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Subunit neighbor interactions in enzyme kinetics: Half-of-the-sites reactivity in a dimer

(steady state/Michaelis–Menten kinetics/negative cooperativity/enhanced reactivity)

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ABSTRACT **We consider an isologous enzyme dimer in which the subunits, if operating independently, would obey Michaelis–Menten kinetics. However, because of neighbor interactions, the rate constants of the kinetic cycle in either subunit depend on the state (E or ES) of the other subunit. The steady-state behavior of this dimer system, with interactions, is investigated. In what is probably the most important special case, ES·ES is destabilized considerably by the neighbor interaction compared to E·ES. This leads to half-of-the-sites reactivity (one subunit is in state ES; the other subunit cycles between E and ES), negative cooperativity, and a considerable enhancement of enzyme activity relative to the activity of independent subunits.**

Our object in this paper is to study the theoretical steady-state properties of an isologous dimer composed of two identical enzyme subunits that interact with each other. The interactions are such that the kinetic rate constants of either one of the subunits depend on the state of the other subunit. The novel feature of our analysis is that we relate explicitly the rate constants of the steady-state cycle to the intersubunit interactions, without the usual quasi-equilibrium assumption about the state of the subunits at steady state.

As will be seen from our examples, we anticipate that these results will be particularly useful in future studies of negative cooperativity and half-of-the-sites reactivity (1). However, the method is much more general than this; it can be applied to multi-enzyme complexes or to one- or two-dimensional enzyme lattices, with interacting components, of arbitrary size and with arbitrary kinetic cycles (2–6). Thus, to some extent, the present paper can serve as an illustrative example of interacting enzyme systems. A quite different example has been treated recently: the cooperative regulated tropomyosin–troponin–actin–myosin ATPase one-dimensional lattice system (7).

The model and its general steady-state properties

To reduce the number of parameters on which the final results depend, we treat the important special case (Michaelis–Menten) shown in Fig. 1*a*. In this figure we give the rate constants for each of the subunits *when they function independently of each other* (the "unperturbed" enzyme). The enzyme E catalyzes S → P, where EP is a transient intermediate. Thus we are concerned, in effect, with a two-state enzymatic cycle (the two states are E, or 1, and ES, or 2, as in Fig. 1). The rate constant inverse to k_3, in the two state cycle, is k_{-3}. We assume, however, that k_{-3} is very small; hence it is omitted from the figures (though it appears below in the text).

All three rate constants in Fig. 1*a* are first-order; k_1 is pseudo-first-order, and is proportional to [S], the concentration of free substrate.

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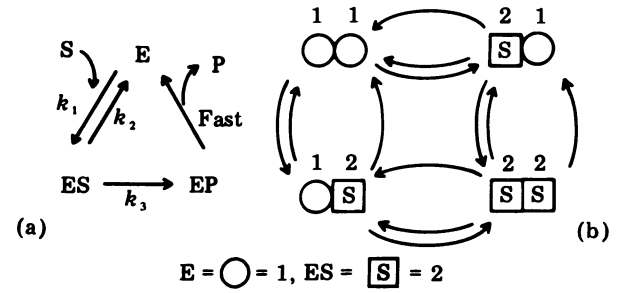

FIG. 1. (*a*) Michaelis–Menten cycle and rate constants used for an unperturbed enzyme subunit. (*b*) Corresponding kinetic diagram needed for a dimer of these subunits (omitting state EP). Interactions between subunits are associated with the different conformations, ○ and □.

The steady-state rate of production of P (the reaction velocity or flux), per enzyme subunit, is

$$V_o = k_1k_3/(k_1 + k_2 + k_3). \quad [1]$$

The subscript here on V refers to the unperturbed enzyme. The maximum value of V_o (when $k_1 \to \infty$) is $V_{om} = k_3$.

The thermodynamic force X driving the reaction S → P is (8)

$$e^{X/RT} = k_1k_3/k_2k_{-3}, \quad X = \mu_S - \mu_P, \quad [2]$$

where μ_S is the chemical potential of S in solution at [S], etc.

In the dimer, each subunit makes use of the same two-state cycle as in Fig. 1*a*, as indicated schematically by the arrows in Fig. 1*b*. However, there are four states in the kinetic diagram of the dimer; also, the rate constants are different. That is, we assume that when the two subunits are brought together to form the dimer, interactions between the subunits lead to rate constants of the enzymatic cycle of either of the subunits that depend on the state (1 or 2) of the other subunit. The subunit interactions presumably originate in a conformation change in the enzyme molecule (schematically ○ → □ in Fig. 1*b*) that is an immediate consequence of the binding of substrate S (however, the argument below does not depend on this interpretation).

