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A model for switch-like phenomena in biological systems

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Abstract

We present a model for the activity of protein clusters based on a simultaneous desorption of an activator (agonist, substrate molecule, etc.) and an inactivator (antagonist, inhibitor, etc.) caused by the collision or interaction between two effector molecules (e.g. receptors, enzymes). This model gives rise to switch-like dose–response curves, which are difficult to explain by ordinary co-operativity. It fits with recent experimental results obtained on single cells. Some other interesting aspects of the model are also pointed out. The model is similar to the model used to explain steep 'dose–response curves' in heterogeneous catalysis, caused by the reaction between two different molecules or atoms on the surface of the catalyst.

Keywords: Protein clusters; Dose–response curves; Large Hill coefficients; Enzymatic activity; Substrate and inhibitor; Agonist and antagonist

1. Introduction

Several biological ligand–receptor controlled reactions show very steep dose–response curves. Such curves are often described by an empirical equation called the Hill equation, which relates the response to the stimuli through an equation of the form

$$R = \frac{S^n}{S^n + S_0^n} \tag{1}$$

where n is the Hill exponent, S and R the stimuli and response, respectively, and S_0 the concentration of the stimuli giving half maximum response.

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A curve with $n = 1$ describes, e.g. a response determined by a first order binding isotherm or a Michaelis–Menten enzyme kinetics, while $n > 1$ describes a sigmodial dose–response curve. The size of n determines the steepness, or the ultrasensitivity [1] of the sigmodial response curve. One familiar example of such ultrasensitivity is found in co-operative enzymes or co-operative binding reactions. The Hill coefficient for oxygen binding by hemoglobin is, e.g. 2.8 [2]. Several mechanisms have been suggested to explain even larger Hill coefficients observed in many systems as discussed particularly by Koshland et al. [3–5] and Ferrell et al. [6–8]. These are related to stoichiometric inhibition, saturation effects and to cascade reactions. It has also been suggested that enzymatic feedback reactions in combination with such effects can yield switch-like dose–response curves with very large Hill coefficients [9,10]. Recently, it was suggested that solute molecule-induced changes in the lateral pressure profile of a cell membrane could lead to a shift in the conformational equilibrium of membrane spanning proteins. The dose–response predictions of this mechanism fit the Hill equation rather well with large Hill coefficients [11]. We have been looking for other simple mechanisms that could yield a switch-like behavior for both membrane and cytoplasm located biological interactions taking place in clusters of proteins. One reason for this has been the observation that signal transduction and amplification often occur in molecular clusters, and involve activating as well as inactivating molecules.

It is well known from heterogeneous catalysis that so-called kinetic phase transitions may occur where the surface coverage of the catalyst shifts between two species, e.g. between hydrogen and oxygen [12,13]. This drastically changes the activity of the catalyst and gives rise to switch-like 'dose–response' curves, with very large 'apparent' Hill coefficients. Interestingly, with some simple assumptions about the properties of the interacting molecules, similar all or none situations can be obtained also in biological systems. Here we thus suggest a possible mechanism for switch-like behavior in biological systems based on an induced simultaneous desorption of activating and inactivating molecules bound to, e.g. receptors or enzymes, a mechanism that resembles the kinetic phase transitions mentioned above. The processes leading to the switch-like phenomena are driven by metabolic energy.

The hypothesis above was also presented at a recent meeting of the Swedish Biophysical Society [14].

2. Model for switch-like dose–response curves

Two of the simplest possibilities to obtain switch-like dose–response curves are shown in Fig. 1. The first one can be called collision-induced desorption, which may occur both in the cytoplasm and in cell membranes (Fig. 1a). In this case we assume that a molecule M is activated by molecule A, and inactivated by a second molecule B. A and B compete for the same binding site on M. It is assumed that upon collision of two molecules M, one occupied by A and one by B, there is a substantial increase in the probability that A and B desorb simultaneously.

