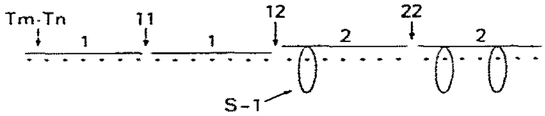

Fig. 1. Model showing, very schematically, tropomyosin–troponin (Tm–Tn) in state 1, which does not allow S-1 binding to actin sites. Tm–Tn is moved into state 2 by binding one or more S-1 molecules. The types of Tm–Tn boundaries are indicated (11, etc.). See text for details.

binding constant K_2/L), subsequent S-1's can bind more easily, without doing any further work moving Tm–Tn relative to the actin sites. In this model, this is the 'umbrella' effect referred to in the title. The binding constant in this case (under the 'umbrella') is K_2, with $K_2 \geqslant K_2/L$ (i.e. $L \geqslant 1$). Fig. 2 shows, *very schematically*, the physical basis for the difference between K_2/L and K_2. Clearly, if ΔG is the (positive) free energy required to move Tm–Tn relative to the actin sites enough to allow binding, then $L \equiv e^{\Delta G/kT} \geqslant 1$. Note that K_1 in ref. [1] is eliminated here as an independent parameter.

In the present model there is *not* a dynamic equilibrium between empty (no S-1 bound) Tm–Tn units in states 1 and 2. Correspondingly, L is not itself an isomeric equilibrium constant as it is in ref. [1]. Tm–Tn is in state 1 if and only if no S-1 is bound to the unit; Tm–Tn is in state 2 if and only if one or more S-1 are bound to the unit. Transitions occur between states 1 and 2 only when the first S-1 is bound to a unit or the last S-1 is released from a unit.

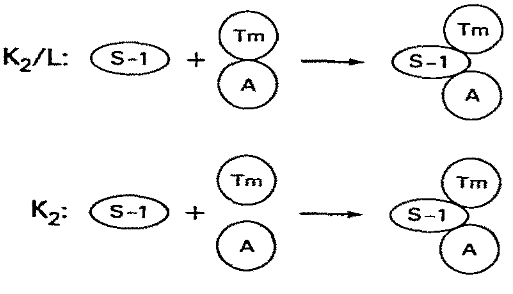

Fig. 2. Top: binding of first S-1 in a unit, with binding constant K_2/L, where $L > 1$. Bottom: easier binding of a subsequent S-1 in the same unit, with binding constant K_2.

The assumption that the *entire* Tm–Tn (over seven sites) is moved by the binding of one S-1 on any one of the seven sites is modified in section 3.

The ends of the Tm–Tn units are assumed to interact with each other, by touching or overlapping [1,4]; the interaction differs according to the states of the two units involved. Three of the four kinds of neighbor pairs are included in fig. 1. These neighbor interactions contribute to the cooperativity of the system (the other source of cooperativity is the feature that all seven sites of a unit change state as a group). On the other hand, neighboring bound S-1 molecules are assumed not to interact directly with each other [1]. Let w_{ij} be the interaction free energy for an ij pair ($i, j = 1, 2$) of Tm–Tn ends. The w_{ij} are presumably all negative quantities, relative to separated ends, though this is not essential. Then we define [1] $x_{ij} = \exp(-w_{ij}/kT)$ and $Y = x_{11}x_{22}/x_{12}x_{21}$. $Y > 1$ contributes positive cooperativity. It is not necessary here, but to simplify algebra in the next section, we make the reasonable assumptions that $w_{11} = w_{22}$ (the two Tm–Tn ends are at the same 'level' in both cases) and $w_{12} = w_{21}$. With this simplification, $Y = (x_{11}/x_{12})^2$. One would expect $-w_{11} > -w_{12}$ and $Y > 1$ (i.e. the attractive interaction is presumably stronger if the two ends are at the same level rather than at different levels).

The formal treatment of the above described model is very similar to that of the previous case [1], so some details can be omitted. The complete grand partition function for one seven-site unit is

$$\xi = 1 + 7(K_2/L)c + 21(K_2/L)K_2c^2 + 35(K_2/L)K_2^2c^3 + \ldots + (K_2/L)K_2^6c^7, \quad (1)$$

where c is the S-1 concentration. For the two separate states of a unit,

$$\xi_1 = 1, \qquad \xi_2 = L^{-1}[(1 + K_2c)^7 - 1], \quad (2)$$

with $\xi = \xi_1 + \xi_2$. The 2×2 matrix for a long chain of units, including pair interactions, is

$$\begin{pmatrix} \xi_1x_{11} & \xi_1x_{12} \\ \xi_2x_{22} & \xi x_{22} \end{pmatrix}. \quad (3)$$

The larger eigenvalue γ_m (m means maximum) of this matrix is given by

$$2\gamma_m = a_1 + a_2 + \left[(a_1 - a_2)^2 + 4a_1 a_2 Y^{-1}\right]^{1/2}, \quad (4)$$

where $a_1 = \xi_1 x_{11}$ and $a_2 = \xi_2 x_{22}$. The equilibrium fraction θ of actin sites occupied by S-1 molecules is found to be

$$\theta = \tfrac{1}{7}\, \partial \ln \gamma_m / \partial \ln c = p_2 \theta_2, \quad (5)$$

$$\theta_2 = K_2 c (1 + K_2 c)^6 / \left[(1 + K_2 c)^7 - 1\right], \quad (6)$$

$$p_2 = 2aY^{-1} / \Upsilon_1 (1 - a + \Upsilon_1), \quad (7)$$

$$a \equiv a_2 / a_1 = \xi_2 x_{22} / x_{11},$$

$$\Upsilon_1 \equiv \left[(1 - a)^2 + 4aY^{-1}\right]^{1/2}, \quad (8)$$

where we have used $\xi_1 = 1$. We shall usually assume (see above) that $x_{11} = x_{22}$ so that $a = \xi_2$. The fraction of units in state 2 is p_2; the fraction of occupied actin sites in state 2 units is θ_2 (of course $\theta_1 = 0$ for state 1 units).

The midpoint of the 1 → 2 transition is $p_2 = 1/2$, which occurs at $a = 1$. If $c \equiv c'$ at the midpoint $p_2 = 1/2$, then the relation (which is independent of Y) between $K_2 c'$ and L is

$$L = (1 + K_2 c')^7 - 1. \quad (9)$$

Also, if $\theta \equiv \theta'$ at $p_2 = 1/2$, then

$$\theta' = \theta_2(c')/2$$

$$= K_2 c' (1 + K_2 c')^6 / 2\left[(1 + K_2 c')^7 - 1\right] \quad (10)$$

$$= \left[(L + 1)^{1/7} - 1\right](L + 1)^{6/7} / 2L. \quad (11)$$

These formulas are useful in establishing parameter values for experimental cases.

Note that $\theta_2 \to K_2 c/(1 + K_2 c)$ for large $K_2 c$ and that $\theta_2 \to 1/7$ for $K_2 c \to 0$ (a state 2 unit has *at least* one S-1 bound). Although $\theta_2 \to 1/7$, $\theta \to 0$ when $K_2 c \to 0$:

$$p_2 \to a/Y \to 7K_2 c/LY \qquad \text{and} \qquad \theta \to K_2 c/LY. \quad (12)$$

Thus the initial slope of $\theta(c)$ is K_2/LY.