The free energy of interaction of two subunits in the dimer in states i and j ($i,j = 1,2$), relative to infinite separation of the subunits in the solution, is denoted by w_{ij}. Also, we introduce the notation $y_{ij} \equiv e^{-w_{ij}/kT}$. Because of symmetry, $w_{12} = w_{21}$ and $y_{12} = y_{21}$.

Dependence of Rate Constants on Interactions. Because of symmetry, there are really only two different sets of (three) rate constants in Fig. 1*b*. The distinction here is whether the neighboring subunit of the "active" subunit is in state 1 or in state 2. In the former case, k_1, k_2, and k_3 become k_1', k_2', and k_3', because of interactions with the neighbor in state 1, while in the latter case k_1, k_2, and k_3 become k_1'', k_2'', and k_3''.

Consider first k_1' and k_2' in Fig. 2*a*. From simple equilibrium considerations,

$$k_1'/k_2' = (k_1/k_2)(y_{12}/y_{11}) \text{ or } K_b' = K_b(y_{12}/y_{11}). \quad [3]$$

Here, k_1/k_2 is equal to K_b[S], where K_b is the unperturbed equilibrium binding constant of S to E, whereas k_1'/k_2' is equal to K_b'[S], where K_b' is the binding constant of S to E in the presence of a neighbor in state 1 (E). The factor y_{12}/y_{11} contains the effects of interactions. In general, the interactions will also influence k_1' and k_2' *separately*, but necessarily in such a way as to be consistent with Eq. 3. We can represent this formally by (2)

$$k_1' = k_1(y_{12}/y_{11})^f, \quad k_2' = k_2(y_{11}/y_{12})^{1-f}, \quad [4]$$

where f is a constant that usually, but not necessarily, would have a value between 0 and 1. Actually, if we assume simple diffusion-controlled binding, we should take $f = 0$ (as is done below). This puts all of the equilibrium interaction effect into k_2'.

In the same way we can write

$$k_3' = k_3(y_{11}/y_{12})^g, \quad k_{-3}' = k_{-3}(y_{12}/y_{11})^{1-g} \quad [5]$$

for the other transition pair of the two-state cycle in Fig. 2*a*. Here g is a different kinetic constant that relates to ES → EP [in general, each transition pair in a cycle has its own constant of this type (2)]. Unlike f, above, $g = 0$ would not be expected. We shall not use k_{-3}' except to note that $k_1'k_3'/k_2'k_{-3}'$ is equal to $e^{X/RT}$, as in Eq. 2. This is to be expected because interactions with the neighboring enzyme subunit, whatever its state, cannot change the overall thermodynamic force X driving the cycle (8); X is a property only of S and P in solution and does not depend on the nature of the enzyme subunit that is catalyzing the transformation S → P.

Similarly, when the perturbing neighbor is in state 2 (Fig. 2*b*), the corresponding relations are

$$k_1'' = k_1(y_{22}/y_{12})^f, \quad k_2'' = k_2(y_{12}/y_{22})^{1-f} \quad [6]$$

$$k_3'' = k_3(y_{12}/y_{22})^g, \quad k_{-3}'' = k_{-3}(y_{22}/y_{12})^{1-g}. \quad [7]$$

We use the same f and g here as in Eqs. 4 and 5 because only the perturbing environment has changed, not the nature of the reactions [this assumption (2), though very plausible, is not essential].

Fig. 3 shows the simplified three-state kinetic diagram we actually work with from this point on. Because, by symmetry, states 12 and 21 in Fig. 1*b* are equivalent, we treat 12 + 21 as a single state in Fig. 3. Also, we take $f = 0$ (see above) and introduce the simplifying notation

$$y \equiv y_{11}/y_{12}, \quad z \equiv y_{12}/y_{22}. \quad [8]$$

The rate constants in Fig. 3 follow from Eqs. 4–8 and symmetry considerations.