In the second case as in Fig. 1b, we have an aggregate or cluster of molecules (in the cytoplasm or in membranes) with the same general assumption as above, namely that A and B molecules compete for the same sites on the molecules M and that a significant increase in the rate of the simultaneous desorption of A and B occurs when they occupy neighboring molecules. It should be noted that it is not necessary that the M molecules are of similar kinds, only that they have the properties given to them above.

The rate equations describing the suggested reaction scheme are

$$\frac{d\theta_A}{dt} = k_A(C_A - \theta_A)(1 - \theta_A - \theta_B) - d_A\theta_A - r\theta_A\theta_B \tag{2}$$

$$\frac{d\theta_B}{dt} = k_B(C_B - \theta_B)(1 - \theta_A - \theta_B) - d_B\theta_B - r\theta_A\theta_B \tag{3}$$

where the concentrations are normalized with the

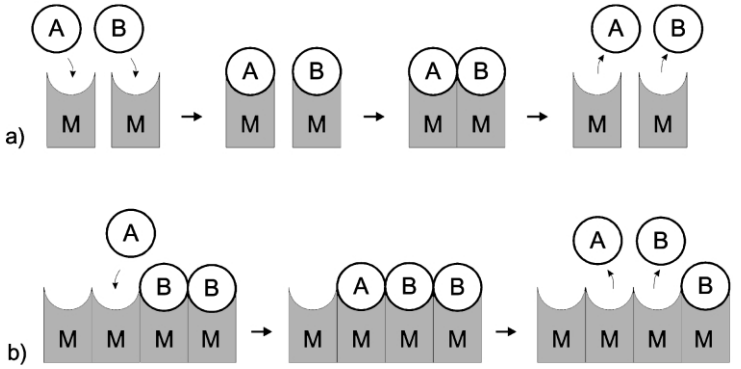

Fig. 1. Two simple situations which can lead to switch-like dose–response curves. (a) Two types of molecules A and B are supposed to compete for the same binding site on a molecule M. Upon collision between two molecules M with molecules of type A and B bound, respectively, A and B desorb simultaneously with a large rate. (b) A similar situation may be obtained in molecular clusters where neighboring bound A and B molecules desorb simultaneously with a large rate. The phenomena in (a) and (b) may occur between molecules both in the cytoplasm and in membranes. The amount of bound molecules A (and B) will show a switch-like behavior at a concentration ratio approximately given by $k_A C_A / k_B C_B$ [see Eqs. (2) and (3)]. If, e.g. molecule A is an agonist of a receptor or a substrate of an enzyme and B an antagonist or inhibitor of M, a switch-like dose–response curve may be obtained around this critical ratio.

concentration of M and the values of θ are the fractional occupancy by molecule A or B of binding sites on M, respectively. The constants k, d and r are rate constants (with the dimension s^{-1}). Eqs. (2) and (3) resemble those for competitive inhibition of an enzyme except for the last term, which is due to the collision or interaction of M molecules occupied by an A and B molecule, respectively, leading to a desorption of both A and B. The $\theta_A \theta_B$ term gives the steady state solution to the equations a switch-like behavior. This is most easily seen by assuming an excess of C_A and C_B (i.e. $\gg 1$) and neglecting the spontaneous desorption described by d_A and d_B. In that case, $1 - \theta_A - \theta_B$ must be zero at steady state (and thus also $\theta_A \theta_B = 0$). The only solutions are therefore $\theta_A = 1$, $\theta_B = 0$ or $\theta_A = 0$ and $\theta_B = 1$ with a transition at the point where $k_A C_A = k_B C_B$. With the desorption mathematically equal to zero the transition is, however, kinetically forbidden, so the example above is only hypothetical. Even with more realistic parameters, however, a sharp transition in the occupancy occurs at or close to the point given by the critical ratio defined above. To illustrate the behavior of the suggested model, we have solved Eqs. (2) and (3) explicitly for the case when C_A and $C_B \gg 1$, $k_A = k_B = k$ and $d_A = d_B = d$.