When $Y = 1$, $p_2 = a/(1 + a)$, with $a = \xi_2$ given by eq. (2). The binding isotherm $\theta(c)$ in this case still shows positive cooperativity for $L > 1$ (because of the seven-site effect mentioned above). When both $Y = 1$ and $L = 1$, however, $\theta = K_2 c/(1 + K_2 c)$ as one would expect, with no cooperativity remaining.

As a numerical illustration, we reexamine the strongly cooperative S-1-ADP binding curve, in

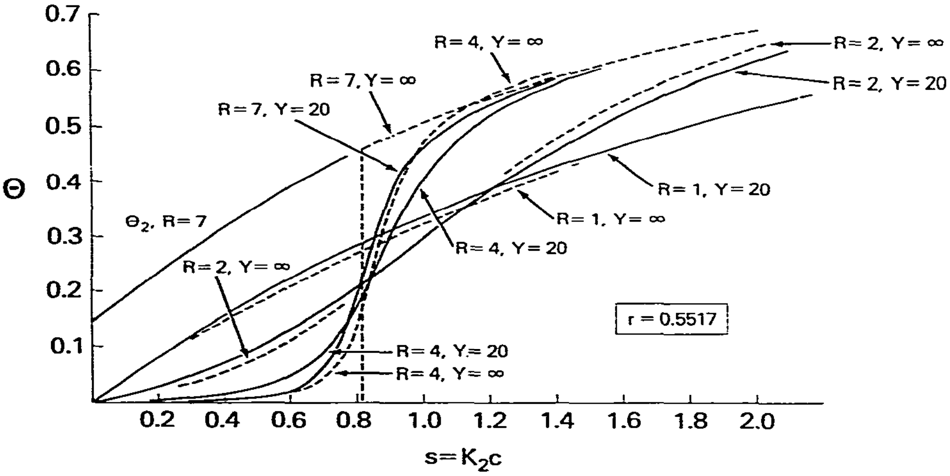

Fig. 3. Effect of varying the range R on binding, $\theta(s)$, into 7-site units. Solid curves (except θ_2) are for $Y = 20$, dashed curves for $Y = \infty$. All curves have $r = 0.5517$. See text for details.

the absence of Ca^{2+}, in fig. 2 of ref. [1]. Parameters used there to fit the data were $K_2 = 0.71$ μM^{-1}, from $\theta(c)$ at large c, and $c' = 1.15$ μM, from the inflection point in $\theta(c)$. If we again adopt these values with the present model, eq. (9) then gives $L = 64.3$ and eq. (11) gives $\theta' = 0.228$. (In ref. [1], $L' = 63.7$ and $\theta' = 0.226$.) The complete curve, taking $Y = 20$ as in ref. [1], is included as the $R = 7$ (notation introduced in section 3), $Y = 20$ curve in fig. 3. This curve is in fact indistinguishable (on this scale) from the corresponding curve in fig. 2 of ref. [1]. In other words, though the earlier model [1] and the present model have somewhat different conceptual bases and equations, they provide essentially identical fits to the experimental data in this case.

3. 'Umbrella' models for seven-site units

We have two purposes in this section. The first is the practical one of investigating whether the observed strong cooperativity in the example just considered can still be accounted for if the assumed full seven-site movement of Tm–Tn, on binding one S-1 molecule, is reduced to a movement over a range of six sites, five sites, etc. The second purpose is to investigate this class of models, in an introductory way, simply as an interesting problem in statistical mechanics that might have future applications elsewhere.

The class of models to be considered here is introduced, by means of an example, in fig. 4. We consider a long chain of symmetrical units, each with $m = 7$ sites for binding a ligand (represented by a circle). Obviously this is itself a special case; the number of sites in a unit could be $m = 1, 2, 3, \ldots$ Eqs. (13)–(25), below, are valid for any m but the remainder of the section is limited to examples with $m = 7$. Section 4 may be regarded as the $m = \infty$ case. The simple special cases $m = 1, 2, 3$ are treated in the appendix.

As in section 2, the sites of a unit are covered by a polymer (Tm–Tn in section 2) which is entirely in state 1 if no ligand is bound to the unit (fig. 4A). When one ligand molecule is bound to the left-hand site of a unit, as in fig. 4B, part of the polymer must be pushed, in the binding process, to a new position or level called state 2. This occurs, in fig. 4, over a range $R = 4$, where R is defined as indicated in the figure. The remainder of the polymer (fig. 4B) is in state 1. If the single bound molecule in fig. 4B happened to be on the central site of the unit, the entire polymer, in this unit, would be in state 2 (the range $R = 4$ applies in both directions; see fig. 4D). Section 2 is concerned with the special case $R = 7$: when $R = 7$, one bound ligand molecule on *any one* of the seven sites causes the entire unit to switch from state 1 to state 2.

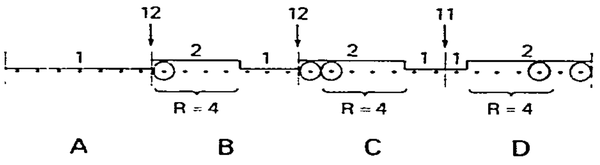

Fig. 4. Illustration of binding of ligand molecules (circles) into 7-site units with range $R=4$. States of sites (1 or 2) and boundaries between units (12, etc.) are labelled. See text for details.

Let K_2 be the binding constant onto a site in a unit that is already entirely in state 2. Further, let us define r as $L^{-1/7}$, or $r^7 = 1/L$. L was defined in section 2 and refers to polymer movement over all seven sites; $r \leqslant 1$ is the factor for a single site. Thus the binding constant for the ligand in fig. 4B would be $r^4 K_2$, because, in the binding process, the polymer over four sites must be moved into state 2. If the single ligand in fig. 4B had been bound onto the central site of the unit, the binding constant would be $r^7 K_2$. Obviously, then, in this example, there is an energetic advantage (with $r < 1$) to binding at the ends of the unit compared to the middle.

In fig. 4C, if the left-hand molecule is bound first ($r^4 K_2$), then the binding constant for the right-hand molecule would be rK_2. The second molecule is bound largely under the state 2 'umbrella' of the first molecule and need move additional polymer into state 2 over only one site (the factor r in rK_2). Thus there is also an energetic advantage (with $r < 1$) to binding next to or near to an already bound molecule; this is a kind of cooperative effect that favors clustering of bound molecules even though the bound molecules do not interact *directly* with each other. If, in fig. 4C the right-hand molecule were bound first ($r^5 K_2$), the left-hand molecule would be bound next (K_2) with a complete umbrella effect (no polymer movement is necessary).

There are nearest-neighbor interactions between the ends of units (fig. 4), just as in section 2 (with the same notation). Here the interaction is determined by the state (1 or 2) of the two *end sites* involved. When $R < 7$, we have the new complication that the two end sites of the same unit may not be in the same state (fig. 4). When $R = 7$ (section 2), the entire unit is always either in state 1 or in state 2.

We turn now to the analytical treatment of this class of models, with the reminder that we are particularly interested in the cases $R = 6, 5, \ldots$ as possible alternatives to the $R = 7$ case already introduced as a model for Tm–Tn.