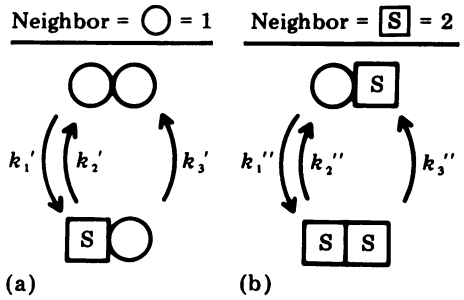

FIG. 2. Modified rate constant notation for the two-state cycle when the neighboring subunit is in: state 1 or E (*a*); or state 2 or ES (*b*).

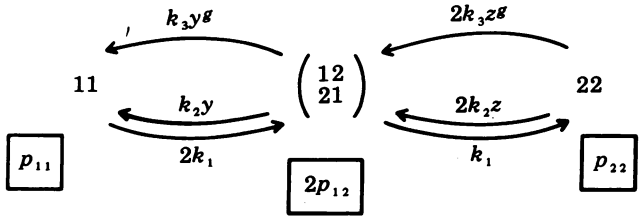

FIG. 3. Explicit kinetic diagram and rate constants used for the dimer, including neighbor-interaction effects on the rate constants. See text for details.

The parameter y expresses the intermolecular interaction contribution to the relative thermodynamic stability of the dimer in state 11 compared to 12 or 21, and, similarly, z refers to the stability of 12 or 21 compared to 22. For example, if $w_{11} < w_{12}$, state 11 is more stable than 12 (insofar as intermolecular interactions are concerned), and $y > 1$. For example, if $y = 100$, $w_{12} - w_{11} = 2.73$ kcal mol^{-1} (at 25°C) (1 kcal = 4.184 kJ).

It should be noted that $y/z = y_{11}y_{22}/y_{12}^2$ is the well-known interaction parameter (9) that appears in the theoretical treatment of equilibrium cooperative systems at the molecular level. When $y/z > 1$, one obtains positive cooperativity; and when $y/z < 1$, one encounters negative cooperativity (9). Clearly, y/z is related to the relative stability (see above) of 11 + 22 compared to 12 + 21 (9).

General Steady-State Properties of the Dimer. We now derive the steady-state properties of the model in Fig. 3. Transient properties could also be considered, but we omit this subject in the present analysis. The parameters are k_2, k_3, y, z, g, and $k_1 \sim$ [S]. The steady-state probabilities of the three states are designated as shown in the boxes in Fig. 3, with $p_{11} + 2p_{12} + p_{22} = 1$. The p_{ij} are easy to obtain because the linear arrangement of states in the diagram leads to simulated "detailed balance" (4) relations:

$$2k_1p_{11} = F_y \cdot 2p_{12}, \quad k_1 \cdot 2p_{12} = 2F_zp_{22}, \quad [9]$$

where

$$F_y \equiv k_2y + k_3y^g, \quad F_z \equiv k_2z + k_3z^g. \quad [10]$$

Then we find

$$p_{11} = 1/\Sigma, \quad p_{12} = Y/\Sigma, \quad p_{22} = YZ/\Sigma, \quad [11]$$

where

$$Y \equiv k_1/F_y, \quad Z \equiv k_1/F_z, \quad \Sigma \equiv 1 + 2Y + YZ. \quad [12]$$

As can be seen from Fig. 3, the reaction velocity per subunit is

$$V = V_1 + V_2; \quad V_1 = k_3y^gp_{12}, \quad V_2 = k_3z^gp_{22}, \quad [13]$$

where V_1 is the contribution from 12 + 21 → 11 and V_2 is the contribution from 22 → 12 + 21. If V_2/V_1 is either very large compared to unity, or very small, "half-of-the-sites" reactivity is obtained (10). In the former case, only one subunit cycles at a time while the other is almost always in state 2; in the latter case, the noncycling subunit is usually in state 1.

More explicitly, the reaction velocity is given by

$$V = k_3Y(y^g + z^gZ)/\Sigma. \quad [14]$$

Because both Y and Z are proportional to k_1, when k_1 is very large, $V \to k_3z^g$. This is usually the maximum velocity V_m (see below). From Eqs. 11 and 13, an explicit expression for V_2/V_1 is

$$V_2/V_1 = k_1/(y/z)^gF_z. \quad [15]$$

Thus V_2/V_1 is always proportional to k_1 (or to [S]). Another

quantity of importance is V/V_o (from Eqs. 1 and 14). We are particularly interested in cases in which V/V_o is large compared to unity; in such cases the catalytic efficiency of the dimer is much higher than that of the separated subunits.

