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A Mechanism for Exact Sensory Adaptation Based on Receptor Modification

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We provide a theoretical explanation for the observation that in many sensory systems a step increase in stimulus triggers a response that goes through a maximum and then returns to the basal level. Considered here is a receptor molecule that in the absence of ligand can be found in either of two states R and D. Two more states, RL and DL, are formed upon the addition of ligand L. It is assumed that the receptor triggers activity in a sensory system, and that the activity is proportional to a weighted combination of the fractions of molecules that are in each of the four states. It is shown that judicious choice of the weights can provide both an adequate response and exact adaptation to step increases in stimuli. The interconversion between states may operate without energy expenditure or through covalent modification. In both cases, adaptation is associated with receptor modification that acts as a counterweight to changed external conditions. Application to cAMP secretion in *Dictyostelium discoideum* and to chemotaxis in bacteria is discussed.

1. Introduction

Many sensory systems have evolved the property of adaptation to external stimuli. Whenever a stimulus is increased the physiological response goes through a maximum and then returns to pre-stimulus behavior despite the continued presence of the external signal. Such an eventual attenuation or even nullification of the effect of the changed conditions must be associated with an internal modification of the responding system (Koshland, 1980).

We shall concentrate our attention on sensory systems in single cells. One well studied case involves adaptation of swimming behavior in bacteria to changes in

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the cells' chemical environment (Adler, 1978; Koshland, 1979; Macnab, 1980). These bacteria normally swim in a fairly straight line for some time, by virtue of their flagellar rotation. They then go through a brief period of uncoordinated flagellar action called *tumbling* after which they are randomly reoriented for the next period of straight line swimming. Positive *chemotaxis*, motion toward attracting chemicals, is achieved by a reduction in tumbling frequency. It has been found that if the concentration of an attractant is elevated in a spatially uniform fashion and then held constant, the tumbling frequency is reduced for a time but eventually returns to its original value. The molecular mechanism of this adaptation is known in considerable detail; it is connected with the methylation of a receptor molecule that is specific for the attractant under consideration (Springer & Koshland, 1977; Parkinson & Revello, 1978; Goy *et al.*, 1979; Russo & Koshland, 1983).

Another adapting system occurs in the cellular slime mold *Dictyostelium discoideum.* Cells synthesize cyclic AMP (cAMP) upon binding of extracellular cAMP to a membrane receptor. This synthesis is the central element in an intercellular signalling system that plays a key role in the cells' morphogenetic movements and subsequent differentiation (Loomis, 1975; Devreotes, 1982; Gerisch, 1982). When the extracellular cAMP concentration is raised to a higher constant value, the intracellular cAMP synthesis passes through a maximum before returning to its pre-stimulus level (Dinauer *et al.*, 1980*a*, *b*). It is not yet certain whether this adaptation is connected with some receptor modification, although there is considerable evidence favoring this view (Klein, 1979; Juliani & Klein, 1981; Lubs-Haukeness & Klein, 1982; Klein *et al.*, 1984, 1985; Devreotes & Sherring, 1985).

Adaptation of cAMP-induced cGMP synthesis has also been demonstrated in *D. discoideum* (Van Haastert & Van der Heijden, 1983). Adaptation at the level of single cells occurs in other sensory systems such as hormone stimulated adenylate cyclase (Su *et al.*, 1980; Stadel *et al.*, 1983), light activated rod outer segments (Kühn, 1981), mechano-sensitive hair cells in the inner ear (Hudspeth, 1983) and acetylcholine receptors in the neuromuscular junction (Katz & Thesleff, 1957). Varying degrees of evidence suggest that in these cases too adaptation is a consequence of receptor modification.

Various investigators have proposed mechanisms for how adaptation can be brought about by receptor modification (see Discussion). The purpose of this paper is to present a new possible form of exact adaptation via receptor modification, for which the adapting quantity is a *linear combination* of receptor states. Although each of these states depends in a fairly complex way on the stimulus concentration, we obtain the counterintuitive result that there exists a class of linear combinations whose steady state is independent of the stimulus level. This permits exact adaptation. We show how the model also can account for other properties of sensory systems such as dependence of adaptation time and extent of receptor modification on stimulus level, recovery after removal of stimulus, and additivity of responses to successive stimuli. We propose ways to implement the model in molecular terms and we discuss briefly how predictions of the model conform with experimental data concerning cAMP secretion in *D. discoideum* and chemotaxis in bacteria. More detailed comparison of theory and experiment can be found in Knox *et al.* (1986).

2. The Model

THE ADAPTING BOX

Our model for an adapting receptor is based on the classical notion (Katz & Thesleff, 1957) that in the absence of ligand the receptor can be found in either of two states. We denote these states (and their concentrations) by R and D. Each of these states can bind a ligand L, in which case they are denoted respectively by X ($\equiv RL$) and Y ($\equiv DL$). The states R and D, as well as X and Y, can be interconverted. Figure 1 depicts the four states together with the rate constants that are associated with the various binding and interconversion steps.

The following kinetic analysis holds when interconversion occurs by conformational changes or by covalent modification.

KINETICS

The kinetic equations are

$$dR/dt = -k_1 R + k_{-1} D - k_r R \times L + k_{-r} X \tag{1a}$$

$$dX/dt = -k_2 X + k_{-2} Y + k_r R \times L - k_{-r} X \tag{1b}$$

$$dY/dt = k_2 X - k_{-2} Y + k_d D \times L - k_{-d} Y \tag{1c}$$

$$dD/dt = k_1 R - k_{-1} D - k_d D \times L + k_{-d} Y. \tag{1d}$$

Addition of these equations gives

$$R + X + Y + D = R_T \tag{2}$$

where the constant R_T represents the total amount of receptor.

We assume that the binding steps $R \Leftrightarrow X$ and $D \Leftrightarrow Y$ are fast compared to the other two steps. Suppose that the system (1) is stimulated by shifting the ligand concentration L from zero to some fixed value L_1. Then, after an initial transient, the fast steps will attain equilibrium, i.e.

$$R \times L_1 = K_R X, \qquad D \times L_1 = K_D Y \tag{3}$$

where

$$K_R \equiv k_{-r}/k_r, \qquad K_D = k_{-d}/k_d. \tag{4}$$

With the abbreviations

$$\gamma \equiv L_1/K_R, \qquad c \equiv K_R/K_D \tag{5}$$

the equilibrium relations (3) become

$$R = X/\gamma, \qquad D = Y/c\gamma. \tag{6}$$

Substitution of (6) into (2) yields

$$R_T = \theta X + \psi Y, \tag{7a}$$

where

$$\theta = 1+\gamma^{-1}, \qquad \psi = 1+(c\gamma)^{-1}. \tag{7b}$$

The "quasi-steady state" relations (6) and (7) will hold for all times after the initial transient.

The values of the concentrations just after the fast transient can now be obtained, once it is realized that during the transient period R will interchange with X only, and D only with Y. Thus, during this period,

$$R(t)+X(t)=R_0, \qquad D(t)+Y(t)=D_0$$

where R_0 and D_0 are the concentrations of R and D in the unstimulated system (see (16)). It follows that during the fast transient

$$X(t)\to\frac{\gamma}{1+\gamma}R_0, \qquad R(t)\to\frac{1}{1+\gamma}R_0, \qquad Y(t)\to\frac{c\gamma}{1+c\gamma}D_0, \qquad D(t)\to\frac{1}{1+c\gamma}D_0. \tag{8}$$

To study the slow interconversions we add (1a) and (1b), obtaining an equation with only slow rate constants (see Appendix 1)

$$\frac{\mathrm{d}}{\mathrm{d}t}[R+X]=-k_1R+k_{-1}D-k_2X+k_{-2}Y. \tag{9}$$

Employing (6) and (7) (which are valid after the initial fast binding step) to eliminate all variables except X, we find

$$\frac{\mathrm{d}X}{\mathrm{d}t}=V-WX \tag{10}$$

where the constants V and W are given by

$$V=\frac{R_T}{\theta\psi}\left[k_{-2}+\frac{k_{-1}}{c\gamma}\right], \qquad W=\theta^{-1}\left(\frac{k_1}{\gamma}+k_2\right)+\psi^{-1}\left(k_{-2}+\frac{k_{-1}}{c\gamma}\right). \tag{11a, b}$$

The solution of (10) is

$$X=\frac{V}{W}+\left(X_i-\frac{V}{W}\right)\mathrm{e}^{-Wt} \tag{12a}$$

where

$$X_i=R_0\gamma/(1+\gamma) \tag{12b}$$

is the value of X at the end of the fast transient period. Let $\bar{X}$ denote the asymptotic value of X when L is held at the value L_1. This value, V/W, can be written

$$\bar{X}(\gamma)=\frac{R_T\gamma}{Q(1+\gamma)}, \qquad Q\equiv 1+\left[\frac{k_1+k_2\gamma}{k_{-1}+k_{-2}c\gamma}\right]\frac{1+c\gamma}{1+\gamma}. \tag{13a, b}$$

The kinetic behavior of the other concentrations can be found from (7) and (6). In particular the other asymptotic steady state values for $L = L_1$ are given by

$$\bar{R}(\gamma) = \frac{R_T}{Q(1+\gamma)}, \qquad \bar{Y}(\gamma) = \frac{c\gamma}{1+c\gamma}\frac{Q-1}{Q}R_T, \qquad \bar{D}(\gamma) = \frac{Q-1}{Q}\frac{R_T}{1+c\gamma}. \quad (14a, b, c)$$

RECEPTOR ACTIVITY AND PHYSIOLOGICAL RESPONSE

We now make the hypothesis that the "activity" A generated by our system is given by a *linear combination* of the concentrations in each of the four receptor states

$$A(t) = a_1 R(t) + a_2 X(t) + a_3 Y(t) + a_4 D(t). \quad (15a)$$

For the time being, "activity" should be regarded as a loose measure of how strongly ligand binding to the receptor is contributing to the induction of the physiological response. Later we show how (15a) could be implemented and linked to a physiological response, for example by differential binding of an intracellular signalling molecule. In this case the a_i are association constants and hence are non-negative. We thus assume

$$a_i \geq 0, \qquad i = 1, 2, 3, 4. \quad (15b)$$

We stress the distinction between the activity of the receptor states and a selected physiological response controlled by the receptor. We assume that the response is a function of the activity that is defined by (15a). This function incorporates the molecular events that link the receptor state to the final response. We show later that under reasonable assumptions (e.g. that the concentration of the signalling molecule is sufficiently small) possible molecular implementations of (15a) yield expressions for response as a function of activity.