As in eq. (1), when $m = 7$, the grand partition function ξ for one unit includes a total of $2^7 = 128$ possible states. Whereas, in eq. (2), the terms in ξ are divided into two classes (two states), here we must divide ξ into four classes, according to the states of the two *end sites* of the unit:

$$\xi = \xi_{11} + \xi_{22} + \xi_{12} + \xi_{21}. \tag{13}$$

The subscripts ij here refer to the states of the left-hand site and of the right-end site, respectively. For example, the four units in fig. 4 contribute, respectively, the terms 1 to ξ_{11}, $r^4 K_2 c$ to ξ_{21}, $r^5 (K_2 c)^2$ to ξ_{21}, and $r^6 (K_2 c)^2$ to ξ_{12}. The explicit expressions for the ξ_{ij} depend on the choice made for R. These expressions (for $m = 7$) will be presented below. But we can proceed here with general properties that do not require the explicit ξ_{ij} (which are polynomials in r and $K_2 c$). However, we might note that, for any R, $\xi_{12} = \xi_{21}$ because the units are assumed (for simplicity) to be symmetrical. Also, in the relatively simple $R = 7$ case, $\xi_{12} = \xi_{21} = 0$ and $\xi_{11} = \xi_1$, $\xi_{22} = \xi_2$, where ξ_1 and ξ_2 are given in eq. (2).

Direct neighbor interactions between bound ligand molecules *within the same unit* could easily be included in the ξ_{ij}, if desired, but we omit such interactions here.

We let ν_k represent the state of the left-end site of unit k, in a long chain of units, and ν_{k-1} the state of the left-end site of unit $k + 1$. The 2×2 matrix corresponding to eq. (3) is then

$$\begin{array}{r|ll} & \nu_{k-1} = 1 & 2 \\ \hline \nu_k = 1 & \xi_{11} x_{11} + \xi_{12} x_{21} & \xi_{11} x_{12} + \xi_{12} x_{22} \\ 2 & \xi_{21} x_{11} + \xi_{22} x_{21} & \xi_{21} x_{12} + \xi_{22} x_{22}. \end{array} \tag{14}$$

Thus, in the first row, the left-end site of unit k is always in state 1 but the two terms in each entry allow the right-end site of unit k to be in either state 1 or state 2. The interaction parameter x_{ij} for the interaction between units k and $k + 1$ depends on the state of the right-end site of unit k (this is i in x_{ij}) and of the left-end site of unit $k + 1$ (this is j in x_{ij}, specified by ν_{k+1}). Thus all x_{ij} in column 1 have $j = 1$ and all x_{ij} in column 2 have $j = 2$. Eq. (14) reduces to eq. (3) when $R = 7$ ($\xi_{12} = \xi_{21} = 0$).

If there are M units in a long chain,

$$\ln \Xi = M \ln \gamma_{\mathrm{m}}, \qquad \theta = (1/m)(\partial \ln \gamma_{\mathrm{m}} / \partial \ln s), \tag{15}$$

where Ξ is the grand partition function of the whole chain, γ_{m} is the larger eigenvalue (+sign on λ_2) of the matrix in eq. (14), $s \equiv K_2 c$, and θ is the fraction of all sites that are occupied by ligand. We can also calculate α, the fraction of sites (not units) in state 2, from

$$\alpha = (1/m)(\partial \ln \gamma_{\mathrm{m}} / \partial \ln r). \tag{16}$$

The explicit expression for γ_{m} is ($\xi_{12} = \xi_{21}$)

$$2\gamma_{\mathrm{m}} = \xi_{11} x_{11} + \xi_{12} x_{21} + \xi_{21} x_{12} + \xi_{22} x_{22} + \lambda_2, \tag{17}$$

where

$$\lambda_2 = \left[(\xi_{11} x_{11} + \xi_{12} x_{21} - \xi_{21} x_{12} - \xi_{22} x_{22})^2 + 4(\xi_{11} x_{12} + \xi_{12} x_{22})(\xi_{21} x_{11} + \xi_{22} x_{21}) \right]^{1/2}.$$

We then find

$$\theta = p_{11}\theta_{11} + p_{22}\theta_{22} + p_{12}\theta_{12} + p_{21}\theta_{21}, \tag{18}$$

$$\alpha = p_{11}\alpha_{11} + p_{22}\alpha_{22} + p_{12}\alpha_{12} + p_{21}\alpha_{21}, \tag{19}$$

where

$$\theta_{ij} = (1/m)\, \partial \ln \xi_{ij} / \partial \ln s, \qquad \alpha_{ij} = (1/m)\, \partial \ln \xi_{ij} / \partial \ln r. \tag{20}$$

In these equations, p_{ij} is the equilibrium fraction of units of type iij (left-end site in state i, right-end j), θ_{ij} is the fraction of occupied sites in units of type ij, and α_{ij} is the fraction of sites in state 2 for units of type ij. The explicit expressions found from eq. (20) (these are quotients of polynomials in r and s) of course depend on the value of R, because the ξ_{ij} depend on R. Since $\xi_{12}=\xi_{21}$, we also have $\theta_{12}=\theta_{21}$. The θ_{ij} and α_{ij} are single unit properties that do not depend on the interaction parameters x_{ij}.

Turning now to the p_{ij}, only if $x_{12}\neq x_{21}$ will we have $p_{12}\neq p_{21}$. To save space, we give explicit formulas for the p_{ij} only for the special case $x_{11}=x_{22}$ and $x_{12}=x_{21}$. If, in this case, we define

$$b_1\equiv\xi_{11}/\xi_{12},\qquad b_2\equiv\xi_{22}/\xi_{12},$$
$$y\equiv Y^{1/2}=x_{11}/x_{12},\tag{21}$$

we can express the p_{ij} as follows:

$$p_{11}=\left[b_1y\mathscr{V}_3+(b_1-b_2)b_1y^2+2b_1(b_2+y)\right]/\mathscr{W}\mathscr{V}_3,\tag{22}$$

$$p_{22}=\left[b_2y\mathscr{V}_3+(b_2-b_1)b_2y^2+2b_2(b_1+y)\right]/\mathscr{W}\mathscr{V}_3,\tag{23}$$

$$p_{12}=p_{21}=\left[\mathscr{V}_3+(b_1+b_2)y+2y^2\right]/\mathscr{W}\mathscr{V}_3,\tag{24}$$

where

$$\mathscr{W}=2+(b_1+b_2)y+\mathscr{V}_3,$$
$$\mathscr{V}_3=\left[(b_1-b_2)^2y^2+4(b_1+y)(b_2+y)\right]^{1/2}.$$

In the special case $y=1$ (all x_{ij} equal), eqs. (22)–(24) reduce, as expected, to $p_{ij}=\xi_{ij}/\xi$. That is, the interactions between units no longer have any influence on the distribution among substates within a unit. When $y\neq 1$, inter-unit interactions tend to perturb the distribution among the ξ_{ij} in such a way as to create more favorable neighbor interactions.

The midpoint of the transition (as s increases) can conveniently be defined as the point $s=s'$ at which $p_{11}=p_{22}$. It is easy to see from eqs. (22) and (23) that this occurs when $b_1=b_2$, that is, when $\xi_{11}(s',r)=\xi_{22}(s',r)$. This equation gives s' as a function of r. This condition is independent of the value of Y. The midpoint condition $a=1$, or $\xi_2=1$, in section 2 is a special case. At $s=s'$, with $b_1=b_2$,

$$p_{11}=p_{22}=b_1/2(1+b_1),$$
$$p_{12}=p_{21}=1/2(1+b_1).\tag{25}$$

These expressions are also independent of Y. Thus θ and α will have values at $s=s'$ that are independent of Y.