We designate the fractional extent of binding of S to E, in the dimer at steady state, by θ_{ss}. This is given by

$$\theta_{ss} = p_{12} + p_{22} = Y(1 + Z)/\Sigma. \quad [16]$$

The dependence of θ_{ss} on k_1 can be quite different than that of V/V_m (1, 11), as will be seen in the examples. For comparison, if we put $k_3 = 0$, we obtain the equilibrium extent of binding

$$\theta_{eq} = \lambda[(z/y)^{1/2} + \lambda]/[1 + 2\lambda(z/y)^{1/2} + \lambda^2], \quad [17]$$

where $\lambda \equiv k_1/k_2(yz)^{1/2}$. Here $\lambda \sim [S]$ is defined in such a way that $\theta_{eq} = 1/2$ at $\lambda = 1$. The steady-state θ_{ss} has this same dependence on λ, with k_2 replaced by $k_2 + k_3$ in the definition of λ, in the special case $g = 1$. But in general, when $g \neq 1$, θ_{ss} does not have this equilibrium form.

If $z > 1$ (the same comments can be made about y), it is apparent from Fig. 3 that the equilibrium binding constant of S to 12 or 21 to form 22 is reduced from the unperturbed value because of the larger dissociation rate constant $2k_2z$. This is a reflection of increased thermodynamic instability of 22 (w_{22}) relative to 12 or 21 (w_{12}). But it should be noted that this instability has at the same time an enhancing effect on the reaction velocity through the rate constant $2k_3z^g$.

On examining $\partial V/\partial k_1 = 0$, from Eq. 14, one finds that $V(k_1)$ passes through a maximum at *finite* k_1 if and only if $G > 2$, where, for convenience below, we define $G \equiv (y/z)^g$. Assuming g is positive, this means that such a maximum cannot occur in negative cooperativity cases ($y/z < 1$) but may occur if there is strong enough positive cooperativity (an example is considered below). The value of k_1 at the maximum is

$$k_1^{max} = \{F_y + F_y[1 + (F_z/F_y)G(G - 2)]^{1/2}\}/(G - 2). \quad [18]$$

In most of the examples studied below, $G < 2$ and V reaches its maximum, $V_m = k_3z^g$, at $k_1 = \infty$. Then, from Eq. 14,

$$V/V_m = Y(G + Z)/\Sigma. \quad [19]$$

The value of k_1 at which $V = V_m/2$ is found to be

$$k_1(1/2) = -F_z(G - 1) + [F_z^2(G - 1)^2 + F_yF_z]^{1/2}. \quad [20]$$

This k_1 can be used to calculate other properties at $V = V_m/2$.

The Special Case $z \gg y$. This is probably the most important special case: there is negative cooperativity, half-of-the-sites reactivity, and a large enhancement of enzymatic activity in the dimer, all as a result of intersubunit interactions.

We note first that $G = (y/z)^g$ is very small and that $F_z \gg F_y$. Then Eq. 20 gives $k_1(1/2) \approx 2F_z$. At $k_1 = k_1(1/2)$,

$$p_{11} \sim 1, \quad 2p_{12} = p_{22} \sim 4F_z/F_y.$$

Consequently p_{11} is very small except when $k_1 \ll k_1(1/2)$. That is, except at the very beginning of the binding of S to the dimer ($k_1 \to 0$), the important states are 12, 21, and 22. Over almost all of the significant range in k_1, k_1 is of order F_z. As a good approximation, then, we have the following simple properties for this system (using $\Sigma = 2Y + YZ$):

$$p_{12} = 1/(2 + Z), \quad p_{22} = Z/(2 + Z) \quad [21]$$

$$V/V_m = Z/(2 + Z), \quad V/V_o = z^gZ/(2 + Z) \quad [22]$$

$$V_2/V_1 = Z/G, \quad \theta_{ss} = (1 + Z)/(2 + Z), \quad [23]$$

where, it will be recalled, $Z = k_1/F_z \sim [S]$. In Eq. 22, V/V_m has essentially the conventional Michaelis–Menten form. As the reader can easily verify, these properties (except for V_2/V_1) can be derived directly as those of a dimer that uses only the right-hand half of the diagram in Fig. 3 (one subunit cycles while the other is in state 2, or ES).