The activity coefficients a_i (Fig. 1) represent the relative contributions of the four receptor states to the overall activity. The *basal activity* obtains when the system is

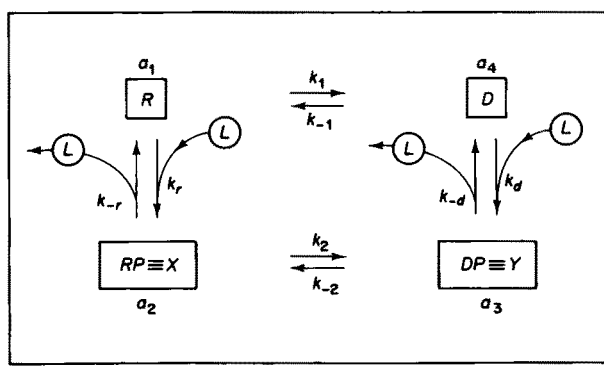

FIG. 1. Model for the sensory system based on receptor modification, showing the four states of the receptor and the rate constants for binding of ligand L and for interconversion. The activity coefficients a_i associated with each receptor state (see eqn (15a)) are also shown.

unstimulated ($L=0$, i.e. $\gamma=0$). Zero subscripts denote the steady state concentrations in this case. The combination of (13) and (14) with $\gamma=0$, $Q=1+K_1^{-1}$ gives

$$X_0=0, \qquad Y_0=0, \qquad R_0=\frac{R_T}{1+K_1^{-1}}, \qquad D_0=\frac{K_1^{-1}}{1+K_1^{-1}}R_T. \tag{16}$$

Here

$$K_1 \equiv k_{-1}/k_1. \tag{17}$$

Thus, from (15), the basal activity A_0 is

$$A_0=a_1R_0+a_4D_0=R_0[a_1+a_4K_1^{-1}]=\frac{R_T}{1+K_1}[a_1K_1+a_4]. \tag{18a}$$

It will prove useful to introduce $B \equiv A_0/R_T$, the basal activity per mole of receptor. The following are two easily verified identities

$$B=\frac{a_1k_{-1}+a_4k_1}{k_{-1}+k_1}, \qquad \frac{a_1-B}{k_1}=-\frac{a_4-B}{k_{-1}}. \tag{18b, c}$$

We define the *initial activity* A_M as the activity that is generated very shortly after the system is subjected to a concentration L_1 of activator, i.e. just when the equilibrium relations (6) have been established but before there has been a shift between the states $R-X$ and $D-Y$. Using a subscript "one" to denote the concentrations that correspond to the initial activity, we find from (8) and (16) that

$$R_1=\frac{R_0}{1+\gamma}, \qquad X_1=\frac{\gamma}{1+\gamma}R_0, \qquad Y_1=\frac{c\gamma R_0}{K_1(1+c\gamma)}, \qquad D_1=\frac{R_0}{K_1(1+c\gamma)}. \tag{19}$$

The initial activity, A_M, obtains when the values from (19) are substituted into (15a). To find the *net initial activity* we subtract the basal activity A_0 from the initial activity A_M

$$A_M-A_0=R_0\left[(a_2-a_1)\frac{\gamma}{1+\gamma}+\frac{(a_3-a_4)}{K_1}\frac{c\gamma}{1+c\gamma}\right]. \tag{20a}$$

In the case $c=1$, (20a) can be written

$$A_M-A_0=[(a_2-a_1)R_0+(a_3-a_4)D_0]\frac{\gamma}{1+\gamma}. \tag{20b}$$

This formula has a clear interpretation: the peak gain in activity is the sum of the individual gains in activity obtained when binding shifts R to X and D to Y, to an extent given by the receptor occupancy $\gamma/(1+\gamma)$. When $c \neq 1$, the two sides of the box must be differently weighted.

EXACT ADAPTATION

Our basic idea is to choose the coefficients a_i of (15a) so that the final steady state activity is equal to the basal activity for all values of L_1 (i.e. for all values of γ)

$$a_1\bar{R}(\gamma)+a_2\bar{X}(\gamma)+a_3\bar{Y}(\gamma)+a_4\bar{D}(\gamma)=A_0. \tag{21}$$

The a_i's must also be chosen such that the net initial activity A_M-A_0 is significant.

Since response is a function of activity, (21) ensures that response will always return to the same value at steady state. (It can be shown that the slow shift of activity from A_M to basal is monotonic, via a single exponential. See Appendix 1.)

In an effort to write (21) in a relatively simple form we note from (13b) that

$$\frac{Q-1}{1+c\gamma}=\frac{k_1+k_2\gamma}{(k_{-1}+k_{-2}c\gamma)(1+\gamma)}, \qquad \frac{1}{Q}=\frac{(1+\gamma)(k_{-1}+k_{-2}c\gamma)}{Q_2(\gamma)} \tag{22}$$

where

$$Q_2(\gamma)\equiv(k_{-1}+k_{-2}c\gamma)(1+\gamma)+(k_1+k_2\gamma)(1+c\gamma) \tag{23}$$

is a quadratic function of γ. Thus, for example, the expression for $\bar{Y}$ can be written compactly as

$$\bar{Y}(\gamma)=c\gamma R_T(k_1+k_2\gamma)/Q_2(\gamma).$$

It thus turns out that (21) implies that

$$(a_1+a_2\gamma)(k_{-1}+k_{-2}c\gamma)+(a_3c\gamma+a_4)(k_1+k_2\gamma)=A_0Q_2(\gamma)/R_T. \tag{24}$$

The problem, then, reduces to the selection of the four activity coefficients so that the quadratic expression for γ on the left side of (24) is identical to the quadratic $A_0Q_2(\gamma)/R_T$. Equating in turn the quadratic, linear, and constant terms on the two sides of (24) we obtain the identity

$$a_1k_{-1}+a_4k_1=a_1k_{-1}+a_4k_1 \tag{25a}$$

together with the equations

$$a_2k_{-1}+a_1k_{-2}c+a_3k_1c+a_4k_2=(k_{-1}+k_{-2}c+k_1c+k_2)\frac{a_1k_{-1}+a_4k_1}{k_{-1}+k_1}, \tag{25b}$$

$$a_2k_{-2}+a_3k_2=(k_{-2}+k_2)\frac{a_1k_{-1}+a_4k_1}{k_{-1}+k_1}. \tag{25c}$$

Equation (25c) can be written as

$$\frac{a_2K_2+a_3}{K_2+1}=\frac{a_1K_1+a_4}{K_1+1} \tag{26a}$$

or in terms of the quantity B of (18b)

$$\frac{a_2-B}{k_2}=-\frac{a_3-B}{k_{-2}}. \tag{26b}$$

Again employing B we can write (25b) in the form

$$(k_2k_{-1}-ck_1k_{-2})\left[\frac{a_2-B}{k_2}-\frac{a_1-B}{k_1}\right]=0. \tag{27}$$

APPLICATION OF MICROSCOPIC REVERSIBILITY

When the modification of the receptor, shown in Fig. 1, is such that k_1, k_{-1} and k_2, k_{-2} relate to the forward and backward steps of the reversible interconversion

of R into D and of X into Y, the principle of microscopic reversibility holds for the "box". (See for example, Wyman, 1975.) Employing this principle, and using the definition of c in (5), we find that

$$k_1 k_d k_{-2} k_{-r} = k_{-1} k_{-d} k_2 k_r, \quad \text{i.e. } k_{-1} k_2 = k_1 k_{-2} c \tag{28}$$

or

$$K_1 = K_2 c \quad \text{where } K_2 \equiv k_{-2}/k_2. \tag{29}$$

In particular, the term in the ordinary parenthesis in (27) vanishes, so that only the single condition (26) is required to give complete adaptation to the basal value!

As we have pointed out, it is not enough to have adaptation; a useful transducing mechanism must also give rise to a significant activity. We characterize the magnitude of the activity by the quotient of the net initial activity of (20a) divided by the basal activity A_0. This *relative net initial activity* $\alpha(L)$ can be written

$$\alpha(L) \equiv \frac{A_M - A_0}{A_0} = \frac{(a_2 - a_1) L R_0 (K_R + L)^{-1} + (a_3 - a_4) L D_0 (K_D + L)^{-1}}{a_1 R_0 + a_4 D_0}. \tag{30a}$$

A particularly simple measure of activity is $\alpha(\infty)$, the value of α that is achieved at saturating ligand concentration

$$\alpha(\infty) = \frac{(a_2 - a_1) R_0 + (a_3 - a_4) D_0}{a_1 R_0 + a_4 D_0} \equiv \frac{a_2 R_0 + a_3 D_0 - A_0}{A_0}. \tag{30b}$$

Note that the net initial activity of (20a) can be increased by any factor merely by multiplying all the coefficients a_i by this factor. This is not the case for $\alpha(L)$, which more suitably compares the activity of the system with the basal activity.

We observe that there is no intrinsic difference between the left and right sides of the "box" in Fig. 1. This symmetry requires that the various conditions be invariant under the following transformation

$$a_2 \leftrightarrow a_3, \qquad a_1 \leftrightarrow a_4, \qquad K_D \leftrightarrow K_R, \qquad K_1 \leftrightarrow 1/K_1, \qquad K_2 \leftrightarrow 1/K_2. \tag{31}$$

Indeed, the expression for $\alpha(L)$ as well as the adaptation requirement (26) and the microscopic reversibility condition (29) are properly invariant. Later formulas have also been checked for suitable invariance.

It follows from Appendix 3 that $\alpha(\infty)$ can be made arbitrarily large compared to unity by choosing

$$K_2 \ll 1, \qquad K_1 \gg 1, \quad \text{so } a_2 B^{-1} \gg 1, \qquad c \gg 1 \tag{32}$$

or

$$K_1 \ll 1, \qquad K_2 \gg 1, \quad \text{so } a_3 B^{-1} \gg 1, \qquad c \ll 1. \tag{33}$$

In Fig. 2(a) we display an example of the activity elicited by three different stimuli, showing exact adaptation in a case where (32) roughly holds. The parameters a_1, a_3, and a_4 are selected: then the value a_2 for exact adaptation, defined as a_2^*, is found from (26).