$$\theta_A = \frac{g_1}{2} \pm \sqrt{\frac{g_1^2}{4} + g_2} \tag{4}$$

and

$$\theta_B = \frac{1 - \theta_A}{1 + d + r\theta_A} \tag{5}$$

where

$$g_1 = \frac{1 - \beta + \frac{d}{r}(1 + \beta) + \frac{d^2}{rkC_B}}{1 - \beta - d/kC_B} \tag{6}$$

and

$$g_2 = \frac{d}{r} \frac{\beta}{\beta - 1 + d/kC_B} \tag{7}$$

with

$$\beta \equiv C_A / C_B$$

The plus sign before the square root in Eq. (4) should be used except when $g_1 > 0$ and $g_2 < 0$,

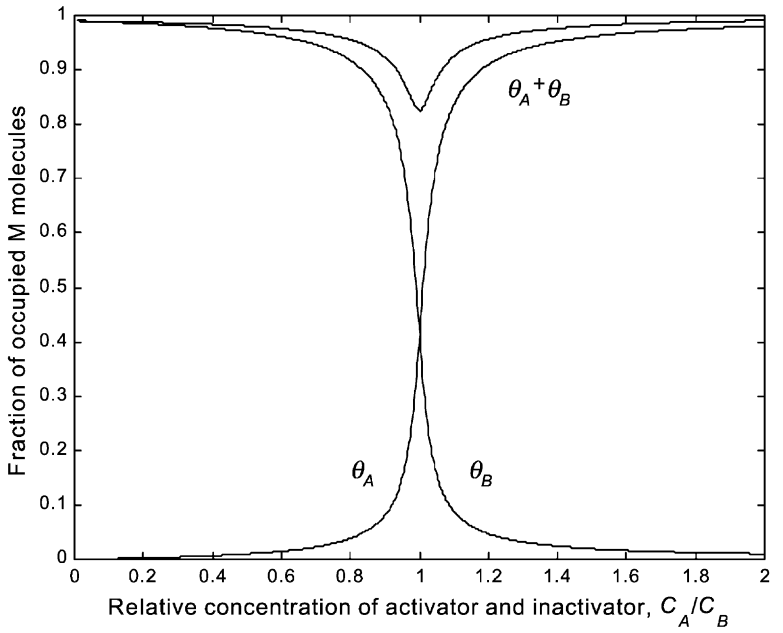

Fig. 2. Fraction of M molecules occupied by A, B, and their sum plotted vs. the relative concentration of A and B for $d = 0.01$ kC_B and $r = kC_B$.

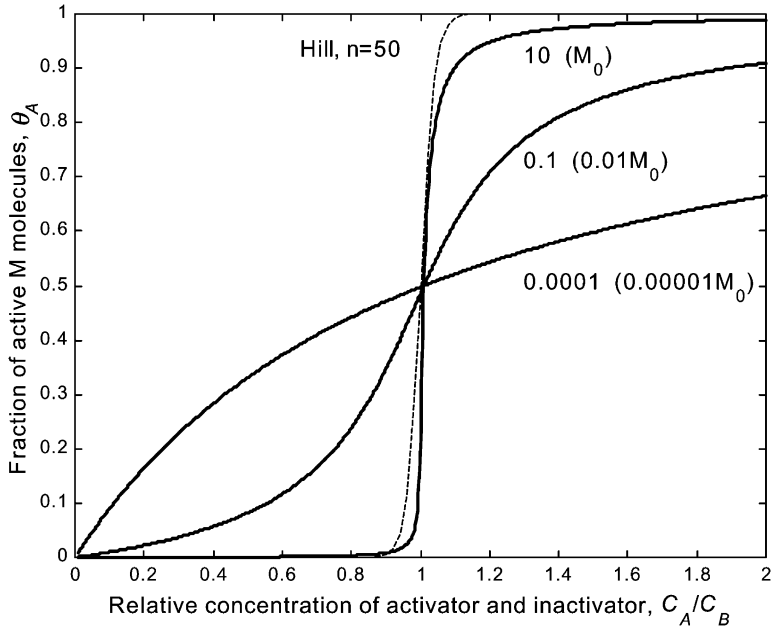

Fig. 3. Model calculations. In the calculations $d = 0.01$ kC_B and $r = r_n kC_B$, with the values of r_n given on the curves. The dashed line is a Hill curve with $n = 50$ and $S_0 = 1$ (and $S = C_A/C_B$). The parameters in parenthesis illustrate how the shape of the response curve may change with the concentration of M, C_M ($C_M = M_0$ is assumed to give a $r_n = 10$).

simultaneously (which may occur for small β-values).