We now limit the discussion to $m=7$ and give the explicit equations for the ξ_{ij} for $R=6,5,\ldots,2$. The case $R=7$ has already been discussed (but note that for this case, p_2 in section 2 is the same as α here); the case $R=1$ will be dealt with separately, below. For each of $R=2$ to 6, all $2^7=128$ substates have to be examined and assigned to one of the ξ_{ij}. Examples (from fig. 4) were given following eq. (13) (but now put $s=K_2c$). We obtain in this way (the appendix gives some simpler examples):

$R=6$:

$$\xi_{11}=1,\qquad \xi_{12}=r^6s,$$
$$\xi_{22}=r^7\left[(1+s)^7-(1+2s)\right];\tag{26}$$

$R=5$:

$$\xi_{11}=1,\qquad \xi_{12}=(r^5+r^6)s+r^6s^2,$$
$$\xi_{22}=r^7\left[(1+s)^7-(1+4s+2s^2)\right];\tag{27}$$

$R=4$:

$$\xi_{11}=1,$$
$$\xi_{12}=(r^4+r^5+r^6)s+(r^5+2r^6)s^2+r^6s^3,$$
$$\xi_{22}=r^7\left[(1+s)^7-(1+6s+6s^2+2s^3)\right];\tag{28}$$

$R=3$:

$$\xi_{11}=1+r^5s,$$
$$\xi_{12}=(r^3+r^4+r^5)s+(r^4+2r^5+3r^6)s^2+(r^5+3r^6)s^3+r^6s^4,$$
$$\xi_{22}=(r^6+8r^7)s^2+r^7(27s^3+33s^4+21s^5+7s^6+s^7);\tag{29}$$

$R=2$:

$$\xi_{11}=1+3r^3s+(2r^4+r^5)s^2+r^5s^3,$$
$$\xi_{12}=(r^2+r^3)s+(r^3+2r^4+3r^5+r^6)s^2+(r^4+3r^5+5r^6)s^3+(r^5+4r^6)s^4+r^6s^5,$$

$$\xi_{22} = (r^4 + 2r^5 + r^6)s^2 + (2r^5 + 6r^6 + 8r^7)s^3 + (3r^6 + 22r^7)s^4 + r^7(19s^5 + 7s^6 + s^7). \tag{30}$$

To illustrate the use of eqs. (20), consider ξ_{12} in eq. (27) (for $R = 5$). We find, for this case,

$$\theta_{12} = (s/7\xi_{12})\, \partial\xi_{12}/\partial s = \left[(r^5 + r^6)s + 2r^6 s^2\right]/7\xi_{12},$$

$$\alpha_{12} = \left[(5r^5 + 6r^6)s + 6r^6 s^2\right]/7\xi_{12}. \tag{31}$$

In some cases (for example, $R = 2$), in numerical calculations, it is probably simpler to differentiate the ξ_{ij} numerically rather than analytically.

When $R = 1$, the above formalism is unnecessary. Only the site occupied by a ligand molecule is converted to state 2 (there is no 'umbrella' effect). Thus $\theta = \alpha$. The sites in a chain of units can be divided into two independent classes: groups of five sites in the middle of units; and groups of two sites at the boundary between two units (one from each unit). These groups bind ligand molecules independently of each other. In fact, in the first kind of group, the five sites bind ligand molecules independently of each other. For one of these sites,

$$\theta \equiv \theta'' = rs/(1 + rs). \tag{32}$$

For a boundary pair of sites, the grand partition function is

$$\xi = x_{11} + 2x_{12}rs + x_{11}r^2s^2, \tag{33}$$

where we have used $x_{11} = x_{22}$ and $x_{12} = x_{21}$. For one of these sites, then,

$$\theta \equiv \theta''' = rs(1 + yrs)/(y + 2rs + yr^2s^2). \tag{34}$$

For the complete chain we have simply

$$\theta = \tfrac{5}{7}\theta'' + \tfrac{2}{7}\theta'''. \tag{35}$$

Note that r and s occur only as the product rs (which is consistent with $\theta = \alpha$).

Numerical examples. We have already discussed, at the end of section 2, the curve $R = 7$, $Y = 20$ in fig. 3. The other parameter used for this curve is $L = 64.3 = r^{-7}$, or $r = 0.5517$. This curve fits a set of strongly cooperative experimental data [1] very well, as already mentioned in section 2. We take this as our reference curve and compare the other curves in fig. 3 with it.

Let us consider first the practical question of whether the reference curve, with its strong cooperativity, can be essentially reproduced if we use values of R smaller than $R = 7$. We take $r = 0.5517$ (this fixes the position of the transition on the s axis, $s = s'$) in all the cases discussed below, but allow variations in Y. The first comment to make is that, in the $R = 6$ case, if we take $Y = 20$ we get a $\theta(s)$ curve (not shown) that is essentially the same (on the scale of fig. 3) as the reference $R = 7$ curve. This is not surprising in view of eqs. (26): when $R = 7$, ξ_{22} contains 127 out of 128 terms; when $R = 6$, the only difference is that two terms (with r^6 instead of r^7) are removed from ξ_{22} and assigned to ξ_{12} and ξ_{21} (these terms correspond to one molecule bound on either end-site of a unit). When $R = 5$, we find (curve not shown) that $Y = 20$ does not give quite enough cooperativity to match the reference curve but that a value $Y \approx 40$ practically reproduces this curve. $Y = 40$ is not at all an extreme cooperativity parameter; it corresponds to only $w_{11} - w_{12} = -1.84\ kT$. Thus we conclude that, at least by this test, the choices $R = 6$ and $R = 5$ cannot be ruled out as alternatives to $R = 7$. However, still smaller values of R (see below) *can* be excluded. Simply because the equilibrium and kinetic equations for $R = 7$ are less complicated than those for $R = 6$ and $R = 5$, and in the absence of any evidence to the contrary, we have adopted the $R = 7$ model as our current working hypothesis for the tropomyosin–troponin–actin system.

The $R = 4$, $Y = 20$ curve is included in fig. 3. The degree of cooperativity is too small (as expected in view of the $R = 5$ case). In fact, we have to increase Y virtually to $Y = \infty$ in order to get the same midslope as in the reference curve: the $R = 4$, $Y = \infty$ curve is shown as a dashed line in the figure. This curve could easily be shifted horizontally by adjusting the value of r but the unrealistic $Y = \infty$ requirement excludes $R = 4$ as a serious model for Tm–Tn. Smaller values than $R = 4$ are then of course also excluded for this purpose. However, for comparison, curves for $R = 1$ and $R = 2$, with $Y = 20$ and $Y = \infty$, are included in fig. 3.

As we have just seen (solid curves in fig. 3), the degree of cooperativity decreases with decreasing R, if we hold Y and r constant. There are two reasons for this. The first is that the range and magnitude of the cooperative 'umbrella' effect, discussed above in connection with fig. 4, are determined by R. This property in pure form (unmodified by unit end effects and nearest-neighbor interactions at unit boundaries) is studied in section 4. The second reason why cooperativity decreases as R decreases is that the correlation between the states of successive units in the chain, owing to interactions at the boundaries between units, is propagated optimally along the chain only when $R = 7$. In this case each unit is fully in one state or the other; one end of a unit knows what the other end is doing, so to speak. In this respect, this is a standard one-dimensional Ising system [see eqs. (7) and (8)]. Both when $R < 7$, and the more so the smaller R, the states of the two end sites of a unit may differ (leading to terms in ξ_{12} and ξ_{21}). This has the effect of attenuating the propagation of the correlation referred to above.