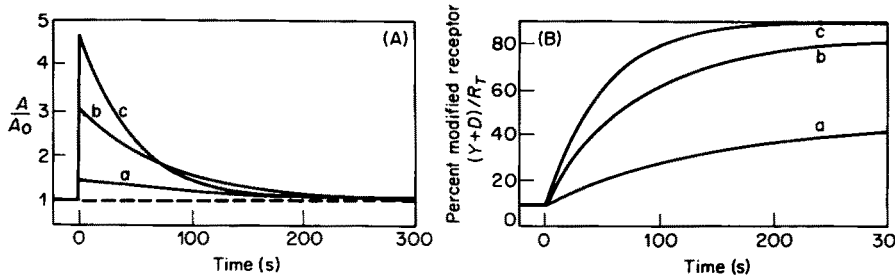

FIG. 2. Exact adaptation in the receptor system subject to microscopic reversibility, in response to three stimuli bringing γ from zero to 0·1 (a), 1 (b) and 10 (c) respectively. Shown in panel A is the time evolution of the activity A relative to the basal activity A_0 for $K_1=10$, $K_2=0{\cdot}1$, $c=100$, $R_T=1$, $k_{-1}=0{\cdot}01\ s^{-1}$, $k_{-2}=0{\cdot}002\ s^{-1}$. Three activity coefficients are arbitrarily assigned the values $a_1=20$, $a_3=10$, $a_4=1$; a_2 is given the value $a_2^*=101$ prescribed by condition (26) for exact adaptation. (The fast binding of ligand (assumed for simplicity) has been approximated so that the activity instantaneously rises to a maximum.) Shown in panel B are the changes in the fraction of modified receptor that accompany adaptation to the three stimuli. N.B. K_1, K_2 and R_T are multiples of any suitable reference concentration.

In the case of Fig. 2(a) the activity produced by the initial formation of the complex X from R is magnified by the large value of a_2. Adaptation is brought about by the later strong shift of X into Y due to the small value of K_2. Figure 2(b) depicts the conversion of the receptor to its modified form for the same three stimuli applied in Fig. 2(a). Note that the conditions $c \gg 1$, required by microscopic reversibility, and $K_2 \ll 1$ favor tight binding of the ligand in the Y-state.

Can our model work if some of the activity coefficients a_i are zero? Consider the case $a_1=0$, $a_4=0$, wherein the basal activity A_0 vanishes, by (18a). Then (26) cannot hold, by the positivity assumption (15b), so that exact adaptation cannot occur. Inspection shows, however, that all properties can be present if just one corresponding pair of free and liganded states is active—for example if $a_3=a_4=0$, $a_1 \neq 0$, $a_2 \neq 0$.

EXACT ADAPTATION IN THE CASE OF COVALENT MODIFICATION

Consider situations wherein the interconversion of R into D and of X into Y occurs through reversible covalent modification. Our kinetic analysis remains valid provided that the enzymes which catalyze the modification and demodification steps operate in the first order kinetic domain. In this case, however, the microscopic reversibility constraint (29) need not be imposed on the constants of Fig. 1 (see Appendix 2). From (27) we see that condition (26) for exact adaptation must now be supplemented by

$$\frac{a_1-B}{k_1}=\frac{a_2-B}{k_2}. \tag{34a}$$

Conditions (26) and (34a) are equivalent to

$$a_2=\frac{a_1(k_2+k_{-1})-a_4(k_2-k_1)}{k_{-1}+k_1}, \qquad a_3=\frac{-a_1(k_{-2}-k_{-1})+a_4(k_{-2}+k_1)}{k_{-1}+k_1}. \tag{34b}$$

Combining (34a), (26b), and (18c), we obtain as the totality of requirements in this case

$$\frac{a_1-B}{k_1}=\frac{a_2-B}{k_2}=-\frac{a_3-B}{k_{-2}}=-\frac{a_4-B}{k_{-1}}. \tag{34c}$$

The relative net initial activity (30a) now becomes

$$\alpha(L)=\frac{(a_1-a_4)[(k_2-k_1)LR_0(K_R+L)^{-1}-(k_{-2}-k_{-1})LD_0(K_D+L)^{-1}]}{(k_{-1}+k_1)(a_1R_0+a_4D_0)}. \tag{35}$$

When, for example, $K_1>K_2$ and $k_{-2}<k_{-1}$, it follows from equations (A3.7a) and (A3.8) of Appendix 3 and from (34c) that maximum initial activity is attained by choosing the following weights

$$a_1=B(1+k_1k_{-1}^{-1}),\qquad a_2=B(1+k_2k_{-1}^{-1}),\qquad a_3=B(1-k_{-2}k_{-1}^{-1}),\qquad a_4=0. \tag{36}$$

If the kinetic coefficients are in suitable ranges, activity can be arbitrarily large (Appendix 3).

Figure 3(a) shows examples of exact adaptation in the present case. For the parameters chosen, the initial change in activity occurs mainly when ligand binding rapidly shifts the receptor from R to X. Adaptation takes place due to the covalent

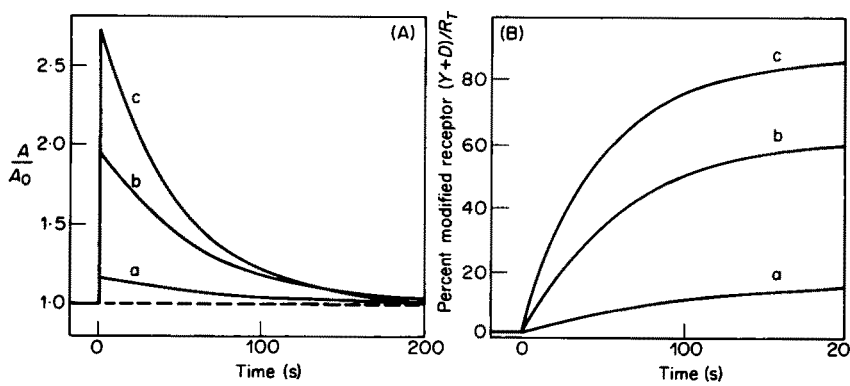

FIG. 3. Exact adaptation in the case of receptor covalent modification, for the three stimuli considered in Fig. 2. Parameter values are $K_1=70$, $K_2=0{\cdot}1$, $c=1$, $R_T=1$, $k_{-1}=0{\cdot}01\ s^{-1}$, $k_{-2}=0{\cdot}002\ s^{-1}$. Two activity coefficients are arbitrarily taken as $a_1=20$, $a_4=1$; a_2 and a_3 are given the values $a_2^*=57{\cdot}2$ and $a_3^*=16{\cdot}0$ prescribed by conditions (34b) for exact adaptation. The figure shows the time course of the relative activity A/A_0 in panel A and of the concomitant variation in the fraction of modified receptor $(Y+D)/R_T$ in panel B.

modification of the receptor into the Y and D states, which depopulates the particularly active X state. Figure 3(b) depicts the time course of the receptor modification. The characteristic time for this modification is the same as that for the adaptation process. The level of receptor modification at steady state increases with the level of stimulus; therefore covalent modification acts as a counterweight to the changing external conditions.

INEXACT ADAPTATION

The question naturally arises, particularly in connection with the possible evolution of an adapting system, as to the extent of adaptation in situations where requirement (26) is only approximately satisfied. To study this matter we define a deviation η in steady state activity $\bar{A}$ by

$$\bar{A} = A_0(1+\eta). \tag{37a}$$

Let us suppose that there is a deviation δ_2 from the exactly adapting value a_2^* of a_2

$$a_2 = a_2^*(1+\delta_2). \tag{37b}$$

From the definition of $A(t)$, we have

$$\bar{A} = a_1\bar{R} + a_2\bar{X} + a_3\bar{Y} + a_4\bar{D} = (a_1\bar{R} + a_2^*\bar{X} + a_3\bar{Y} + a_4\bar{D}) + \delta_2 a_2^*\bar{X} = A_0 + \delta_2 a_2^*\bar{X},$$

so that

$$\eta = (\bar{A}/A_0) - 1 = [(A_0 + \delta_2 a_2^*\bar{X})/A_0] - 1 = \delta_2 a_2^*\bar{X}/A_0. \tag{38}$$

Hence the relation between η and δ_2 is a straight line, passing through the origin, with a slope $(a_2^*\bar{X}/A_0)$.

In the case where adaptation is brought about by covalent modification, a_3 can also deviate. The deviation, η, is given by the analogous formula $\eta = \delta_3(a_3^*\bar{Y}/A_0)$ where $\delta_3 = (a_3/a_3^*) - 1$. As illustrated in Fig. 4, in the absence of exact adaptation the activity can tend to a value above or below the basal level, depending on whether the activity coefficient is above or below the precise value required for exact adaptation.

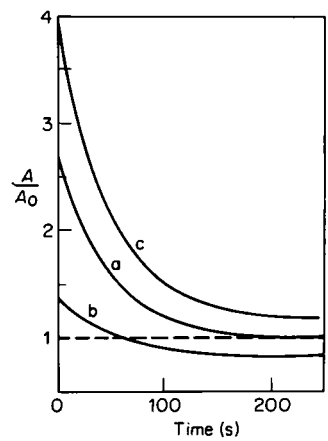

FIG. 4. Exact vs inexact adaptation. The activity of the sensory system is shown under the conditions of Fig. 3 for the stimulus $\gamma = 0 \to 10$. Whereas exact adaptation occurs for $a_2 = a_2^* = 57{\cdot}197$ (a), inexact adaptation obtains for $a_2 = a_2^*/2$ (b) or $a_2 = 1{\cdot}5a_2^*$ (c) with a final steady state activity which is some 20% below or above A_0, respectively.

ALTERNATIVE DERIVATION

Our derivation of the equations for exact adaptation was a straightforward search for conditions that would permit accomplishment of our goal. We now present an

alternative derivation, one that is less straightforward but that is briefer and is more closely related to the biophysics of the problem.

Recall the relationship $A_0 = BR_T$ between the basal activity A_0, the basal activity per mole of receptor B, and the total amount of receptor R_T. Using the conservation requirement (2) to replace R_T we can write our requirement that steady state activity is always equal to basal activity

$$a_1\bar{R} + a_2\bar{X} + a_3\bar{Y} + a_4\bar{D} = BR_T \tag{39a}$$

in the form

$$\tilde{a}_1\bar{R} + \tilde{a}_2\bar{X} + \tilde{a}_3\bar{Y} + \tilde{a}_4\bar{D} = 0 \tag{39b}$$

where

$$\tilde{a}_i = a_i - B; \qquad i = 1, 2, 3, 4. \tag{40}$$

Comparing (39a) with (39b) we see that with no loss of generality we can restrict consideration to a situation where basal activity is zero. Any conclusions we draw about the activity coefficients $\tilde{a}_i$ in the latter case can, by (40), at once be transformed into conditions on the general activity coefficients a_i.

