Chemical model of reaction cascades induced by activated enzymes or catalysts

Two-step cascades in visual transduction

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ABSTRACT A dissipative system is approximated by a nonlinear rate equation: $\dot{z} \simeq \kappa_1 z - \kappa_2 z^3 (\kappa_2 > 0)$, in which the right side is derived from $-\delta G/\delta z$ of Taylor's series of the thermodynamic potential given by Gibbs' function $G_{(T_C, P_C)}(z)$ at about the critical point $C(T_C, P_C)$ of the control variables (parameters) T and P. The stability or instability of the system is treated by the changes in the control parameters. In the case that $\dot{T} \neq \dot{P} \neq 0$ in the steady state, $\dot{z} = 0$, and T and P pass the point C, κ_1 becomes negative. By this change, the G function is convex at $z = 0$ and each product is created rapidly with concentration or number of the molecules $z = (|\kappa_1|/\kappa_2)^{1/2}$. This dynamic theory is applied to enzyme cascades. Based on cyclic GMP (cGMP) hypothesis in visual transduction, the cascade hydrolysis of cGMP of vertebrates is analyzed by dividing it into two-step reaction cascades: The initial process is that metarhodopsin II catalyzes the exchange of GDP for GTP by transducin (G_{td}) and that GTP-G_{td} complex is hydrolyzed to GDP-G_{td} complex. In the following cascade cGMP is hydrolyzed with amplification of phosphodiesterase (PDE) activated by the removal of the small inhibitory subunit. The quantity of the hydrolysis of cGMP is estimated as $\sim 5 \times 10^{4-5}$ molecules per photolyzed rhodopsin semiempirically, and this coincides well with experiments.

INTRODUCTION

Biological systems exist in open surroundings and are organized by each internal self-condition under the circumstances. Because many of the reactions are driven by a relatively small stimulus, these systems are in quasi-equilibrium, a metastable state. At present it is difficult to derive simple and useful models applicable to biological systems from general nonequilibrium theories (1–4). To investigate such reactions, we have recently proposed simple dynamic theories by modeling an overdamped anharomonic oscillator (5–7) and a chemical network reaction simplified by a di-molecule scheme (8–11). These models are described by a set of equations for a system that stabilizes the initial state (the reactant) rapidly against external forces, and for the other system which controls the above reaction slowly. The two systems with the interactions to the external forces regulate mutually each reaction through feedback processes and a dissipative system is formed.

Here, we discuss a reaction driven by an activated enzyme or catalyst and apply the chemical model to the light-induced cascade hydrolysis of guanosine 3′, 5′ cyclic-monophosphate (cGMP) in visual transduction of vertebrates by dividing it into two-step cascades based on cGMP hypothesis. Further, we estimate the amount of the hydrolysis semiempirically.

SIMPLE CHEMICAL NETWORK MODEL

To treat a reaction cascade, a chemical system with interactions to the circumstances is investigated under the following conditions: The thermodynamic potential is given by Gibbs' function $G(T, P, z)$ which is expanded by Taylor's series of z in the vicinity of the critical point $C(T_C, P_C)$ of the dynamic variables (the control parameters) T and P, and has the local minimum of G at $z = z_0$. Pressure P means an internal driving force in the system, which is due to the interactions with external systems. The system in equilibrium at z_0 satisfying $\dot{z} = \dot{T} = \dot{P} = 0$ can be controlled by the control parameters and the instability occurs when T and P pass the point C under $\dot{T} \neq \dot{P} \neq 0$ in the steady state, $\dot{z} = 0$. Such a system is stable to perturbations smaller than the reaction threshold value decided by the coefficient (the function) $f(T, P)$ of the z^2 term of the Taylor series of G. When a rate equation of the system is approximated by a nonlinear equation: $\dot{z} \simeq -\kappa_1 z - \kappa_2 z^3 (\kappa_2 > 0)$, the right side is derived from $-\delta G/\delta z$ of the Taylor expansion because $\dot{z} = -\partial G/\partial z (= \eta)$ if the chemical potential η is not zero. The stability or instability of the system in the steady state, $\dot{z} = 0$, is discussed by $\kappa_1(T, P)$ which describes the G curvature at $z = 0$. Because κ_1 is a function of $T - T_C$ and $P - P_C$, that is, the G function is convex at $z = 0$ for $\kappa_1 < 0$, a phase transition appears when $\dot{T} \neq \dot{P} \neq 0$ and κ_1 passes zero. For $\kappa_1 < 0$, the products are created rapidly