The attenuation effect just mentioned is seen quite clearly when we consider the limit $Y \to \infty$. The dashed curve for $R = 7$ in fig. 3 has $\theta = 0$ ($p_2 = 0$) for $s < 0.817$ and $\theta = \theta_2$ ($p_2 = 1$) for $s > 0.817$, with a discontinuous vertical jump in θ at $s = s' = 0.817$. However, even in the limit $Y \to \infty$, the corresponding jump is not quite vertical for $R = 6$ and $R = 5$ (not shown); there is some rounding off at the two ends of the jump and a finite slope at $s = s'$. The $R = 4$, $Y = \infty$ curve is included in fig. 3, as are pieces of the $Y = \infty$ curves for $R = 1$ and $R = 2$. When $R = 1$, attenuation is complete and there is no propagation at all of boundary interaction effects; the boundaries are all independent of each other.

We can illustrate the role of mixed units (terms in ξ_{12} and ξ_{21}), referred to above, with the following numerical example. In the $R = 2$, $Y = \infty$ case, at $s = 0.7$, we calculate

$$\theta = 0.149, \qquad \alpha = 0.310,$$
$$p_{11} = 0.619, \qquad p_{12} = p_{21} = 0.167, \qquad p_{22} = 0.047. \tag{36}$$

There are significant amounts (the p_{ij}) of all four kinds of units, despite the fact that $Y \to \infty$ requires that essentially no *boundaries* be of types 12 or 21. This can be accomplished, however, by combining the three phases A, B, and C shown in fig. 5, in none of which are there any 12 or 21 boundaries (also, the phases can be mixed without creating any such boundaries). The entire, smooth $R = 2$, $Y = \infty$ curve can be accounted for by varying the proportions of these three phases. In contrast, the discontinuous $R = 7$, $Y = \infty$ curve uses only phase B for $s < 0.817$ and only phase C for $s > 0.817$ (mixing these two phases necessarily creates 12 and 21 boundaries).

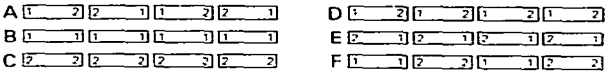

Fig. 5. Possible phases with extreme cooperativity: $Y = \infty$ (A, B, C); and $Y = 0$ (D, E, F). A rectangle represents a unit and the numbers (1 or 2) indicate the states of the end sites. See text.

Some of the above comments can be expressed analytically by examining eqs. (22)–(24) in the limit $Y \to \infty$. We find

$$p_{11} = [b_1 \mathscr{X}_4 + (b_1 - b_2)b_1]/(b_1 + b_2 + \mathscr{X}_4)\mathscr{X}_4, \tag{37}$$

$$p_{22} = [b_2 \mathscr{X}_4 + (b_2 - b_1)b_2]/(b_1 + b_2 + \mathscr{X}_4)\mathscr{X}_4, \tag{38}$$

$$p_{12} = p_{21} = 2/(b_1 + b_2 + \mathscr{X}_4)\mathscr{X}_4, \tag{39}$$

where

$$\mathscr{X}_4 = [(b_1 - b_2)^2 + 4]^{1/2}.$$

Eqs. (37) – (39) determine the relative amounts of the phases A, B, and C (fig. 5). Phase A makes a significant contribution whenever $\xi_{12} = \xi_{21}$ is finite. If $\xi_{12} = \xi_{21} \to 0$, as in the $R = 7$ case, b_1 and b_2 become infinite, $p_{12} = p_{21} \to 0$, and

$$p_{11} \to 1, \quad p_{22} \to 0, \quad \text{if } b_1 > b_2 \quad \text{(phase B)},$$
$$p_{11} \to 0, \quad p_{22} \to 1, \quad \text{if } b_2 > b_1 \quad \text{(phase C)}. \tag{40}$$

Fig. 6 reproduces the $\theta(s)$ curves for $r = 0.5517$, $Y = 20$ and $R = 1$, 2, 4, and 7 from fig. 3, but also includes the corresponding $\alpha(s)$ curves ($\theta = \alpha$ for

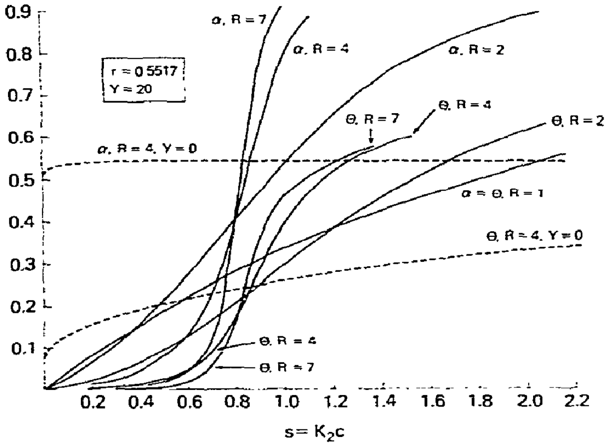

Fig. 6. Solid curves compare $\alpha(s)$ with $\theta(s)$ for $r = 0.5517$, $Y = 20$, and several values of the range R. Dashed curves give $\theta(s)$ and $\alpha(s)$ for $R = 4$ and $Y = 0$. See text.

$R = 1$), for comparison. Necessarily, because of the 'umbrella', $\alpha(s) > \theta(s)$ when $R > 1$.

For completeness, we mention the $Y \to 0$ case briefly. In this case, boundaries can be of types 12 or 21 but not 11 or 22. The three phases D, E, and F in fig. 5 are acceptable for this purpose; also these phases can be mixed without creating 11 or 22 boundaries. Necessarily $p_{11} = p_{22}$ (phase F), as well as $p_{12} = p_{21}$ (symmetry). When $\xi_{12} = 0$, as in the two-state Ising problem ($R = 7$, section 2), only phase F is possible (extreme negative cooperativity; an 'antiferromagnet'). Quantitatively, from eqs. (22)–(24), when $Y \to 0$,

$$p_{11} = p_{22} = (b_1 b_2)^{1/2}/2\left[1 + (b_1 b_2)^{1/2}\right], \tag{41}$$

$$p_{12} = p_{21} = 1/2\left[1 + (b_1 b_2)^{1/2}\right]. \tag{42}$$

When $\xi_{12} = 0$, b_1 and b_2 are infinite, $p_{11} = p_{22} = 1/2$, and $p_{12} = p_{21} = 0$.

Fig. 6 includes $\theta(s)$ and $\alpha(s)$ for $Y = 0$, $R = 4$, $r = 0.5517$ (dashed curves). Note that the two θ curves ($Y = 20$ and $Y = 0$) for $R = 4$ cross at the same s value ($s = s' = 0.86$) as do the two α curves for $R = 4$ [see the discussion above eq. (25)]. The same is true of the two θ curves ($Y = 20$ and $Y = \infty$) for $R = 4$ in fig. 3.