Consider

$$J = k_1\bar{R} - k_{-1}\bar{D}. \tag{41}$$

From Fig. 1 we see that J is the net clockwise flux of molecules around the box at steady state. At steady state the clockwise and counterclockwise fluxes must be equal and opposite, so that we can write, for example

$$-J = k_2\bar{X} - k_{-2}\bar{Y}. \tag{42}$$

We solve (41) and (42) for $\bar{X}$ and $\bar{R}$ respectively, and insert the results in (39b). This yields

$$J\left(\frac{\tilde{a}_1}{k_1} - \frac{\tilde{a}_2}{k_2}\right) + k_{-1}\bar{D}\left(\frac{\tilde{a}_1}{k_1} + \frac{\tilde{a}_4}{k_{-1}}\right) + k_{-2}\bar{Y}\left(\frac{\tilde{a}_2}{k_2} + \frac{\tilde{a}_3}{k_{-2}}\right) = 0. \tag{43}$$

The coefficient of $\bar{D}$ vanishes, by expression (18c) that relates a_1 and a_4 to the basal activity. At equilibrium, when microscopic reversibility holds, J also vanishes. In this case, therefore the sole condition for perfect adaptation is

$$\frac{\tilde{a}_2}{k_2} + \frac{\tilde{a}_3}{k_{-2}} = 0. \tag{44}$$

In the case of covalent modification, J is nonzero and independent of $\bar{Y}(\gamma)$, so that in addition to (44), perfect adaptation for all γ requires that

$$\frac{\tilde{a}_1}{k_1} - \frac{\tilde{a}_2}{k_2} = 0. \tag{45}$$

Using (40), we see that (44) and (45) are equivalent to the previously derived conditions (26b) and (34a). Note from (34c) that in the essentially general case of $B = 0$, the four weighting coefficients are inversely proportional to the adapting half-lives of each of the four states.

3. Properties of the Model Related to Adapting Systems

In addition to adaptation, sensory systems are known to possess a variety of other dynamic and steady state properties. We examine here whether our model can account for such properties, emphasizing trends rather than details of particular experiments. We shall treat in turn (a) the dependence of the adaptation time on stimulus level, (b) the dependence of the extent of receptor modification at steady state on stimulus level, (c) the change in activity due to stimulus removal, (d) the additivity of activity changes brought about by successive stimuli.

Our earlier results have been established for the elevation of the ligand concentration L from zero to a fixed value L_1. In order to carry out the program just outlined we have to consider the more general situation of changing L from L_1 to L_2. We stress that the conditions for exact adaptation remain unaltered, for this is a steady state property.

If the ligand concentration L is held at L_1, the concentrations of the receptor species will reach steady state values $\bar{R}(\gamma)$, $\bar{X}(\gamma)$, $\bar{Y}(\gamma)$, $\bar{D}(\gamma)$ given by (13) and (14). If L is then suddenly elevated to L_2, then (generalizing (8)) during the fast transient

$$X(t) \to \left(\frac{\gamma'}{1+\gamma'}\right)[\bar{R}(\gamma)+\bar{X}(\gamma)] \equiv X_i(\gamma'), \quad \text{where } \gamma' = \frac{L_2}{K_R} \tag{46}$$

that is,

$$X_i(\gamma') = \bar{X}(\gamma)\left(\frac{1+\gamma}{\gamma}\right)\left(\frac{\gamma'}{1+\gamma'}\right). \tag{47}$$

The corresponding values $R_i(\gamma')$, $D_i(\gamma')$, and $Y_i(\gamma')$ are found from (6) and (7) upon replacing γ by γ'. The initial activity in this case is

$$\begin{aligned} A_M^{\gamma\to\gamma'} &= a_1 R_i(\gamma') + a_2 X_i(\gamma') + a_3 Y_i(\gamma') + a_4 D_i(\gamma') \\ &= \left[\left(\frac{a_1}{\gamma'}+a_2\right) - \frac{\theta(\gamma')}{\psi(\gamma')}\left(a_3+\frac{a_4}{c\gamma'}\right)\right]\frac{1+\gamma}{\gamma}\frac{\gamma'}{1+\gamma'}\bar{X}(\gamma) + \left(a_3+\frac{a_4}{c\gamma'}\right)\frac{R_T}{\psi(\gamma')}. \end{aligned} \tag{48a}$$

The corresponding relative net initial activity can be found from

$$\alpha^{\gamma\to\gamma'} = \frac{A_M^{\gamma\to\gamma'} - A_0}{A_0}. \tag{48b}$$

During the slow interconversion to the final steady state, generalization of (12a) shows that the activity is given by

$$A^{\gamma\to\gamma'}(t) = A_0 + (A_M^{\gamma\to\gamma'} - A_0)\exp[-W(\gamma')t] \tag{49}$$

where $W(\gamma')$ is given by (11b) with γ replaced by γ'.

TIME FOR ADAPTATION

One of the properties of adapting systems that is most readily measurable is the time required for adaptation. The simplest theoretical expression that characterizes

this time is the half-time $t_{1/2}$. From (49) it follows that $t_{1/2} = \ln 2/W$, or

$$t_{1/2}(\gamma) = \frac{(\ln 2)(1+\gamma)(1+c\gamma)}{(1+c\gamma)(k_1+k_2\gamma)+(1+\gamma)(k_{-1}+k_{-2}c\gamma)}. \tag{50}$$

Note from (49) that because of our assumption of fast ligand binding, the adaptation time $t_{1/2}$ to a new stimulus level is independent of the initial level of stimulus, i.e. in notation like that of (48) and (49),

$$t_{1/2}^{\gamma'\to\gamma} = t_{1/2}^{0\to\gamma} \quad \text{where } t_{1/2}^{0\to\gamma} \equiv t_{1/2}(\gamma). \tag{51a, b}$$

Of particular interest are the two limiting values of $t_{1/2}$, occurring for small and saturating stimuli

$$\lim_{\gamma\to 0} t_{1/2}(\gamma) = \frac{\ln 2}{k_1+k_{-1}}, \qquad \lim_{\gamma\to\infty} t_{1/2}(\gamma) = \frac{\ln 2}{k_2+k_{-2}}. \tag{52a, b}$$

From (51a) it follows that (52a) also gives the half-time for adaptation when the stimulus level is decreased to zero from any initial value. Depending on the relative size of k_1+k_{-1} and k_2+k_{-2}, the model provides either an overall increase or decrease in response time with increasing stimulus (see Figs 2 and 3) and a smaller or larger half-time for deadaptation as compared to adaptation. We note in the case of covalent modification that the conditions that favor large activity (see (A.3.11)) also favor a decrease in recovery time with increasing stimulus and hence faster adaptation than deadaptation. (See Appendix 3 for further comment.)

It is also possible to choose parameters such that $t_{1/2}(\gamma)$ as given in (50) is independent of γ. Proceeding in the same fashion as we did in deriving the conditions (25) we find that $t_{1/2}$ is indeed independent of stimulus level if and only if

$$c = 1 \quad \text{and} \quad k_1+k_{-1} = k_2+k_{-2} \tag{53}$$

or

$$k_1 = k_2 \quad \text{and} \quad k_{-1} = k_{-2}. \tag{54}$$

In the latter case, however, $K_1 = K_2$ and there is no response. (See Appendix 3.)

Another feature of interest is additivity in response times. By this is meant that the time for the response to recover from elevation in ligand concentration from 0 to L_1 plus the corresponding time for a further elevation to L_2 equals the time to recover from an elevation in L from 0 to L_2. In terms of half-times this would require that

$$t_{1/2}^{0\to\gamma_1} + t_{1/2}^{\gamma_1\to\gamma_2} = t_{1/2}^{0\to\gamma_2}. \tag{55}$$

But it follows from (51) that the activity half-times cannot reproduce the additivity property. It is quite possible that a rough additivity would be found if the recovery time were defined as the time for response to approach within some small fraction of the final value (as considered by Goldbeter & Koshland, 1982), but we shall not pursue this matter further. There is also the possibility that if physiological response is not proportional to activity (see Discussion) the time course of the response is not simply related to the time course of activity.

EXTENT OF RECEPTOR MODIFICATION

Another experimentally measurable quantity is the dependence on receptor occupancy of the extent of receptor modification at steady-state. From (14), the fraction of occupied receptor at steady state is

$$\frac{\bar{X}+\bar{Y}}{R_T}=\frac{1}{Q}\left[\frac{\gamma}{1+\gamma}+\frac{(Q-1)c\gamma}{1+c\gamma}\right]. \tag{56}$$

In general this expression is the quotient of two quadratics in γ and thus has a non-Michaelian character. We will restrict our discussion to the case when $c=1$, so that

$$\frac{\bar{X}+\bar{Y}}{R_T}=\frac{\gamma}{1+\gamma}. \tag{57}$$

Then binding is Michaelian with half-saturation when $\gamma=1$.

When $c=1$, the increase in the fraction of modified receptor at steady state is given by

$$\frac{\bar{D}+\bar{Y}-D_0}{R_T}=\left[\frac{k_2(k_{-1}+k_1)-k_1(k_{-2}+k_2)}{(k_{-1}+k_1)(k_2+k_{-2})}\right]\frac{\gamma}{(k_{-1}+k_1)/(k_2+k_{-2})+\gamma}. \tag{58}$$

Like (57), this expression is Michaelian. When appropriately normalized to their maximum, the graphs of (57) and (58) coincide if their half-saturation constants are identical, that is if

$$k_1+k_{-1}=k_2+k_{-2}.$$

By (53) this condition also guarantees that half-times for adaptation are independent of the stimulus level. Furthermore, comparison of (57) and (58) indicates a link between adaptation time and receptor modification. If the time course for adaptation is an increasing (decreasing) function of stimulus level then the midpoint of the normalized curve for the fraction of modified receptor lies to the right (left) of the midpoint of the curve for receptor occupancy.