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with concentration, number of the molecules, $z = (|\kappa_1|/\kappa_2)^{1/2}$, through the potential bifurcation.

The above reaction system is derived from a network system formed by two systems (8–11). We assume a chemical reaction in open surroundings as follows:

$$A \underset{k_2}{\overset{\overset{(+\text{stimulus})}{k_1}}{\rightleftharpoons}} A^* \underset{k_4}{\overset{k_3}{\rightleftharpoons}} B + C_\Delta, \qquad (1)$$

where k_1 and k_3 are variables induced by a stimulus, and k_2 and k_4 are variables which stabilize rapidly the reactant A against the stimulus. When the intermediate A* and each product, B and C, are created a little with concentrations x and Δx, respectively, x is decreased by k_2 and Δx is decreased by k_4. The concentration Δx corresponds to z. The behavior of the reaction (Eq. 1) is represented by a relaxation equation:

$$\frac{dx}{dt} \simeq -k_2 x + k_4(\Delta x)^2. \qquad (2)$$

When the system (Eq. 1) begins to interact with an inactivated enzyme or catalyst under the circumstances, the hidden variables k_1 and k_3 in Eq. 2 are increased from near zero, while k_4 is decreased slowly and k_2 is decreased slightly. The system (Eq. 1) regulates the enzyme or catalyst and obstructs these changes. The balance of the system is broken by the interaction and the enzyme or catalyst controls slowly the concentrations x and Δx increases slightly. Such an enzyme system can be approximately described by a relaxation equation as follows:

$$\frac{d(\Delta x)}{dt} \simeq -\alpha \Delta x - \gamma x(\Delta x). \qquad (3)$$

The enzyme couples with the system (Eq. 1) under the condition $k_2 \gg \alpha$ (the adiabatic condition [2]) through the feedback processes. A rate equation of the coupled system is obtained by substituting the solution of Eq. $2 \simeq 0$ into Eq. 3:

$$\frac{d(\Delta x)}{dt} \simeq -\alpha \Delta x - \frac{\gamma k_4}{k_2}(\Delta x)^3, \qquad (4)$$

where α is the variable corresponding to κ_1, which decreases Δx for $\alpha > 0$ and increases Δx for $\alpha < 0$, and $\gamma(>0)$ is the variable which reduces x and Δx. The coupling system (Eq. 4) is an open system with the dissipative process as mentioned before and α, $k_4(>0)$, and γ decrease, whereas $k_2(\gg 0)$ decreases only slightly through the mutual feedback processes. If k_2 decreases considerably under the condition satisfying Eq. 4 after the coupling of the two systems, the products are created a little by $\Delta x = (|\alpha| k_2/\gamma k_4)^{1/2}$. Further, if $k_1 > k_2$ by the coupling, the usual enzyme kinetics is applied. The system (Eq. 4) proceeds in nonequilibrium and a metastable state appears at a relatively small $\alpha(>0)$. This state is decided by the internal self-condition of the system under the circumstances; that is, the system is organized by itself into the metastable state. By the stimulus over the reaction threshold, α is changed to negative and the products are created. Therefore, we defined the coupled system as a self-organized system and α as a transition parameter (7–11). On the other hand, Eq. 4 is similar to that of an overdamped anharmonic oscillator (2, 6, 7), and hence the oscillation period of the system becomes infinity at $\alpha = 0$; therefore, the fluctuation, the symmetry breaking instability, appears. Consequently, a phase transition occurs with a jump of α (7) at the threshold decided by the function (T, P) (11).