4. 'Umbrella' models for a simple infinite chain

We consider here the special case of section 3, $m = \infty$. That is, we have a very long chain of identical binding sites, with a parallel very long polymer molecule that blocks the binding sites, as above (state 1). In order to bind, an isolated ligand molecule must push the polymer away from the sites (into state 2) over a range $R' = 1, 2, 3, \ldots$ (fig. 7A). The relation of R' to R, above, is $R' = 2R - 1$. Note that we include half-integer values of R here, e.g. $R' = 2$ and $R = 1/2$ (this could have been done, as well, in section 3). There are no boundaries between units, as above. Therefore there are no neighbor interactions at boundaries. Direct nearest-neighbor interactions between bound ligand molecules can easily be included in the treatment below but for clarity and simplicity we omit these interactions (as we did in sections 2 and 3). What is left is the pure 'umbrella' effect which itself amounts to a multiple neighbor interaction (see below).

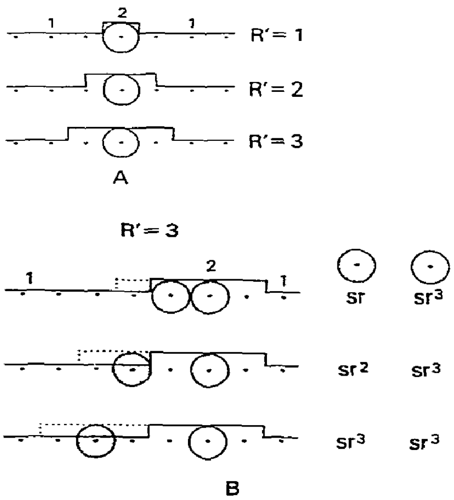

Fig. 7. (A) Definition of the range R', useful when $m = \infty$, and which is related to R by $R' = 2R - 1$. States 1 and 2 of sites are indicated. (B) Grand partition function factors, when $R' = 3$ and $m = \infty$, for first binding (open circles) and second binding (shaded circles) under 'umbrella' of first binding. The dotted lines show additional $1 \to 2$ state change needed on second binding, for first, second, and third neighbors.

Thermodynamically, this system (also sections 2 and 3) involves adsorption in which the adsorbed molecules perturb the adsorbent. Though more complicated than adsorption on an inert adsorbent, this is a very common situation [5,6]. There may be a number of real one-dimensional binding systems that belong formally to the class of models of the present section (in this sense, section 3 is clearly more specialized).

The one-dimensional models of this section can be generalized to two dimensions in obvious ways (depending on the lattice used). The term 'umbrella' becomes then even more appropriate.

Let us introduce the problem by considering details for the case $R' = 3$. We use the s and $r(\leqslant 1)$ notation of section 3, with the same definitions (the x_{ij}, Y and y do not appear here). Binding of one ligand molecule on an isolated site introduces a term sr^3 in the grand partition function (open circle, fig. 7B). The factor r^3 arises from the necessity of 'lifting' polymer over three sites into state 2. Binding of a subsequent molecule (shaded circle) onto a nearest-neighbor site (top row of fig. 7B) is then represented by sr (instead of sr^3), because polymer over only one site (dotted line) need be lifted. There is a 'saving' of a factor $r^2 \leqslant 1$, which is equivalent to a nearest-neighbor interaction parameter of $r^{-2} \geqslant 1$. That is, the grand partition function term for the *pair* of molecules in the top row of fig. 7B can be regarded as $(sr^3)^2 r^{-2}$. In similar fashion, binding on a second-neighbor site (middle row in fig. 7B), if the intervening site is empty, is associated with an effective interaction parameter r^{-1}, and on a third- or higher-neighbor site (bottom row) with $r^0 = 1$.

Incidentally, throughout this paper we have been interested in systems with $r \leqslant 1$. The opposite case, with $r \geqslant 1$ (i.e. subsequent binding on neighboring sites, under the 'umbrella', is more difficult rather than easier), is of course also possible.

If we examine the equivalent of fig. 7B for arbitrary R', we find that the effective interaction parameter for nth neighbor binding is simply $r^{n-R'}$, with $1 \leqslant n \leqslant R'$. For $n \geqslant R'$, the parameter is $r^0 = 1$. Thus our problem is mathematically equivalent to a one-dimensional Ising problem with an intrinsic (isolated site) binding factor $sr^{R'}$, and with interaction parameters $r^{1-R'}$, $r^{2-R'}, \ldots, 1$ for first, second,..., R'th neighbors. However, it must be emphasized that the factors for second- and higher-neighbor interactions apply only if the intervening sites are all empty. Thus, three consecutive occupied sites, with empty sites on both sides, count as two nearest-neighbor pairs and *zero* second-neighbor pairs.

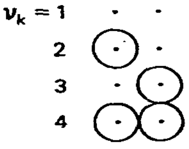

Fig. 8. Definition of states for 2-site mathematical unit, when $m = \infty$. ν_k refers to state of kth unit in chain.

We return now to details for the case $R' = 3$. In applying the matrix method to this Ising problem, we have to consider a mathematical unit (not to be confused with the physical units in section 3) of two sites in order to include second-neighbor interactions between occupied sites as a first-neighbor interaction between these units [7]. Fig. 8 shows the numbering of the four possible states of the kth unit. The matrix analogous to eqs. (3) and (14) is then

$$\begin{pmatrix} 1 & 1 & 1 & 1 \\ sr^3 & sr^2 & sr^3 & sr^2 \\ sr^3 & sr & sr^2 & sr \\ s^2r^4 & s^{r}r^2 & s^2r^3 & s^2r^2 \end{pmatrix}. \tag{43}$$

The entries 22, 24, 33, and 43 include a factor r^{-1} for second-neighbor interactions between occupied sites; the entries 32, 34, 42, and 44 include a factor r^{-2} for first-neighbor interactions between occupied sites that are in successive units; and the entires 41, 42, 43, and 44 include a factor r^{-2} for first-neighbor interactions between occupied sites that are in the same unit.

The largest eigenvalue γ_m of this 4×4 matrix is the largest root of the cubic equation (one eigenvalue is zero)

$$\gamma^3 + a_2\gamma^2 + a_1\gamma + a_0 = 0, \tag{44}$$

where

$$a_2 = -(1 + A_2), \qquad a_1 = A_2 - sr^3(2 + sr),$$
$$a_0 = -(1 - r)^2 r^4 s^2, \qquad A_u \equiv r^u[(1 + s)^u - 1] \tag{45}$$

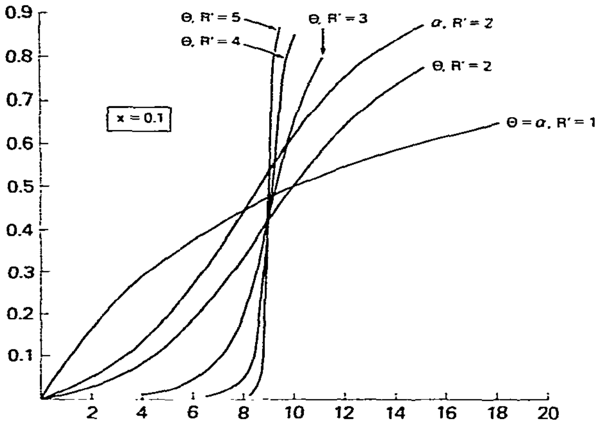

Fig. 9. $\theta(s)$ and $\alpha(s)$ for $r=0.1$, $m=\infty$ and various values of the range R'. See text for details.

and u refers to the number of sites in a unit (here, $u=2$; other values of u are used below). The grand partition function Ξ of the system is given by $\ln \Xi = M \times \ln \gamma_m$, where M is the number of u-site units in the chain. Then

$$\theta = u^{-1}\, \partial \ln \gamma_m / \partial \ln s, \tag{46}$$

$$\alpha = u^{-1}\, \partial \ln \gamma_m / \partial \ln r, \tag{47}$$

where θ is the fraction of sites occupied by ligand molecules and α is the fraction of sites in state 2.