STIMULUS REMOVAL

When stimulus is removed one would expect a deadaptation to prestimulus behavior. This occurs as the receptors progressively return to their prestimulus condition. Figure 5 shows the response to successive changes in level of ligand γ: $0\to 1$, $1\to 10$, $10\to 0$, $0\to 10$. Also depicted are the concomitant changes in the extent of receptor modification. The removal of stimulus results in a sharp drop of activity below the basal level and a later slow recovery to the unstimulated state. This change in activity is associated with a decrease in the level of modified receptor.

ADDITIVITY OF ACTIVITIES RESULTING FROM SUCCESSIVE STIMULI

Another measurable property sometimes found in adapting systems is additivity of response magnitudes. That is, the response following an elevation in ligand L to

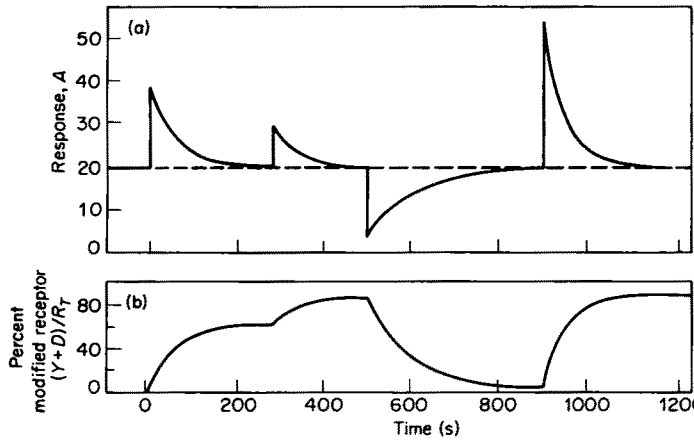

FIG. 5. Response of the receptor system to successive stimuli, $\gamma = 0 \to 1$, $1 \to 10$, $10 \to 0$, $0 \to 10$, in the conditions of Fig. 3. The time course of the activity A (eqn 15a) is plotted in panel (a), rather than the ratio A/A_0, in order to show more clearly the response to a removal of stimulus. Shown in panel (b) is the related change in the fraction of modified receptor. The curve for the time evolution of the activity is obtained by means of eqns (6), (7), (12a) and (15a); for the initial conditions, eqns (12b) or (47) are used, depending on whether or not the background stimulus is nil.

a level L_1, plus the response elicited by a further elevation of L to a level L_2, is equal to the response induced by a single increase in L from 0 to L_2.

To examine the additivity property in terms of activities, we identify the change in activity A_T with the integrated activity above basal as given by (49)

$$A_T^{\gamma \to \gamma'} = \int_0^\infty A^{\gamma \to \gamma'}(t)\,\mathrm{d}t = (A_M^{\gamma \to \gamma'} - A_0)/W(\gamma'). \tag{59}$$

We shall restrict our study of additivity to the cases of equations (53) or (54), wherein $W(\gamma)$ is independent of γ. With this, the additivity requirement

$$A_T^{0 \to \gamma} + A_T^{\gamma \to \gamma'} = A_T^{0 \to \gamma'}$$

becomes a requirement on the initial activity

$$(A_M^{0 \to \gamma} - A_0) + (A_M^{\gamma \to \gamma'} - A_0) = A_M^{0 \to \gamma'} - A_0 \tag{60a}$$

or on the relative net initial activity

$$\alpha^{0 \to \gamma} + \alpha^{\gamma \to \gamma'} = \alpha^{0 \to \gamma'}. \tag{60b}$$

A straightforward albeit somewhat lengthy calculation shows that (60) indeed holds when (53) and the covalent adaptation conditions (34c) are assumed (so that $a_1 - a_4 = a_2 - a_3$). Figure 6 illustrates both the independence of adaptation time on stimulus level and also additivity.

Additivity is certainly not a general property of our model. Let us consider for example the situation where (53) holds together with the adaptation condition (26) for microscopically reversible transformations, instead of (34c). In this case, the

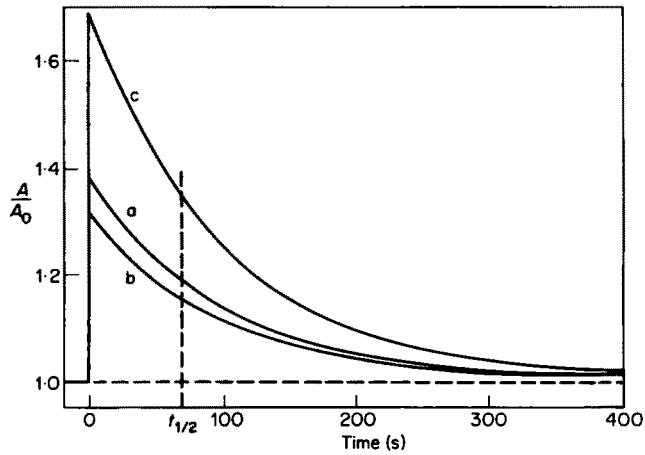

FIG. 6. Independence of adaptation time on stimulus level and additivity of integrated activities resulting from successive stimuli. The curves are obtained for the stimuli $\gamma = 0 \to 1$ (a), $1 \to 10$ (b), and $0 \to 10$ (c) for the parameter values $K_1 = 70$, $k_{-1} = 0{\cdot}01\ s^{-1}$, $k_{-2} = 0{\cdot}002\ s^{-1}$, $R_T = 1$, $c = 1$; K_2 is taken so as to yield equality of $k_1 + k_{-1}$ and $k_2 + k_{-2}$. Thus $K_2 = 0{\cdot}2456$, $k_1 = 0{\cdot}00014\ s^{-1}$, and $k_2 = 0{\cdot}00814\ s^{-1}$. Two activity coefficients are arbitrarily taken as $a_1 = 20$, $a_4 = 1$; a_2 and a_3 are given the values $a_2^* = 35{\cdot}0$ and $a_3^* = 16{\cdot}0$ which satisfy conditions (34b) for exact adaptation. As indicated, all curves possess the same half-time, $t_{1/2}$.

right side of the additivity equation (60a) contains the additional term

$$\frac{R_T}{1+\gamma_2}(a_1 - a_2 + a_3 - a_4)\left[\frac{1}{Q(\gamma_1)} - \frac{K_1}{K_1+1}\right].$$

This term will be small, and therefore total additivity will be observed, only in the cases that γ_1 is small or γ_2 is large.

4. Discussion

Besides the present analysis, other authors have considered exact adaptation of receptor-induced activities. One way of accomplishing this was outlined by Macnab & Koshland (1972). (Also see Koshland, 1977.) In their mechanism, binding of the stimulus to the receptor enhances both the synthesis of a response regulator and its degradation, but with different time courses. This leads to a transient increase in the level of the regulator that governs the physiological response. Since the level of the response is proportional to the difference between synthesis and degradation rates, exact adaptation can be obtained by arranging that the steady state values of these rates depend in identical fashion on the stimulus.

Another approach to exact adaptation via receptor-mediated activities was exemplified in a model by Goldbeter & Segel (1977, 1980) for the cAMP signalling system in *Dictyostelium*. When the model was applied to the effect of a step increase in extracellular cAMP concentration, it was found that intracellular cAMP rose and then returned to its initial value—as a result of the assumed constant input of the substrate ATP. Substrate degradation acts here as a counterweight to increased

stimulus and renders possible exact adaptation. Similar conclusions are reached in a related model where a sizeable portion of the counterweight effect is associated with receptor modification (Martiel & Goldbeter, 1984; Goldbeter *et al.*, 1984).

A detailed study of a model of covalent receptor modification and its application to bacterial chemotaxis was made by Goldbeter & Koshland (1982). With regard to exact adaptation, ratios of two receptor species or of modification rates, or differences between modification rates, were considered as the quantity linked to the response. On the other hand, Block *et al.* (1983) showed how exact adaptation occurs in a model based on receptor modification when the response is proportional to the difference between the fractions of unmethylated and methylated receptor with bound attractant.

We have shown that a sensory system based on receptor modification, with an activity given by a linear combination (15) of four receptor states, can respond and then adapt exactly to constant stimuli. We have investigated interconversion between different conformational states under the constraint of microscopic reversibility as well as interconversion through covalent modification. In all of our analytic work we have assumed (as seems very reasonable for slime mold and bacteria at least) that the formation on ligand binding of X from R and Y from D is fast compared to the $R-D$ and $X-Y$ interconversions. Under study are extensions of our work where this assumption is not made.

In the covalent case, two conditions (34b) on the four activity coefficients a_1, a_2, a_3 and a_4 insure exact adaptation of the activity $A(t)$ to its basal level. It turns out that if these two conditions are assumed to hold, then the amount by which the activity A exceeds its basal level A_0 is completely determined up to a multiplicative constant. To demonstrate this we note that a combination of (34b) and (15) yields

$$A(t)-A_0=\frac{a_1-B}{k_1}[k_1R(t)+k_2X(t)-k_{-2}Y(t)-k_{-1}D(t)]. \tag{61}$$

Indeed, all the remaining arbitrariness in the model is incorporated into A_0 and the multiplicative constant $(a_1-B)/k_1$. The activity would be completely determined (by (18) and (A3.7)) if its basal and maximum level could be measured.

Equation (61) can be interpreted as showing that

$$A(t)-A_0\sim v_m-v_d,$$

where $v_m\equiv k_1R+k_2X$ is the rate of modification and $v_d\equiv k_{-1}D+k_{-2}Y$ is the rate of demodification. Our model can thus be regarded as a molecular implementation of one of the mechanisms analyzed by Goldbeter & Koshland (1982) in which v_m-v_d was considered as the adapting quantity. Two other connections of our model with rates can be obtained by combining (61) and (1) to obtain

$$A(t)-A_0\sim\frac{\mathrm{d}}{\mathrm{d}t}(D+Y)=-\frac{\mathrm{d}}{\mathrm{d}t}(R+X).$$

We stress that our theory requires the perfectly adapting receptor to have at least three different conformations. That is, binding of the ligand must significantly alter

at least one of the two basic interconverting configurations of the free receptor R and D. If not, then $a_1 = a_2$ and $a_3 = a_4$. In the covalent case, this implies $k_1 = k_2$ and $k_{-1} = k_{-2}$ by (34c), and hence no activity, by (35). In the reversible case, the absence of activity follows immediately from (30a).