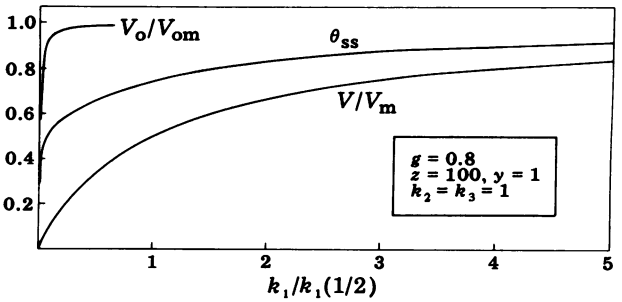

FIG. 4. Reaction velocity (V/V_m) and substrate saturation (θ_{ss}) as a function of substrate concentration (proportional to k_1), in an important special case (see box). The abscissa is chosen so that it has the value 1 when $V = V_m/2$. The upper-left curve (V_o/V_{om}) refers to the unperturbed enzyme, using the same abscissa scale as for the other curves. See text for details.

With z large and G small, V/V_o and V_2/V_1, above, are both large compared to unity. In fact, at $k_1 = k_1(1/2)$, where $Z = 2$, we have simply $V/V_o = z^g/2$ and $V_2/V_1 = 2(z/y)^g$. The apparent experimental correlation (1) between enhanced reactivity (V/V_o) and half-of-the-sites reactivity (V_2/V_1) may thus be attributable to large values of both z and z/y.

An explicit comparison of V_m and $k_1(1/2)$ with the values for the unperturbed enzyme is instructive. Here ($z \gg y$) we have

$$V_m = k_3z^g, \quad k_1(1/2) \approx 2(k_2z + k_3z^g), \quad [24]$$

whereas, for the unperturbed enzyme,

$$V_{om} = k_3, \quad k_1^o(1/2) = k_2 + k_3. \quad [25]$$

If $z \gg 1$, the dimer exhibits a much higher V_m than the independent (unperturbed) enzyme, but at the same time the value of k_1 required to reach $V = V_m/2$ is also much higher in the dimer with interacting subunits.

Finally, we note that V/V_m and θ_{ss} behave rather differently here. Half-saturation of the sites ($\theta_{ss} = 1/2$) is attained at very low k_1, before V/V_m reaches a significant value. Then saturation of the second site and V/V_m proceed together, according to $Z/(2 + Z)$.

The numerical case in the next section provides an explicit example of the above properties.

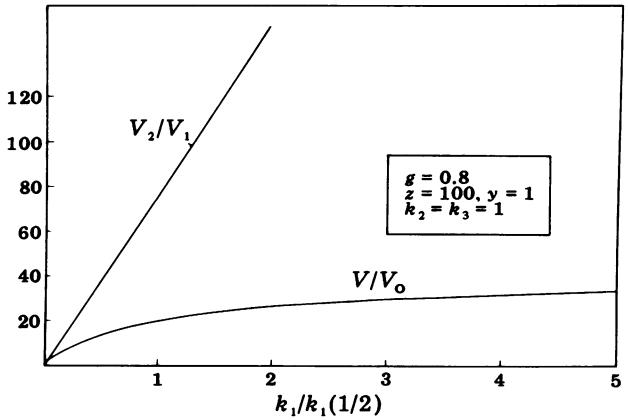

FIG. 5. Half-of-the-sites index (V_2/V_1) and dimer activity enhancement (V/V_o), plotted as in Fig. 4, and for the same case (see box).

Table 1. Effect of variation of g (at $V = V_m/2$)

g	V_2/V_1	V/V_o	θ_{ss}
0.30	6.01	2.02	0.713
0.50	18.10	5.05	0.736
0.65	38.1	10.06	0.742
0.80	77.9	20.05	0.746
0.90	124.6	31.7	0.747
1.00	198.5	50.3	0.748

The Special Case $z = y$. In this case, $y/z = 1$, though differential intermolecular interactions may be present (i.e., $y \neq 1, z \neq 1$), these interactions will not be apparent operationally because we have

$$F_y = F_z, \quad Y = Z = k_1/F_y, \quad k_1(1/2) = F_y \qquad [26]$$

$$V/V_m = \theta_{ss} = Y/(1 + Y) \qquad [27]$$

$$V_2/V_1 = Y, \quad \theta_{eq} = \lambda/(1 + \lambda). \qquad [28]$$

That is, the behavior is of the simple Michaelis–Menten type (12), with no distinction between V/V_m and θ_{ss}; the interactions between subunits do not result in cooperative behavior either in substrate binding or in catalytic activity.

