.

In terms of the present analysis, this can be presented as follows. First, to separate out the U factors in K_s and K_2, we define K_s^0 and K_2^0 by [see eqs. (13), (17), and (22)]

$$K_s \equiv K_s^0 e^{-U/kT}, \quad K_2 \equiv K_2^0 e^{-2U/kT}, \tag{28}$$

where K_2^0 includes $e^{-W/kT}$. In the presence of any one of the above mentioned perturbations, the experimental observation is that

$$K_s = K_s^0 e^{-U/kT}\alpha, \quad K_2 = K_2^0 e^{-2U/kT}\alpha^2, \tag{29}$$

where α is the perturbation effect on K_s. We can deduce from eqs. (29) that not only K_s^2/K_2 but also K_s^{02}/K_2^0 is unperturbed. If we *assume* that K_s^0 and K_2^0 are *separately* unperturbed, then we can conclude from eqs. (29) that these perturbations alter the strength of binding, U, only. This would seem to be a reasonable possibility.

It has also been observed [21,23; see, however, 19] that K_s^2/K_2 is independent of temperature, at least from 5°C to 25°C. The implication of this, from eqs. (28), is that K_s^{02}/K_2^0 is also independent of temperature, and hence that

$$2\,d\ln K_s^0/dT = d\ln K_2^0/dT. \tag{30}$$

However, because of the complexity of the temperature dependence of K_s^0 and K_2^0 [eqs. (13), (17), and (22)], this has to be regarded as fortuitous; there is no fundamental reason to expect it.

Appendix

We show here that the value for K' in table 1 is reasonable from a kinetic point of view. If we write K' as a quotient of first-order rate constants, $K' = k_{on}/k_{off}$, and use the experimental S-1 value of about $0.2\ s^{-1}$ [7] as a presumably good approximation to k_{off}, then $k_{on} = 1.7 \times 10^6\ s^{-1}$ (broad range case). The approximate calculation below demonstrates that this is the expected order of magnitude for k_{on}.

The proper way (in principle) to calculate k_{on} can be sketched as follows. With the actin site in the optimal position relative to the cross-bridge, the complementary

binding site on either head of the unattached cross-bridge can move through a certain restricted volume v_h in the interfilament space (part of the restriction arises from the other head). There is a non-uniform probability distribution for different locations of the binding site within v_h. For each such location as starting point in a diffusional random walk, one calculates the mean first passage time to "capture" within a hemispherical shell of radius R ("activated complex"; $R > R_b$) about the actin site [3]. This time is then averaged over all possible starting locations, using the above-mentioned probability distribution. The reciprocal of the averaged time is $k_{on}/2$ (because there are two heads) [24].

The simple approximate argument we use, in the above spirit, is the following. The second-order rate constant for diffusional capture of one head within the hemispherical shell is $2\pi DRf$ [3], and the corresponding (pseudo) first-order rate constant is $2\pi DRfc_h$, where D is the diffusion coefficient of a head that is part of an unattached cross-bridge, f is the probability that the rotational orientation is suitable for binding when the binding site crosses the hemispherical shell, and c_h is the effective concentration of heads in the diffusional process. For c_h we use $1/v_h$ (above), because $1/c_h$ is the volume per head and there is one head in v_h. Thus, we have the approximate formula

$$k_{on}/2 \approx 2\pi DRf/v_h \,. \tag{31}$$

The value of k_{on} is given above; for D we estimate 4×10^{-7} cm^2 s^{-1}; $R = 4$ Å [3]; and we take $f = 1/10$. From these we calculate $v_h = (22.9\ \text{Å})^3$ (or a sphere of radius 14.2 Å) for the volume available to a binding site on the head of an unattached cross-bridge. This is the anticipated order of magnitude, in view of the myofilament geometry.

An alternative but similar approximate approach is the following. Suppose the center of v_h (see the "proper way", above) is a distance r from the actin site. Let this be the only starting point (instead of using a distribution of starting points). In time t, the mean-square diffusion distance from the center of v_h is $\overline{x^2} = 6Dt$. We are interested in the case $\overline{x^2}^{1/2} = r$, so that $r^2 = 6Dt_r$. Then $1/t_r = 6D/r^2$ can be used as the first-order rate constant for the head reaching the spherical shell of radius r about the starting point. Let p be the probability that the point reached on the shell is within a distance R of the actin site, and that the rotational orientation is suitable. Then

$$k_{on}/2 \approx 6Dp/r^2 \,. \tag{32}$$

One can show that $p \approx fR^2/4r^2$ and that eqs. (31) and (32) differ by a factor R/r (about 1/3).

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References

[1] T.L. Hill, Nature 274 (1978) 825.
[2] L. Greene and E. Eisenberg, J. Biol. Chem. 255 (1970) 549.
[3] T.L. Hill, Proc. Natl. Acad. Sci. U.S. 72 (1975) 4918.
[4] T.L. Hill and E. Eisenberg, Biochemistry 15 (1976) 1629.
[5] D.M. Crothers and H. Metzger, Immunochemistry 9 (1972) 341.
[6] S.S. Margossian and S. Lowey, Biochemistry 17 (1978) 5431.
[7] E.W. Taylor, CRC Crit. Revs. Biochem. 6 (1979) 103.
[8] T.L. Hill, Introduction to statistical thermodynamics (Addison-Wesley, 1960).
[9] E. Eisenberg, and T.L. Hill, Prog. Biophys. Mol. Biol. 33 (1978) 55.
[10] E. Eisenberg, T.L. Hill and Y. Chen, Biophys. J. 29 (1980) 195.
[11] R.A. Mendelsohn and K.M. Kretzschmar, Biophys. J. 25 (1979) 20a (Abstract).
[12] G. Offer and A. Elliott, Nature 271 (1978) 328.
[13] S. Marston and A. Weber, Biochemistry 14 (1975) 3868.
[14] S. Highsmith, Arch. Biochem. Biophys. 180 (1977) 404.
[15] H.D. White and E.W. Taylor, Biochemistry 15 (1976) 5818.
[16] L. Greene and E. Eisenberg, J. Biol. Chem. 255 (1980) 543.
[17] L. Stryer, Biochemistry (Freeman, 1975) p. 147.
[18] T.L. Hill and J.W. Drenan, J. Chem. Phys. 17 (1949) 775.
[19] S. Highsmith, Biochemistry 17 (1978) 22.
[20] L. Greene and E. Eisenberg, Proc. Natl. Acad. Sci. U.S. 75 (1978) 54.
[21] L. Green (submitted).
[22] R.A. Mendelsohn and P.H. Cheung, Biochemistry 17 (1978) 2139.
[23] R.C. Woledge, in: Applications of calorimetry in life sciences, ed. B. Lanprecht (deGruyter, Berlin, 1977) p. 183.
[24] T.L. Hill and G.M. White, Proc. Natl. Acad. Sci. U.S. 61 (1968) 514.