The behavior of the solution above is illustrated in Fig. 2 where θ_A, θ_B, and $\theta_A + \theta_B$ are plotted vs. C_A/C_B for a given set of parameters. Since $k_A = k_B$, a sharp transition from B to A occupancy of M occurs at $C_A/C_B = 1$ for sufficiently large r. For the smallest r, the result is close to a normal Langmuir like dose–response curve for competitive inhibition obtained with $r = 0$ in Eqs. (2) and (3). Fig. 3 shows the fraction of active M molecules (i.e. θ_A) for different relative sizes of k, r and d. In this figure, a hypothetical Hill curve, Eq. (1), with $n = 50$, $S_0 = 1$ and $S = C_A/C_B$ is also shown.

3. Other consequences of the model

The rate of collision between two molecules M is proportional to C_M^2, where C_M is the concentration of M. The desorption rate r in Eqs. (2) and (3) related to the coverages is therefore, proportional to C_M (since the equations are normalized by C_M). This means that not only the absolute size of the response ($\sim C_M\theta_A$), but also its shape will change with C_M as indicated in Fig. 3.

A fine tuning of the response can be obtained through a regulator molecule R. This can be achieved in several ways as illustrated in Fig. 4. In all cases, we assume that one or two molecules R are involved in the collision or interaction leading to the rapid desorption of A and B. For a given concentration of M the desorption rate depends now on C_R, the concentration of R. This, in turn, influences the shape of the dose–response curve. As a consequence, at constant concentrations of M, A and B, the coverage of θ_A (the activity) may be fine tuned by the concentration of the regulator R as illustrated in Fig. 5.

Another interesting property of the model is that it can predict a very narrow peak in the rate of production of a product in the case the protein cluster needs both M–A and M–B to be active. The total activity depends then on $\theta_A\theta_B$ which leads to a narrow activity peak at the stoichiometric ratio of A and B if r is sufficiently large, as

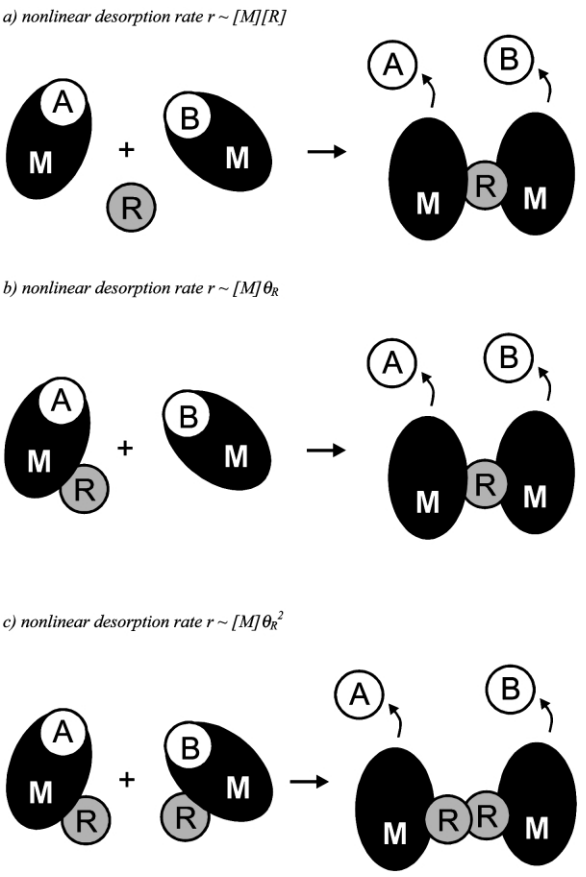

Fig. 4. (a) Induced desorption through three molecular collision. (b) and (c) Regulator molecule adsorption to M (other site than for A and B) triggers desorption upon collision or interaction between two M molecules. For simple non-competitive adsorption of R, $\theta_R = C_R/(C_R + K)$. K is the concentration of R giving half the maximum coverage of R on M.

shown in Fig. 6. It is observed that a similar activity curve controlled by ordinary competitive adsorption is much broader. The assumption of a 'non-linear' desorption thus leads to the possibility to control the activity of a protein cluster in a very narrow concentration interval.