Numerical calculations in a reference example

In this section we calculate various properties in a special case with plausible parameters. In the following section we shall then use this case as a standard or reference in a study of the effect on several properties at $k_1 = k_1(1/2)$ of varying one parameter at a time.

In the present example, we take $z = 100$, $y = 1$, $k_2 = k_3 = 1$, and $g = 0.8$. This corresponds to negative cooperativity. The magnitude chosen for k_2 and k_3 is unimportant; this merely sets the scale for k_1 and V, as can be seen from Eqs. **10, 12, and 14.** The choice $g = 0.8$ is rather arbitrary. For reference, we note that the physical significance of a value $g = 0$ would be that the interaction free energy $w_{22} - w_{12} > 0$ that destabilizes the bound state 22 or ES·ES (see the paragraph following Eq. **17**) is also fully present in the activated complex that is intermediate between the states ES·ES and ES·EP. In this case ($g = 0$), we would have $k_3'' = k_3 z^g = k_3$. If this interaction free energy *in the activated complex* were $(w_{22} - w_{12})/2$, that is, reduced by a factor of two, then $g = 0.5$, etc. Larger values of g (say, between 0.5 and 1.0) give larger values of V_2/V_1 and V/V_o (see the next section).

With the above parameters we find $k_1(1/2) = 273.6$ and $V_m = 39.8$. By contrast, in the unperturbed enzyme, $k_1°(1/2) = 2$ and $V_{om} = 1$. Fig. 4 shows plots of V_o/V_{om}, θ_{ss}, and V/V_m against $k_1/k_1(1/2) \sim [S]$, as calculated from Eqs. **1, 16, and 19,** using $k_1(1/2) = 273.6$ in all curves. The behavior of V/V_m and θ_{ss} is as predicted by the approximate Eqs. **22** and **23:** both V/V_m and $\theta_{ss} - 1/2$ are close to Michaelian. But the full θ_{ss} curve shows strong negative cooperativity. Both θ_{ss} and V_o/V_{om} rise much more quickly than V/V_m, which is delayed by the interactions ($z = 100$). Incidentally, θ_{eq} differs very little from θ_{ss} (with horizontal scales adjusted to agree at $\theta_{ss} = \theta_{eq} = 1/2$) because $g = 0.8$ is near to $g = 1$ (see the discussion of Eq. **17**).

Table 2. Effect of variation of y (with $z = 100$) (at $V = V_m/2$)

y	V_2/V_1	$2(z/y)^g$	V/V_o	$z^g/2$	θ_{ss}
0.1	500.6	502.4	20.05	19.91	0.749
1.0	77.9	79.6	20.05	19.91	0.746
10.0	11.04	12.62	20.07	19.91	0.720
100.0	1.000	2.000	20.19	19.91	0.500
237.8	0.404	1.000	20.26	19.91	0.322

Table 3. Effect of variation of z (with $y = 1$) (at $V = V_m/2$)

z	V_2/V_1	$2(z/y)^g$	V/V_o	$z^g/2$	θ_{ss}
0.4204	0.391	1.000	0.945	0.250	0.320
1.0	1.000	2.000	1.000	0.500	0.500
10.0	11.06	12.62	3.38	3.16	0.720
100.0	77.9	79.6	20.05	19.91	0.746
1000.0	500.6	502.4	125.7	125.6	0.749

Fig. 5 shows V_2/V_1 and V/V_o plotted against $k_1/k_1(1/2)$. Note the vertical scale. Both of these quantities are large, thus indicating half-of-the-sites reactivity and strong dimer enhancement of reactivity, for k_1 greater than about $0.4k_1(1/2)$.

Variation of parameters in reference examples

In this section we examine the effect, at the characteristic point $V = V_m/2$, $k_1 = k_1(1/2)$ in the $V(k_1)$ curve, of varying parameters in the above example one at a time.

In Table 1, g is varied, keeping $z = 100$, $y = 1$, $k_2 = k_3 = 1$. Of course, for each g, $k_1(1/2)$ is recalculated (Eq. **20**) and used for the k_1 value. We see that both V_2/V_1 (half-sites criterion) and V/V_o (dimer enhancement) increase with g and are fairly large for $g \gtrsim 0.5$. The boxed values are the reference values from the previous section (see Figs. 4 and 5). Also, $\theta_{ss} \approx 3/4$ because $2p_{12} \approx p_{22} \approx 1/2$ (see Eqs. **16** and **21**).