TWO-STEP CASCADES IN VISUAL TRANSDUCTION

An enzyme cascade which results in hydrolysis of $>10^5$ guanosine 3′, 5′ cyclic monophosphate (cGMP) molecules per photon has recently been found (12, 13) to be induced by the excitation of photopigment rhodopsin in polymerized bilayer membranes. This cascade has been considered to appear through the relaxation process (14) of the distortion of the protein portion in rhodopsin and it was found that cGMP regulated directly the gating of Na^+ channels in the plasma membrane of rod outer segment (ROS) (15). In the visual system, the reaction cascade is induced by a chemical energy lower than the irradiated photoenergy. From this, this system is considered to be in a metastable state in the dark.

Here, we apply the chemical network theory to the photochemical transduction based on the cGMP hypothesis (12, 13, 15–18) in photoreceptor cells of vertabrates. The complex chain reactions of cGMP hydrolysis are assumed to be composed of two-step reaction cascades. The main reactions are as follows: In illumination, rhodopsin is excited and isomerized. In the process converted from photoenergy to chemical energy, metarhodopsin II (mRh II) catalyzes the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) by transducin (G_{td}) and GTP-G_{td} complex is hydrolyzed to GDP-G_{td} complex. Finally, the removal of the small inhibitory subunit (γ) from phosphodiesterase (PDE) leads to activation of the same number as PDE (19, 20), and successively cGMP is hydrolyzed with cascade by the activated PDE (PDE*).

Such reactions are described by

$$A \overset{+E^*}{\rightleftharpoons} A^* \rightleftharpoons B + C, \qquad (5)$$

where E* is an activated enzyme. In the visual transduction the initial process consists of a coupled system

between the system A, which stabilizes GDP rapidly, and an inactivated enzyme, rhodopsin, which regulates GDP slowly in dark. Such a coupled system forms a dissipative one in disc membrane (Fig. 1 *a*). Dark-adapted GDP is in a metastable state because the photoreaction cascade is elicited by a relatively small chemical energy converted from photoenergy as mentioned before. From these results, the initial cascade is represented by

$$\text{GDP-G}_{td} \overset{+\text{mRh II}}{\rightleftharpoons} \text{GTP-G}_{td} \rightleftharpoons \text{GDP-G}_{td} + \text{Pi}, \qquad (6)$$

and the following cascade is described by

$$\text{cGMP} \overset{+\text{PDE*}}{\rightleftharpoons} \text{cGMP*} \rightleftharpoons \text{5'-GMP} + \text{H}^+, \qquad (7)$$

where cGMP* means cGMP at the local maximum (Δx =

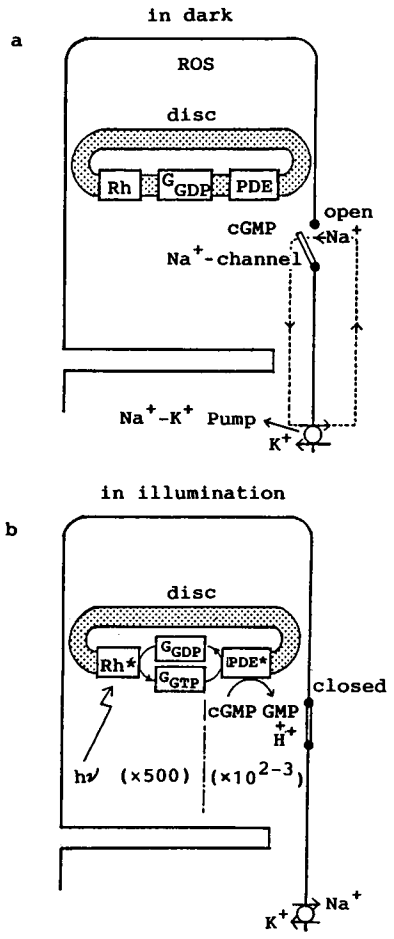

FIGURE 1 Schematic drawings of cGMP hypothesis in visual transduction. In dark (*a*), cGMP in cytoplasm acts to open Na^+ channels in rod outer segment (ROS). G_{GDP} means GDP-transducin (G_{td}) complex. In illumination (*b*), the hydrolysis of cGMP occurs with the two-step cascades through the enzyme actions and Na^+ channels are closed by the release of cGMP bound to the Na^+ channels. G_{GTP}, GTP-G_{td} complex; Rh*, photoexcited rhodopsin (mRh II).