4. Discussion

We have presented a simple model for reactions in protein clusters, either in solution or in membranes. The model is based on an increased

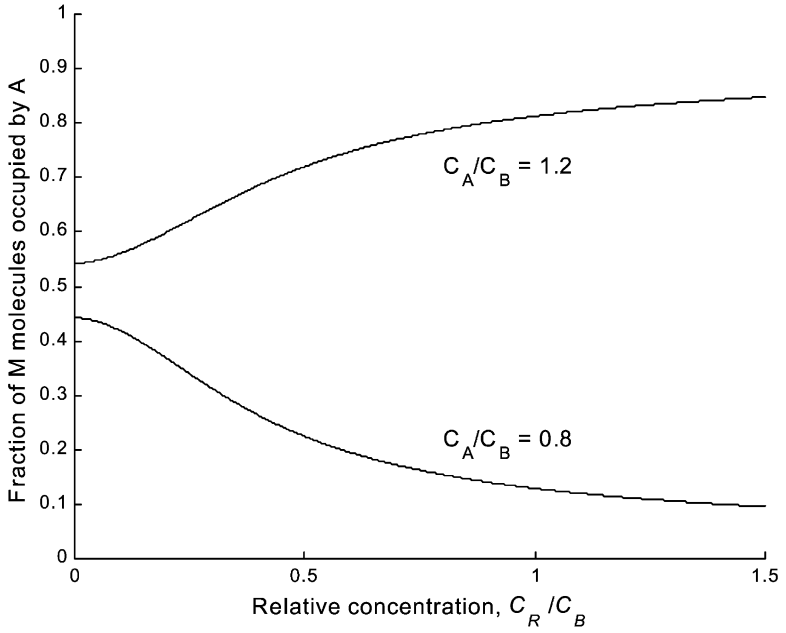

Fig. 5. Control of activity: the drawing shows θ_A for the situation in Fig. 4c. In the calculations $r = 10\theta_R^2$, $d = 0.01kC_B$, and $\theta_R = (C_R/C_B)/(C_R/C_B + 1)$.

non-linear desorption caused by the interaction between the molecules in the clusters. It is remarkable how such a simple model can predict phenomena, which otherwise need large co-operativity, cascade reactions or feedback loops to be explained.

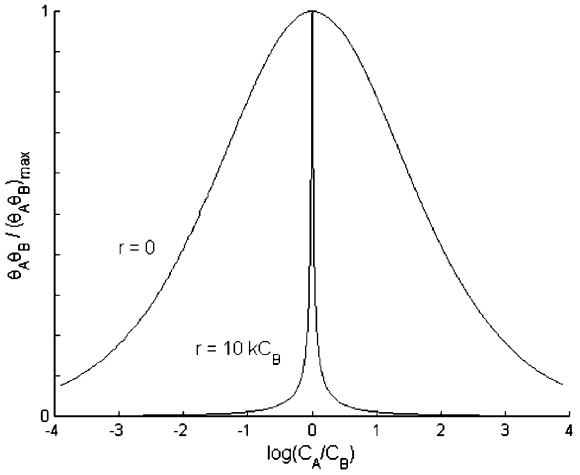

Fig. 6. The drawing shows $\theta_A\theta_B/(\theta_A\theta_B)_{\max}$ for the case when $d = 0.01kC_B$ and $r = 10kC_B$ (large non− linear desorption), and $r = 0$ (ordinary competitive adsorption/desorption).