We now briefly examine to what extent the model proposed here is relevant to such well studied cases of adaptation as those found in bacterial chemotaxis, and *D. discoideum* cAMP secretion (Springer *et al.*, 1979; Dinauer *et al.*, 1980*a*, *b*). (Detailed comparison with experiment will be provided elsewhere (Knox *et al.*, 1986).) In addition to adaptation, the main features of these systems are (i) the dependence of adaptation time on receptor occupancy (Berg & Tedesco, 1975; Spudich & Koshland, 1975; Devreotes & Steck, 1979), (ii) the additivity of net response to incremental stimuli (Rubik & Koshland, 1978; Devreotes & Steck, 1979), (iii) the dependence of the extent of receptor modification on receptor occupancy (Goy *et al.*, 1979; Klein *et al.*, 1984), (iv) the reversal of response and initiation of deadaptation upon removal of stimuli (Kleene *et al.*, 1979; Koshland, 1979; Dinauer *et al.*, 1980*a*). In section 3 we demonstrated that all these major trends can be mimicked by the present model. Obtaining exact correspondence of the model with experimental observations may require further refinement of the model. For instance, receptors could be multiply modified or different receptor classes may interact. Bacterial chemoreceptors are known to be multiply methylated (De Franco & Koshland, 1980). The role of such multiple modification has recently been considered in a two-state receptor model for exact adaptation proposed by Asakura & Honda (1984).

The question arises as to how one might implement in molecular terms the requirement of our model that the activity be proportional to a linear combination of the receptor states. One way to do this is to assume that the activity coefficients a_i of (15) represent affinities of binding of an effector molecule M to each of the receptor forms. On stimulation, the effector is removed from the intracellular medium by virtue of differential binding to receptor states. (Alternatively, the effector could be released upon stimulation.) Later the effector returns to its initial value because of the rearrangement of receptor states accompanying adaptation. The transient decrease (or increase) in M is assumed to trigger the behavioral response (Macnab, 1980).

To show explicitly how such a scheme might work, we assume that effector binding to the receptor is fast, so that the amounts of the four different effector–receptor complexes would be given by the following equilibrium relationships

$$RM = a_1 R \times M, \qquad XM = a_2 X \times M, \qquad YM = a_3 Y \times M, \qquad DM = a_4 D \times M. \quad (62)$$

Let M_T denote the total amount of M. Then conservation of M together with (15) and (62) implies

$$M_T = M + RM + XM + YM + DM, \quad \text{i.e. } M_T = M + MA(t). \quad (63)$$

Two possibilities now arise. A relevant physiological response **R** could be an increasing function of either of the concentrations M and $M_T - M$ of free and

bound effector, i.e. from (63)

$$\mathbf{R}=f\left(\frac{M_T}{1+A}\right) \quad \text{or} \quad \mathbf{R}=g\left(\frac{M_T A}{1+A}\right). \tag{64a, b}$$

The former possibility might find application in the liberation, upon stimulation, of one of the *che* gene products in bacterial chemotaxis. In the second possibility the activity coefficients may represent coupling constants between receptor and an intracellular enzyme, e.g. adenylate cyclase in the case of *D. discoideum.* Whatever the functions f and g in (64), in all situations, since $A(t)$ always returns to the same value at steady state, so will $M(t)$.

In case (64b), if $A \ll 1$

$$R \approx g(0)+g'(0)M_T A$$

and the response **R** above basal is proportional to the amount of activity above basal. Consider, however case (64b) in situations wherein stimulus induces a peak activity A_M that is much larger than unity. Here, although activity decreases exponentially from A_M, response **R** will saturate and remain constant over an extended period of time before it finally returns to its basal level. Generally speaking understanding of the activity A generated by the receptor may be only the first (essential) step in discerning the perhaps numerous links between receptor activity and physiological response. See Segel *et al.* (1985) for further discussion of this issue.

In the analysis of section 2 we did not take into account any binding of an intracellular effector to the four receptor species. This is justified if the total amount of effector M_T is small compared to the total amount of receptor R_T. Such an assumption may not be unrealistic, since sufficiently small M_T would naturally lead to stochastic fluctuations of the type thought to play a role in the random occurrence of tumbling in bacterial chemotaxis (Koshland, 1977).

Another way to implement our basic assumption (15a) is to assume that the activity coefficients represent the conductances of an ion channel. The "resting" concentration of ion in the cytoplasm would be determined by the balance between the influx due to the basal response of the adapting box and an efflux due to an active pumping mechanism (not considered here). Binding of stimulus to receptor would cause a transient increase in conductance and cytoplasm ion concentration. The cytoplasmic concentration would eventually return to the resting level, since the net conductance due to the box would return to its basal state. The activity coefficients may also represent relative enzymatic activities in the synthesis of an effector molecule, when the receptor possesses a catalytic site.

We found that the relative affinities of the two receptor states, R and D, had to differ significantly to obtain a strong increase in activity and also exact adaptation in the microscopically reversible case. Our requirement for a tight binding state in an adapting receptor system subject to microscopic reversibility was also found by Swillens & Dumont (1976) in their related study of receptor desensitization in response to hormone stimuli.

In contrast to the reversible case, if exact adaptation occurred via covalent modification a strong increase in activity could be obtained even if the two receptor

states had identical affinities for stimulus. Examples of both adaptation mechanisms exist. Desensitization of acetylcholine receptors is accompanied by a shift to a high affinity state (Heidmann & Changeux, 1978) whereas, in bacteria, adaptation to chemotactic stimuli appears not to bring about a change in affinity. The latter mechanism requires expenditure of energy to methylate the receptor.

Why should energy be expended in order to maintain a given binding affinity? Suppose that the receptor is bifunctional, i.e. in addition to its role as a sensor, which was investigated herein, it also carries out a second function. If the binding step were rate limiting in this second activity, the binding affinity change required for adaptation in the absence of covalent modification could have a deleterious effect on the second function. For example, in bacteria, some of the chemoreceptors are also transporters of the chemoattractants. If the affinity increased greatly as a result of adaptation, transport might be greatly reduced. Thus, the mechanism which had effectively directed the bacteria toward higher concentrations of attractant would render them incapable of utilizing it. Therefore, the energy required by covalent modification seems justified in this case.

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APPENDIX 1

Resolution of Receptor Kinetics Into Fast Binding and Slow Modification Steps

The text provides an informal treatment of the kinetic equations (1) and the conservation condition (2). The kinetic equations contain a mixture of fast and slow terms referring respectively to binding of the ligand and interconversion of the receptor states. A possible way to obtain approximate equations for the slow interconversion, a way different from that of the text, is to assume that the fast steps are in equilibrium and therefore that the binding terms are negligible compared to the slower interconversion terms. This alternative approach leads to the same steady state but to a different time course for the adaptation process. Analysis of particular simple models indicates that the second approach is not correct. To clarify this matter more fully we now show how a fairly standard application of singular perturbation theory (see for example Rubinow 1975, Section 2.3) can be used to justify the approach in Section 2 to equations (1) and (2). Cha (1968) gives a procedure to deal with kinetic schemes containing both slow and fast steps—but no indication of a proof is supplied.

We first note that eqns (1) and (2) are valid for $t > 0$, after ligand concentration has been elevated to the fixed value L_1. Initially the system is at the basal level, i.e.

$$\text{at } t = 0; \quad R = R_0, \quad X = 0, \quad Y = 0, \quad D = D_0 \tag{A1.1}$$

where R_0 and D_0 are given explicitly in (16).

Let us introduce the dimensionless variables

$$r \equiv \frac{R}{R_T}, \qquad x = \frac{X}{R_T}, \qquad y = \frac{Y}{R_T}, \qquad \delta = \frac{D}{R_T}, \qquad \tau = \frac{t}{1/k_{-r}}. \tag{A1.2}$$

In terms of these variables, the equations (1) and (2) and the initial conditions (A1.1) take the form

$$\mathrm{d}r/\mathrm{d}\tau = -\varepsilon l_1 r + \varepsilon l_{-1}\delta - \gamma r + x, \tag{A1.3a}$$

$$\mathrm{d}x/\mathrm{d}\tau = -\varepsilon l_2 x + \varepsilon l_{-2} y + \gamma r - x, \tag{A1.3b}$$

$$\mathrm{d}y/\mathrm{d}\tau = \varepsilon l_2 x - \varepsilon l_{-2} y + \lambda\gamma c\delta - \lambda y, \tag{A1.3c}$$

$$\mathrm{d}\delta/\mathrm{d}\tau = \varepsilon l_1 r - \varepsilon l_{-1}\delta - \lambda\gamma c\delta + \lambda y, \tag{A1.3d}$$

$$r + x + y + \delta \equiv 1, \tag{A1.3e}$$

$$\tau = 0: \qquad r = r_0, \quad x = 0, \quad y = 0, \quad \delta = \delta_0. \tag{A1.3f}$$

Here we have employed the new dimensionless parameters

$$\begin{gathered} \varepsilon l_1 \equiv \frac{k_1}{k_{-r}}, \qquad \varepsilon l_{-1} \equiv \frac{k_{-1}}{k_{-r}}, \qquad \varepsilon l_2 \equiv \frac{k_2}{k_{-r}}, \qquad \varepsilon l_{-2} \equiv \frac{k_{-2}}{k_{-r}}, \qquad \lambda \equiv \frac{k_{-d}}{k_{-r}}, \\ r_0 = \frac{R_0}{R_T}, \qquad \delta_0 = \frac{D_0}{R_T}. \end{gathered} \tag{A1.4}$$

We are interested in the situation wherein k_{-1}, k_1, k_{-2} and k_2 are very much smaller than k_{-r}, k_r, k_{-d} and k_d. For this reason we introduced the very small dimensionless number ε into the definitions of the dimensionless parameters that are constituted of the ratio of a rate constant from the first group to a rate constant from the second group. We suggested earlier that certain other parameters might be small or large; recall for example the conditions on c in (32) and (33). We assume that nonetheless the overriding difference in parameter magnitudes is expressed in ε.