Tables 2 and 3 show the effect of varying either y or z, holding $g = 0.8$, $k_2 = k_3 = 1$, and either $z = 100$ or $y = 1$, respectively. The third and fifth columns give approximate values for V_2/V_1 and V/V_o, respectively, when $z \gg y$ (see above). The approximate formulas $2(z/y)^g$ and $z^g/2$ make it clear why V_2/V_1 is very sensitive to both y and z, whereas V/V_o depends essentially only on z. The limiting values $y = 237.8$ and $z = 0.4204$ that appear in these tables correspond to the upper limit $G = 2$ (see Eq. **18**). When $G > 2$, $k_1(1/2)$ has to be calculated in a different way.

Turning now to the variation in k_2 and k_3, we remark first that V_2/V_1, V/V_o, and θ_{ss} all depend, at $k_1 = k_1(1/2)$, on the ratio k_2/k_3 only (i.e., not on k_2 and k_3 separately). Furthermore, when k_2/k_3 is varied from 10^{-3} to 10^3, these three quantities all change by less than 0.5% from the reference values (at $k_2/k_3 = 1$).

A numerical example with positive cooperativity

In this example we reverse the values of y and z; $y = 100$, $z = 1$, $k_2 = k_3 = 1$, and $g = 0.8$. We include this example for contrast; it is unlikely to be important for real systems. The choice of y and z corresponds to positive cooperativity; also, V passes through a maximum (see Eq. **18**), $V_m = 2.63$ (compare $V_{om} =$

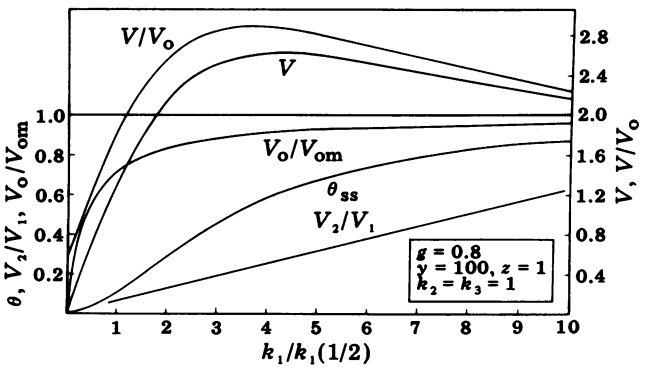

FIG. 6. An example with positive cooperativity (see box), in which V passes through a maximum as a function of substrate concentration. Note left and right scales. See text for details.

1) at $k_1^{max} = 21.2$. The value of $k_1(1/2)$ (i.e., at $V = V_m/2$) is 5.04. This is not much larger than the unperturbed value $k_1^o(1/2) = 2$.

Fig. 6 gives plots of V, V/V_o, V_2/V_1, V_o/V_{om}, and θ_{ss} against $k_1/k_1(1/2)$. Positive cooperativity is evident in the θ_{ss} curve. Again (see Fig. 4) θ_{eq} and θ_{ss} (with adjusted horizontal scales) are very close to each other because $g = 0.8$ is near to $g = 1$. On the other hand, the curves θ_{ss} and V (or V/V_m, not shown) are again quite different in shape.

The reaction velocity drops down from $V_m = 2.63$ to the asymptotic value $V = 1$ as $k_1 \to \infty$. The velocity enhancement ratio V/V_o is never very large; the maximum value is 2.9. Also, $V_2/V_1 = 0.266$ at $V = V_m$, so that we do not have clean-cut half-of-the-sites reactivity in this example.

Recapitulation

We believe that the most important case here is $z \gg 1$ and $z/y \gg 1$. Interactions between subunits destabilize state 22 of the dimer compared to 12 + 21. There is strong negative cooperativity and half-of-the-sites reactivity: only one subunit cycles significantly while the other is in state ES or 2. Quantitatively, this is indicated very simply by $V_2/V_1 \approx 2(z/y)^g$ at $V = V_m/2$. The considerable enhancement of reactivity in this case is also given by a simple relation: $V/V_o \approx z^g/2$ at $V = V_m/2$.

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