One of the first observations is that the presented model may give dose–response curves with very large equivalent Hill coefficients. The details of the proposed switching mechanism are determined by the rate constants, eventual diffusion limitations, and other left out possibilities like increased desorption of two As (or Bs) on colliding or neighboring M molecules. Furthermore, there is no reason why the rate constants for A and B should be the same, as assumed in the calculations presented. This will not influence the qualitative conclusions made, however, as long as the collision (or interaction)-induced simultaneous desorption of A and B dominates. The model requires thus that molecules with bound A and B, respectively, have conformations which upon collision (interaction) induce a large desorption of A and B.

We also assume that solely the number of active Ms determine the dose–response curve.

This is not necessarily always the case, which could influence the shape of the dose–response curve. The situation at hand corresponds, e.g. to an enzyme M with substrate A (and inhibitor B) in a large excess of M.

A comparison between cellular phenomena and heterogeneous catalysis may be farfetched. Interestingly, however, switching phenomena similar to that proposed here occur also in heterogeneous catalytic reactions, where the coverage of catalyst surface changes between two types of molecules at a specific concentration ratio of the molecules given by the reaction conditions. The reaction between oxygen and hydrogen on a platinum surface is a simple example, which shows such a switching phenomenon [12,13]. This gives for example, an all-or-none response to hydrogen of a semiconductor device with a platinum gate as shown in Fig. 7a. Without pushing the analogy too far, a 'recombination' between activators and inactivators on neighboring molecules as suggested in this paper, would be the biochemical equivalent as illustrated in Fig. 7b.

One of the largest experimentally determined Hill exponents so far was found in the response of individual *Xenopus laevis* oocytes to the maturation-inducing hormone progesterone [9]. A small change in the concentration of progesterone was converted into an all-or-none response very similar to the curve with $r_n = 10$ in Fig. 3. The analysis of individual oocytes showed that the response of the mitogen activated protein kinase (MAPK) to progesterone was equivalent to a Hill equation with a coefficient of at least 35 [9]. The all-or-none response was explained by the intrinsic ultrasensitivity of the MAPK cascade and a positive feedback loop in the cascade. Also, this mechanism requires the interaction between several molecules in the biological system. We thus suggest that another possible mechanism for the creation of switch-like behavior in cellular responses is the collision or interaction between two or more molecules, one occupied by an activator and another by an inactivator. The only assumption is that the rate of desorption of these increases considerably upon collision, as illustrated in Fig. 1. The driving force for this behavior could be the conformational