The dimensionless problem (A1.3) is appropriate for the early development of the solution where $1/k_{-r}$ is a suitable time scale. (See Segel (1984, Chapter 4) for a discussion, in a biochemical context, of the crucial choice of time scale.) As a first approximation we equate to zero the terms multiplied by ε. This yields

$$\frac{\mathrm{d}r}{\mathrm{d}\tau} = -\gamma r + x, \qquad \frac{\mathrm{d}x}{\mathrm{d}\tau} = \gamma r - x, \qquad \frac{\mathrm{d}y}{\mathrm{d}\tau} = \lambda\gamma c\delta - \lambda y, \qquad \frac{\mathrm{d}\delta}{\mathrm{d}\tau} = -\lambda\gamma c\delta + \lambda y. \tag{A1.5}$$

It follows, using (A1.3f), that

$$r + x = r_0, \qquad y + \delta = \delta_0. \tag{A1.6}$$

Consequently,

$$\begin{gathered} r = \frac{r_0}{\gamma+1}[1 + \gamma\,\mathrm{e}^{-(\gamma+1)\tau}], \qquad x = \frac{r_0\gamma}{\gamma+1}[1 - \mathrm{e}^{-(\gamma+1)\tau}], \\ y = \frac{\delta_0\gamma c}{\gamma c+1}[1 - \mathrm{e}^{-\lambda(\gamma c+1)\tau}], \qquad \delta = \frac{\delta_0}{\gamma c+1}[1 + \gamma c\,\mathrm{e}^{-\lambda(\gamma c+1)\tau}]. \end{gathered} \tag{A1.7}$$

The solution thus far approximately describes the relatively fast attainment of a new equilibrium between R and X and between D and Y upon the addition of L. For large τ

$$r \approx \frac{r_0}{\gamma+1} \equiv r_i, \qquad x \approx \frac{r_0\gamma}{\gamma+1} \equiv x_i, \qquad y \approx \frac{\delta_0\gamma c}{\gamma c+1} \equiv y_i, \qquad \delta \approx \frac{\delta_0}{\gamma c+1} \equiv \delta_i. \tag{A1.8}$$

The formulae in (A1.8) are the dimensionless forms of the corresponding relations in (8).

To follow the further development of the solution, we employ the values of (A1.8) as initial conditions in a new calculation on a much slower time scale. The new time scale must permit interchange between R and D and between X and Y. This will occur if we define a new time variable τ_s by

$$\tau_s = \varepsilon\tau.$$

We shall use tildes to distinguish the concentrations in the "slow" layer that we are about to consider. It turns out that these concentrations must be examined to a second approximation. We thus write

$$\tilde{r}(\tau_s) = \tilde{r}_0(\tau_s) + \varepsilon\tilde{r}_1(\tau_s) + \ldots,$$

with similar expressions for $\tilde{x}$, $\tilde{y}$ and $\tilde{\delta}$. The equation for $\tilde{r}$ implies that

$$\varepsilon\frac{\mathrm{d}\tilde{r}_0}{\mathrm{d}\tau_s} + \ldots = -\varepsilon l_1\tilde{r}_0 + \varepsilon l_{-1}\tilde{\delta}_0 - \gamma(\tilde{r}_0 + \varepsilon\tilde{r}_1 + \ldots) + \tilde{x}_0 + \varepsilon\tilde{x}_1 + \ldots.$$

Collecting powers of ε, and equating each to zero in turn, we obtain

$$-\gamma\tilde{r}_0 + \tilde{x}_0 = 0, \qquad \frac{\mathrm{d}\tilde{r}_0}{\mathrm{d}\tau_s} = -l_1\tilde{r}_0 + l_{-1}\tilde{\delta}_0 - \gamma\tilde{r}_1 + \tilde{x}_1, \ldots.$$

The other equations are treated similarly. We thus obtain at lowest order from the equations for $\tilde{r}$, $\tilde{x}$, $\tilde{y}$ and $\tilde{\delta}$

$$-\gamma\tilde{r}_0 + \tilde{x}_0 = 0, \qquad -\gamma\tilde{r}_0 + \tilde{x}_0 = 0, \qquad \gamma c\tilde{\delta}_0 - \tilde{y}_0 = 0, \qquad \gamma c\tilde{\delta}_0 - \tilde{y}_0 = 0.$$

We cannot solve for the four unknown functions at this stage: all we know from the above equations is that these functions are constrained by

$$\tilde{x}_0 = \gamma\tilde{r}_0, \qquad \tilde{y}_0 = c\gamma\tilde{\delta}_0. \tag{A1.9}$$

The terms proportional to ε give

$$\begin{aligned}
\mathrm{d}\tilde{r}_0/\mathrm{d}\tau_s &= -l_1\tilde{r}_0 + l_{-1}\tilde{\delta}_0 - \gamma\tilde{r}_1 + \tilde{x}_1 \\
\mathrm{d}\tilde{x}_0/\mathrm{d}\tau_s &= -l_2\tilde{x}_0 + l_{-2}\tilde{y}_0 + \gamma\tilde{r}_1 - \tilde{x}_1 \\
\mathrm{d}\tilde{y}_0/\mathrm{d}\tau_s &= l_2\tilde{x}_0 - l_{-2}\tilde{y}_0 + \lambda\gamma c\tilde{\delta}_1 - \lambda\tilde{y}_1 \\
\mathrm{d}\tilde{\delta}_0/\mathrm{d}\tau_s &= l_1\tilde{r}_0 - l_{-1}\tilde{\delta}_0 - \lambda\gamma c\tilde{\delta}_1 + \lambda\tilde{y}_1.
\end{aligned} \tag{A1.10}$$

By adding, respectively, the first and second pairs of the equations (A1.10) and

employing (A1.9), we obtain

$$c(1+\gamma)\,\mathrm{d}\tilde{x}_0/\mathrm{d}\tau_s = -c\tilde{x}_0(l_1+l_2\gamma)+\tilde{y}_0(l_{-1}+l_{-2}c\gamma) \tag{A1.11a}$$

$$(1+c\gamma)\,\mathrm{d}\tilde{y}_0/\mathrm{d}\tau_s = c\tilde{x}_0(l_1+l_2\gamma)-\tilde{y}_0(l_{-1}+l_{-2}c\gamma). \tag{A1.11b}$$

The unknown functions $\tilde{r}_0(\tau_s)$, $\tilde{\delta}_0(\tau_s)$, $\tilde{x}_0(\tau_s)$ and $\tilde{y}_0(\tau_s)$ can now be determined by (A1.11), (A1.9) and the initial conditions—from (A1.8)

$$\tilde{r}_0(0)=r_i, \qquad \tilde{\delta}_0(0)=\delta_i, \qquad \tilde{x}_0(0)=x_i, \qquad \tilde{y}_0(0)=y_i. \tag{A1.12}$$

The equations (A1.11) and the boundary conditions imply

$$c(1+\gamma)\tilde{x}_0+(1+c\gamma)\tilde{y}_0=c(1+\gamma)x_i+(1+c\gamma)y_i=c\gamma(r_0+\delta_0)=c\gamma.$$

Elimination of $\tilde{y}_0$ in (A1.11a) yields an equation that can be written

$$\frac{\mathrm{d}\tilde{x}_0}{\mathrm{d}\tau_s}=\tilde{V}-\tilde{W}\tilde{x}_0 \tag{A1.13}$$

where

$$k_{-r}\tilde{V}=\frac{1}{\theta\psi}\left[l_{-2}+\frac{l_{-1}}{c\gamma}\right], \qquad k_{-r}\tilde{W}=\theta^{-1}\left(\frac{l_1}{\gamma}+l_2\right)+\psi^{-1}\left(l_{-2}+\frac{l_{-1}}{c\gamma}\right).$$

This equation is equivalent to (10), which completes the justification of our earlier calculations.

We emphasize that the function x of (A1.7) is an approximation to the dimensionless X concentration during the fast transient period, while the appropriate solution to (A1.13), namely

$$\tilde{x}_0(\tau_s)=\frac{\tilde{V}}{\tilde{W}}+\left(x_i-\frac{\tilde{V}}{\tilde{W}}\right)\mathrm{e}^{-\tilde{W}\tau_s},$$

provides an approximation for the slow readjustment period. Singular perturbation theory shows that an approximation x that is valid throughout both periods is

$$x(t)=x(k_{-r}t)+\tilde{x}_0(\varepsilon k_{-r}t)-\lim_{\tau\to\infty}x(\tau),$$

so that

$$\frac{X(t)}{R_T}=-\frac{r_0\gamma}{\gamma+1}\mathrm{e}^{-(\gamma+1)k_{-r}t}+\frac{\tilde{V}}{\tilde{W}}+\left(\frac{r_0\gamma}{\gamma+1}-\frac{\tilde{V}}{\tilde{W}}\right)\mathrm{e}^{-\varepsilon\tilde{W}k_{-r}t}.$$

There are three independent differential equations in the governing (linear) system (1), so that we expect to observe three different exponentials. The exponents are $(K_R^{-1}L_1+1)k_{-r}$ for the fast initial $R-X$ interconversion, $(K_D^{-1}L_1+1)k_{-d}$ for the concurrent fast D-Y interconversion, and $\varepsilon\tilde{W}k_{-r}$ for the later slow interconversion among all four forms.

It follows from (12a) that during the slow modification phase the activity A decreases (increases) monotonically if the net response to binding of ligand during the fast phase is an increase (decrease) of A. Activity often increases (decreases)

monotonically during the fast binding period as well, but it turns out that there are conditions where a single extremum can exist during this period, for example when

$$\frac{\gamma+c^{-1}}{\gamma+1}<\frac{k_{-1}-k_{-1}}{k_2-k_{-1}}<\frac{k_r}{k_d}. \tag{A1.14}$$

APPENDIX 2

Constraints on the Parameters of the Receptor "Box": the Case of Covalent Modification

When the states R and X are covalently modified into the states D and Y (see Fig. 1) the question arises as to whether the conditions of microscopic reversibility (28) and (29) hold for the receptor "box". We show here that this particular relation does not hold in the general case of covalent modification.

In this general case, the modification of $R(X)$ into $D(Y)$ and the reverse transition from $D(Y)$ to $R(X)$ are catalyzed by two distinct enzymes. For example protein phosphorylation and dephosphorylation are catalyzed by a protein kinase and a protein phosphotase, respectively. Such a situation probably pertains to the cAMP receptor in *D. discoideum.* Similarly, a methyltransferase and a methylesterase catalyze methylation and demethylation of proteins, as observed for bacterial chemoreceptors. Moreover, in these two cases the two modifying enzymes operate with different cofactors (Fig. A1). In the example of phosphorylation-dephosphory-

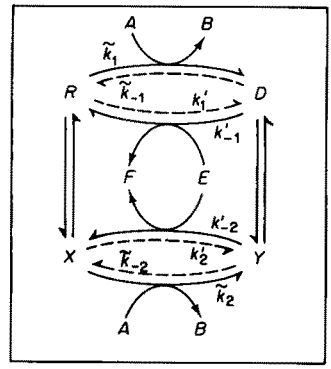

FIG. A1. Covalent modification of the receptor. Solid arrows indicate binding of ligand and predominant reactions for the interconversion of R into D and of X into Y (see text).

lation, which will be treated here for definiteness, A and B refer to ATP and ADP, while E and F refer to H_2O and P_i, respectively.