As an example, the curve labelled $R'=3$ in fig. 9 shows $\theta(s)$ from eq. (46) for $r=0.1$ (the free energy change per site for state $1 \rightarrow 2$ is 2.30 kT). The differentiation has been carried out numerically. The curve is strongly cooperative (recall that the effective nearest-neighbor parameter is $r^{-2} = 100$, and the effective second-neighbor parameter is $r^{-1} = 10$). The approximate location of the midpoint of the transition on the s axis can be established by the following argument (which is more accurate for larger R'; see below). Because $r \ll 1$, eq. (44) simplifies, approximately, to

$$\gamma^2 - (1 + A_2)\gamma + A_2 = 0. \tag{48}$$

This equation has roots $\gamma = 1$ and $\gamma = A_2$. For small s, $\gamma_m = 1$ is the larger eigenvalue; for large s, $\gamma_m = A_2$ is the larger eigenvalue. There is a *sharp* transition at $A_2 = 1$ and $s = s'$, where s' satisfies $A_2(s', r) = 1$ [eq. (45)]. That is, the two eigenvalues intersect at $s = s'$. When $r = 0.1$, $s' = 9.05$ (compare fig. 9). In this *approximation*, $\theta = 0$ for $s < s'$ (from $\gamma_m = 1$) and

$$\theta = s(1+s)/\left[(1+s)^2 - 1\right], \tag{49}$$

for $s > s'$ (from $\gamma_m = A_2$). Similarly, α jumps from $\alpha = 0$ to $\alpha = 1$ at $s = s'$. The approximation, with its sharp transition, becomes exact in the limit $r \rightarrow 0$. Recall that, in section 3, there is *not* a sharp transition in the limit $Y \rightarrow \infty$, if $R < m$.

We now consider other values of R'. When $R' = 1$, there is no umbrella effect. We have simple Langmuir binding:

$$\theta = \alpha = rs/(1 + rs). \tag{50}$$

When $R' = 2$, the intrinsic binding factor is sr^2 and there is a nearest-neighbor interaction parameter r^{-1}. The mathematical unit size is one site ($u = 1$); the matrix is 2×2, as in eq. (3). The characteristic equation is

$$\gamma^2 - (1 + rs)\gamma + rs - r^2 s = 0. \tag{51}$$

Note that $A_1 = rs$ [eq. (45)] and that eq. (51) reduces to eq. (48) if $r \ll 1$, but with A_1 in place of A_2. However, there is no need here to use the approximation. The larger eigenvalue of eq. (51) is given by

$$2\gamma_m = 1 + rs + \Lambda_5, \tag{52}$$

where

$$\Lambda_5 = \left[(1 - rs)^2 + 4r^2 s\right]^{1/2}.$$

This leads to

$$\theta = 2r^2 s / \Lambda_5 (1 - rs + \Lambda_5) \tag{53}$$

and

$$\alpha = \left[rs(rs - 1 + \Lambda_5) + 4r^2 s\right] / \Lambda_5 (1 + rs + \Lambda_5). \tag{54}$$

Eq. (53) is essentially the same as eq. (7) while eq. (54) is a combination of eqs. (14) and (15) of ref. [1]. The midpoint of the transition ($\theta = 1/2$) occurs at $s' = r^{-1}$ for any r. Fig. 9 includes $\theta(s) =$

$\alpha(s)$ for $R' = 1$ [eq. (50)], and $\theta(s)$ and $\alpha(s)$ for $R' = 2$, using $r = 0.1$ in all cases.

When $R' = 4$, the intrinsic binding factor is sr^4 and the effective interaction parameters for first, second, and third neighbors are r^{-3}, r^{-2}, and r^{-1}, respectively. Third neighbors can still be accommodated by a unit of two sites ($u = 2$). The 4×4 matrix again has one eigenvalue that is zero. The cubic equation in γ is in this case

$$\gamma_3 + a_2\gamma^2 + a_1\gamma + a_0 = 0, \tag{55}$$

where

$$a_2 = -(1 + A_2), \qquad a_1 = A_2 - sr^4(2 + sr),$$
$$a_0 = -(1 - r)^2 r^5 s^2.$$

Fig. 9 includes $\theta(s)$ for $r = 0.1$, calculated numerically from eqs. (46) and (55). The approximation, eq. (48), also applies here, when $r \ll 1$, and can be seen from eqs. (44) and (55) to be more accurate than in the $R' = 3$ case (as expected, because the $R' = 4$ curve in fig. 9 is steeper than the $R' = 3$ curve).

When $R' = 5$, the intrinsic binding factor is sr^5 and the effective interaction parameters are $r^{-4}, \ldots, r^{-1}$, the last being for fourth neighbors. To take care of fourth neighbors, we need three sites in a unit ($u = 3$). In fact, $u = 3$ also suffices for fifth neighbors ($R' = 6$). Thus, there are $2^u = 8$ states for a unit, and we have to start with an 8×8 matrix. However, the characteristic equation is again a cubic:

$$\gamma^3 + a_2\gamma^2 + a_1\gamma + a_0 = 0, \tag{56}$$

where

$$a_2 = -(1 + A_3),$$
$$a_1 = A_3 - sr^5(3 + 2sr + sr^2 + s^2r^2),$$
$$a_0 = -s^2r^6(1 - r)^2[r^2 + 4r + 1 + sr(r + 2)].$$

The $\theta(s)$ curve for $r = 0.1$, obtained from eqs. (46) and (56), is included in fig. 9. This curve is quite steep because $r^{-4}, r^{-3}, \ldots$ are large. Eq. (48), with A_3 in place of A_2, is a very good approximation to eq. (56) when $r \ll 1$. The transition occurs when $A_3(s', r) = 1$, or $s' = 9.003$ for $r = 0.1$. Note, from eq. (45), that $A_u = 1$ gives $s' \rightarrow r^{-1} - 1$ if r is small and u is large.

When $R' = 6$, the only change is to replace r^5 and r^6 in eq. (56) by r^6 and r^7, respectively. Eqs. (44) and (55), for $R' = 3$ and $R' = 4$, are related in similar fashion. In fact, it is easy to see that the $R' = 1$ and $R' = 2$ quadratic equations follow the same pattern. The very steep $\theta(s)$ curve for $R' = 6$ is not included in fig. 9.

We have not carried out detailed calculations beyond $R' = 6$. However, in view of its physical interpretation, the approximation $\gamma_m = 1$, A_u is presumably applicable (when $r \ll 1$) for arbitrarily large R'. $\gamma_m = 1$ implies $\theta = \alpha = 0$ for $s < s'$. $\gamma_m = A_u$ implies

$$\theta = s(1 + s)^{u-1}/[(1 + s)^u - 1], \qquad \alpha = 1, \tag{57}$$

for $s < s'$, where $s'(r)$ is determined by $A_u(s', r) = 1$ [eq. (45)]. That is, at the transition, all sites in the chain switch from state 1 to state 2 and every mathematical unit has at least one ligand molecule in it. For the latter property to *guarantee* the former, it is easy to see that the values of u corresponding to $R' = 2, 3, 4, 5, 6, \ldots$ cannot be larger than $1, 2, 2, 3, 3, \ldots$. This is the same relation between R' values and u values already forced on us by the matrix method.