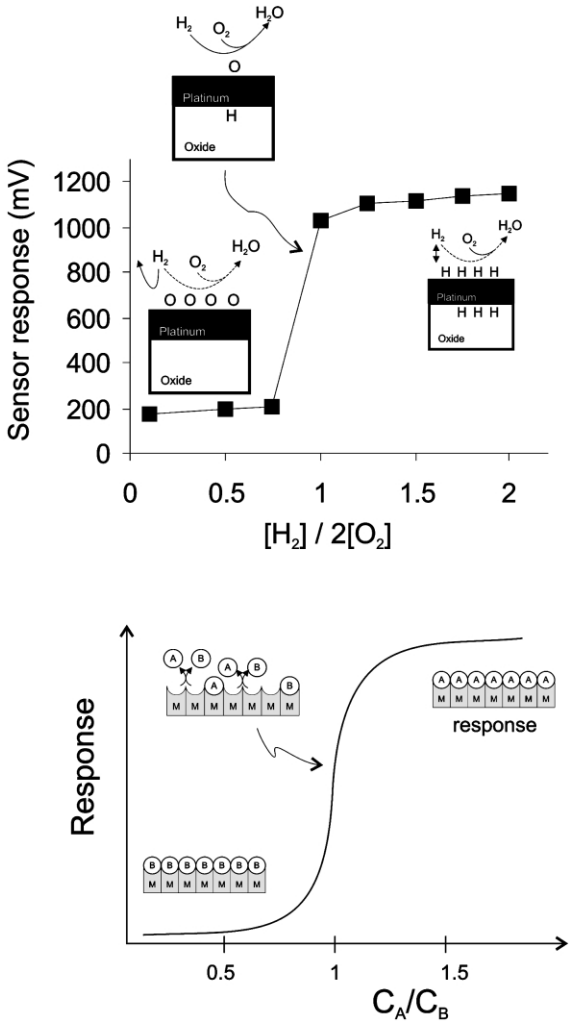

Fig. 7. (a) Experimentally determined response to hydrogen of a Pt–SiO_2–SiC device exposed to hydrogen-oxygen mixtures. Device temperature equals 500°C. The switch-like response is caused by a blocking of oxygen adsorption by hydrogen atoms and vice versa, and an efficient water production ('recombination') in the transition region (data from Baranzahi et al. [13]). The concentration ratio at which the transition occurs depends on the reaction conditions (flow rate, temperature, etc.). (b) Schematics of a molecular 'recombination' mechanism causing a switch-like dose response. The recombination between A and B is only to show that they desorp simultaneously and is not necessarily a real physical phenomenon in this case. The M molecules or the bound A and B molecules are allowed to move freely within the cluster.

changes occurring in the receptor molecules upon binding of an activator and/or an inactivator, or

an interaction between bound activators and inactivators. We believe that the suggested model (and variations thereof) may constitute a general way to give large responses to small relative variations in the concentration of activating molecules. The model may be particularly suitable to explain switch-like responses induced by molecular clusters in cell membranes or in the cytoplasm. It may, however, also be applicable to enzymatic reactions with large Hill coefficients. Such reactions are normally explained by co-operativity between a large number of (substrate) molecules. They could also be explained by the simultaneous desorption of a substrate and inhibitor molecule, as discussed above.

We have not yet addressed the question how metabolic energy is utilized to power the rapid simultaneous desorption of A and B. It is still therefore, a part of our hypothesis that such a possibility exists. There are many possible hypothetical schemes. One of them could work like the following. Metabolic energy puts M in a conformation making binding of A or B possible. This we call the high energy state of M. Binding of A and B gives M, still with high energy, a conformation which upon collision between two occupied molecules causes a release of the energy, i.e. a conformational change back to the low energy form of M, and a simultaneous release of A and B. Metabolic energy takes M from the low to the high energy state, and makes it ready to bind A and B again. This metabolic step will not influence the qualitative results in the paper, but only influence the details of the switch-like behavior. We have, however, excluded the fact that all Ms are not necessarily in the binding conformation to clearly demonstrate the behavior of collision-induced desorption. In principle, if the metabolic step is fast in comparison to other time constants in the process, our kinetic equations are almost 'exact'.

The non-linear desorption model enables the fine tuning of the activity of the molecular clusters through the incorporation of regulator molecules. One property of 'allosteric' enzymes is that other substances interacting with the enzyme can influence the Hill coefficient describing the dose–response curve of the substrate [15]. In our model, this would be explained by a regulating role of the other molecule, changing the rate of the non-linear desorption. Furthermore, clusters, whose activity depends on bound molecules of both 'kinds' (A and B in the model), may show a very narrow peak in activity at a given ratio of the two molecules.

As mentioned above, Ferrell used coupled ultrasensitive reactions (i.e. with a Hill coefficient larger than one) in the MAPK cascade and a positive feedback between two key proteins to describe the all-or-none response of maturing oocytes to progesterone [9,10]. One observation is that this positive feedback cannot be achieved in oocyte extracts, which is explained by the assumption that one of the key proteins is not translated at an appreciable rate. Another possible explanation for such observations as suggested by the model in this paper, is that the key protein and an inhibitor inside the cell compete for the same binding sites and that the organization of the proteins inside the cell allows a non-linear desorption. The 'amplification' is then due to the switch in the response at a critical ratio between the competing molecules. The model proposed by Ferrell et al. is, however, very elegant and plausible. Our intention is only to point out that also the assumption of a non-linear desorption can give rise to steep dose–response curves.

Several of the phenomena easily predicted by the proposed model need otherwise rather complicated coupled reactions to be explained. We conclude that the interaction or collision-induced desorption treated in this paper may be an interesting alternative to explain all or none responses in biological systems. The response is simply triggered at a critical concentration ratio between two key molecules. This may have applicability to biological phenomena like differentiation, oocyte maturation and apoptosis.

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