The rate constants $\tilde{k}_1$, $\tilde{k}_2$ and $\tilde{k}_{-1}$, $\tilde{k}_{-2}$ refer to the direct and reverse steps of the kinase reaction, whereas the rate constants k_1', k_2' and k_{-1}', k_{-2}' relate to the backward and forward steps of the phosphatase reaction.

At equilibrium detailed balance applied to each of the six reactions yields the two conditions

$$(\tilde{k}_{-1}/\tilde{k}_1) = (K_R/K_D)(\tilde{k}_{-2}/\tilde{k}_2) \tag{A2.1}$$

and

$$(k'_{-1}/k'_1) = (K_R/K_D)(k'_{-2}/k'_2). \tag{A2.2}$$

These relations still hold when the system is driven far from equilibrium by changing the (independent) ratios B/A and E/F. Moreover if the cofactor concentrations are regarded as constants one can rewrite (A2.1) and (A2.2) in terms of pseudo first order rate constants

$$(\tilde{k}_{-1}B/\tilde{k}_1A) = (K_R/K_D)(\tilde{k}_{-2}B/\tilde{k}_2A) \tag{A2.3}$$

$$(k'_{-1}E/k'_1F) = (K_{-R}/K_D)(k'_{-2}E/k'_2F). \tag{A2.4}$$

The pair of independent constraints (A2.1) and (A2.2) (or (A2.3) and (A2.4)) reflects the existence of two cycles in the system of Fig. A1, as illustrated in Fig. A2.

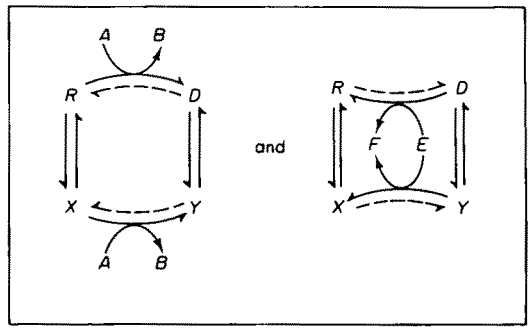

FIG. A2. The two cycles that constitute the scheme of Fig. A1.

Far from equilibrium, the system predominantly operates following the solid arrows in the scheme of Fig. A1, i.e. the phosphorylation of the receptor is catalyzed primarily by the kinase, whereas dephosphorylation is catalyzed principally by the phosphatase. This approximate scheme is identical with that of Fig. 1 when the kinetics parameters are defined as

$$k_1 = \tilde{k}_1A, \qquad k_{-1} = k'_{-1}E, \qquad k_2 = \tilde{k}_2A, \qquad k_{-2} = k'_{-2}E. \tag{A2.5}$$

As shown by eqns (A2.3) and (A2.4) the rate constants k_1, k_{-1}, k_2, k_{-2} of (A2.5) do not have to obey the particular constraint of microscopic reversibility (28) (or (29)) since they are associated with a "hybrid" cycle constructed from two true thermodynamic cycles. Indeed microscopic reversibility constraints exist for each of these two cycles, i.e. conditions (A2.3) and (A2.4), but these do not imply condition (29).

APPENDIX 3

Positivity of Activity Coefficients and Initial Activity

The requirement that activity coefficients be positive (which is implicit in the molecular implementations proposed in the Discussion) leads to constraints on the coefficients. For example, consider (18c). Upon rearrangement, this equation yields

$$a_1 = B + K_1^{-1}(B - a_4).$$

If $a_1 > 0$ then $a_4 < B(1+K_1)$. Similarly, if (18c) is solved for a_4 and the positivity of a_4 is involved, we find that $a_1 < B(1+K_1^{-1})$. Identical treatment of (26b) yields the final result that in the reversible case, the following are conditions for the a_i to be non-negative

$$\frac{a_1}{B} \leq 1 + K_1^{-1}, \qquad \frac{a_2}{B} \leq 1 + K_2^{-1}, \qquad \frac{a_3}{B} \leq 1 + K_2, \qquad \frac{a_4}{B} \leq 1 + K_1. \tag{A3.1}$$

If one of the pair (a_1, a_4) is chosen according to (A3.1) then the other member of the pair, given by (18c), will be non-negative. The same holds for the pair (a_2, a_3), which are related by (26b).

To examine relative initial activity in the reversible case, we focus attention on the terms $a_2R_0 + a_3D_0$ in (30b). Use of (16) and (44) yields

$$a_2R_0 + a_3D_0 = \frac{R_T}{K_1+1}[BK_1(1+K_2^{-1}) + a_3(1 - K_1K_2^{-1})]. \tag{A3.2}$$

There are now two situations. If $K_1 > K_2$ then $a_2R_0 + a_3D_0$ is largest when $a_3 = 0$. If $K_1 < K_2$ we maximize activity by replacing a_3 by its largest possible value as obtained from (A3.1). We thus obtain, using $A_0 \equiv BR_T$, the following bounds on the maximum initial activity

$$\alpha(\infty) \leq \frac{K_1 - K_2}{K_2(K_1+1)} \quad \text{if } K_1 > K_2; \qquad \alpha(\infty) \leq \frac{K_2 - K_1}{K_1+1} \quad \text{if } K_2 > K_1. \tag{A3.3}$$

The equals signs in (A3.3) are appropriate if

$$a_2 = B(K_2^{-1}+1), \qquad a_3 = 0, \quad \text{or} \quad a_3 = B(1+K_2), \qquad a_2 = 0, \tag{A3.4}$$

respectively.

Let us turn to the irreversible case. Given (34c), we find that the requirement that all the a_i's be non-negative leads, instead of (A3.1), to

$$1 - \frac{k_1}{k_2} \leq \frac{a_1}{B} \leq 1 + \min\left(\frac{k_1}{k_{-1}}, \frac{k_1}{k_{-2}}\right), \qquad 1 - \frac{k_2}{k_1} \leq \frac{a_2}{B} \leq 1 + \min\left(\frac{k_2}{k_{-1}}, \frac{k_2}{k_{-2}}\right), \tag{A3.5a, b}$$

$$1 - \frac{k_{-2}}{k_{-1}} \leq \frac{a_3}{B} \leq 1 + \min\left(\frac{k_{-2}}{k_1}, \frac{k_{-2}}{k_2}\right), \qquad 1 - \frac{k_{-1}}{k_{-2}} \leq \frac{a_4}{B} \leq 1 + \min\left(\frac{k_{-1}}{k_1}, \frac{k_{-1}}{k_2}\right). \tag{A3.5c, d}$$

Here by "min (α, β)" we mean the smaller of the quantities α and β. These "min" expressions arise from combining two of the inequalities that are obtained by manipulating the six equations that are included in (34c)—under the requirement

that one or the other of the a's contained in each is positive. If any one of the activity coefficients is chosen according to (A3.5), then the other three, given by (34c), will be non-negative.

Turning to maximum peak activity in the irreversible case, using (16) we observe that

$$(k_2-k_1)R_0-(k_{-2}-k_{-1})D_0=R_Tk_2(K_1-K_2)/(K_1+1). \tag{A3.6}$$

Employing $a_1R_0+a_4D_0=A_0=BR_T$ and (35), we write

$$\alpha(\infty)=\frac{(a_1-a_4)k_2(K_1-K_2)}{(K_1+1)(k_{-1}+k_1)B} \quad \text{if } K_1>K_2; \tag{A3.7a}$$

$$\alpha(\infty)=\frac{(a_4-a_1)k_2(K_2-K_1)}{(K_1+1)(k_{-1}+k_1)B} \quad \text{if } K_2>K_1. \tag{A3.7b}$$

From (18c) and (A3.5a)

$$a_1-a_4=(a_1-B)(1+K_1)\leq\left[\min\left(\frac{k_1}{k_{-1}},\frac{k_1}{k_{-2}}\right)\right]B(1+K_1). \tag{A3.8}$$

It follows from (A3.7a) that

$$\alpha(\infty)\leq\left[\min\left(\frac{k_2}{k_{-1}},\frac{k_2}{k_{-2}}\right)\right]\frac{K_1-K_2}{1+K_1} \quad \text{if } K_1>K_2. \tag{A3.9a}$$

The corresponding formula

$$\alpha(\infty)\leq\left[\min\left(\frac{k_{-2}}{k_1},\frac{k_{-2}}{k_2}\right)\right]\frac{K_2-K_1}{K_2(1+K_1)} \quad \text{if } K_2>K_1, \tag{A3.9b}$$

is an immediate consequence of the alternative to (A3.8) that is obtained by using (18c) to write a_1 in terms of a_4

$$a_4-a_1=(a_4-B)K_1^{-1}(K_1+1)<\left[\min\left(\frac{k_{-1}}{k_1},\frac{k_{-1}}{k_2}\right)\right]B(1+K_1^{-1}). \tag{A3.10}$$

Note that bounds (A3.9a) and (A3.9b) are somewhat more restrictive than their counterparts (A3.3) in the reversible case. Again, an arbitrarily large response can theoretically be achieved. In cases (A3.9a) and (A3.9b), respectively, this requires

$$k_2\gg k_{-1}, \qquad k_2\gg k_{-2}, \qquad K_1 \text{ not small}, \tag{A3.11a}$$

or

$$k_{-2}\gg k_1, \qquad k_{-2}\gg k_2, \qquad K_1 \text{ not large}. \tag{A3.11b}$$

As noted in the text, conditions for large initial activity are associated with certain properties of recovery times. To explore this matter, let us consider the situation of (53) when $t_{1/2}$ is independent of stimulus level. If

$$k_{-1}-k_{-2}=k_2-k_1>0$$

then (A3.9a) reduces to $\alpha(\infty)\leq 1-(k_{-2}/k_{-1})$. Thus in these circumstances $\alpha(\infty)\leq 1$, i.e. $A_M\leq 2A_0$. It follows that the maximum of $\alpha(\infty)$ will be of magnitude unity unless there is a marked dependence of recovery time on stimulus level.