Discussion. If 'free' binding under a complete umbrella is characterized by $s = K_2c$, then initial binding by isolated ligand molecules that have to lift the adjacent polymer into state 2, over a range of R' sites, is characterized by $sr^{R'}$. With $r < 1$, the initial binding is thus inhibited (the effective binding constant is $r^{R'}K_2$). But when c is large enough (s near s'), binding will occur cooperatively, possibly quite suddenly (small r, large R') because subsequent binding can occur relatively easily under the 'umbrellas' set up in the difficult initial binding. In effect, even though the ligand molecules do not attract each other directly, there is a favorable first-neighbor interaction parameter $r^{1-R'}$, a second neighbor parameter $r^{2-R'}$, etc.

The model in section 3 is more complicated in two ways. First, there are physical units of m sites, e.g., $m = 7$ in Tm–Tn, whereas here $m = \infty$. Thus there are significant end effects when m is small (the umbrella influence operates only *within* individual units). Second, there are nearest-neighbor interactions at the boundaries between physical units (parameter Y).

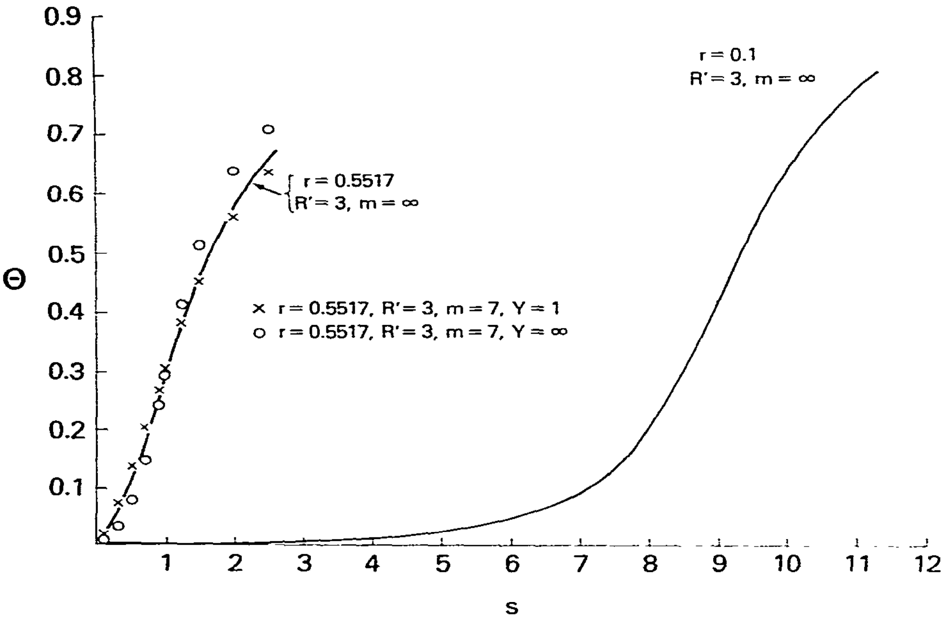

Fig. 10. The two solid curves show the effect of changing the value of r when $m = \infty$. The circles and crosses, compared to the left-hand solid curve, show the effect of finite units ($m = \infty \to m = 7$), and for finite units, the effect of $Y = 1 \to Y = \infty$. See text for details.

The two solid curves in fig. 10 refer to $R' = 3$, $m = \infty$ and show the significant effect of increasing r from 0.1 to 0.5517 (the $r = 0.1$ curve is also in fig. 9). Appreciable binding occurs at much smaller s when r is larger. The crosses near the $r = 0.5517$ solid curve are for the case $R' = 3$, $m = 7$, using this r value and taking $Y = 1$ (no differential boundary interactions). Thus the crosses differ from the solid curve only because of the end effects ($R' = 3$, $m = 7$) referred to in the above paragraph. As expected, the cooperativity is a little stronger for $m = \infty$ than for $m = 7$. The cooperativity order is reversed, however, if we use $Y = \infty$ instead of $Y = 1$ for the boundary interactions (circles in fig. 9). Part of the $Y = \infty$ curve appears as dashed lines in fig. 3 (labelled $R = 2$, $Y = \infty$).

Appendix

We make a few comments on the simplest possible examples of the models treated in section 3.

When $R = m$, for any m, the treatment in section 2 applies with

$$\xi_1 = 1, \qquad \xi_2 = r^m\left[(1+s)^m - 1\right]. \tag{58}$$

When $m = 1$, the only case is $R = 1$. This is the conventional Ising problem with nearest-neighbor interactions between bound ligands. Section 2 applies with $\xi_1 = 1$, $\xi_2 = rs$ [see eq. (58)].

When $m = 2$ and $R = 1$, we have a collection of independent pairs of sites (boundaries) with interactions within the pairs. Eqs. (33) and (34) are applicable. The $m = 2$, $R = 2$ is covered by eq. (58).

When $m = 3$ and $R = 1$, we have a modification of eq. (35):

$$\theta = \tfrac{2}{3}\theta'' + \tfrac{1}{3}\theta'''. \tag{59}$$

When $m = 3$ and $R = 3$, eq. (58) applies.

The case $m = 3$, $R = 2$ is of some interest because it is the simplest case with partial attenuation of boundary correlation propagation. The general equations of section 3 apply but with

$$\xi_{11} = 1, \qquad \xi_{12} = \xi_{21} = r^2 s,$$

$$\xi_{22} = r^3\left[(1+s)^3 - 1 - 2s\right], \tag{60}$$

$$\theta_{11} = \alpha_{11} = 0, \qquad \theta_{12} = 1/3,$$

$$\alpha_{12} = 2/3, \qquad \alpha_{22} = 1,$$

$$\theta_{22} = (1 + 6s + 3s^2)/3(1 + 3s + s^2). \tag{61}$$

Because $\xi_{12} = \xi_{21} \neq 0$, when $Y = \infty$ there is not a sharp transition at the midpoint $s = s'$, where s' is determined by

$$r^{-3} = (1 + s')^3 - 1 - 2s'. \tag{62}$$

For example, if $r = 0.5517$ (as in sections 2 and 3), $s' = 1.09$ and $\theta = 0.338$ at $s = s'$. When $Y = \infty$, $\theta = 0.248$ at $s = 1.0$ and $\theta = 0.441$ at $s = 1.2$. With $Y = 20$, these values are $\theta = 0.282$ and 0.403, respectively. The $\theta(s)$ curve for $Y = \infty$ is somewhat steeper at $s = s'$ than for $Y = 20$, but it is not infinitely steep.

References

[1] T.L. Hill, E. Eisenberg and L. Greene, Proc. Natl. Acad. Sci. U.S. 77 (1980) 3186.

[2] L. Greene and E. Eisenberg, to be published.

[3] T.L. Hill, E. Eisenberg and L. Greene, to be published.

[4] G.N. Phillips Jr., J.P. Fillers and C. Cohen, Biophys. J. 32 (1980) 485.

[5] T.L. Hill, J. Chem. Phys. 18 (1950) 246.

[6] T.L. Hill, Thermodynamics of small systems, part II (Benjamin, New York, 1964) ch. 7.

[7] T.L. Hill, J. Polymer Sci. 23 (1957) 549.