0) of G, which emerges when the potential bifurcates. These reactions are equivalent to Scheme 5 and are analyzed with the chemical system (Eq. 4). When α is changed to negative by illumination, each amount (Δx) of the products is obtained from Eq. 4 = 0. By photon irradiation the GTP or GDP complex is known experimentally to be produced by ~500 molecules per bleached rhodopsin (22). From the result that PDE is activated by GTP complex with the molar ratio of 1:1 (23), the hydrolysis of cGMP is estimated as $\sim 500 \times (|\alpha| k_2/\gamma k_4)^{1/2}$ molecules per photon as the result of successive cascades.

Here, we estimate the variables, $k_i (i = 2, 4)$, α, and γ, in the final stage of the latter cascade. As is evident from Eq. 4, the potential G becomes flat at about $\Delta x = 0$ for $\alpha \sim 0$. The parameters k_1 and k_3 in the reaction (Eq. 1), which are altered to variables through the dissipative mechanism, increase and k_2 decreases only slightly with decreasing α. At $\alpha = 0$, $k_1[A] \simeq k_2[A^*]$, where a lot of the intermediate is produced. From this result, the concentration [A*] is evaluated as $\lesssim$50% of the concentration [A] of cGMP by PDE*. The variable k_1 for $\alpha < 0$ is expected to be nearly equal to the magnitude order of rate constant ($A \rightarrow [A \cdot E]$) of the usual enzyme reactions. From the experimental results (24), the order of the final value of k_2 for $\alpha < 0$ is estimated as $\gtrsim 10^{7-8}$ and by assuming that $\gamma \simeq k_4$, the orders of the final values are given as $\gtrsim 10^{1-2}$. By judging from half-time, ~120 ms (25), of the decrease of cGMP concentration due to the hydrolysis, we evaluate the order of α as $\sim 10^{0-1}$. With these values, Δx is evaluated to be $\sim 10^{2-3}$ molecules per PDE* and $\sim 5 \times 10^{4-5}$ molecules of cGMP per rhodopsin per second are hydrolyzed with amplification. These results agree well with the values obtained by many experiments (12, 13, 15, 26).

DISCUSSION

Visual transduction mechanisms have been widely investigated for a few decades by biochemical and biophysical methods. These mechanisms are described by an internal conversion of photo-excitation energy, which takes place accompanying an amplification of the chemical reactions. We represent such reactions by nonlinear rate Eq. 4, in which the right side is approximated by the Taylor series of Gibbs' function $G_{(T_C,P_C)}(\Delta x)$ around the critical point $C(T_C, P_C)$ of T and P by neglecting the higher terms larger than the sixth order (or $O[6]$). In Eq. 4 the second term is larger than zero, whereas α changes its sign when T and P are driven by activated enzymes. Through this change, the G curvature at $\Delta x = 0$ becomes negative and the products are created with a jump of the reaction energy ΔG induced by that of α from $\alpha \simeq 0$ to a negative (11). The variable α cannot be described exactly as a

function of the control parameters, T and P, at the present stage. However, it is given by an approximate function: $\eta^3 + a\eta + b = 0$, where η is $\Delta G/RT$, a is $3[(T - \beta T_C)/\beta T_C]$, b is $-3(U_{ext}/\beta RT_C)$, β is a shift parameter of T_C (7), and U_{ext} is energy difference between the two states due to the molecule-circumstance (or the solvent-molecule [7]) interactions (11). Using the above function, which is controlled by a and b, the reaction threshold is on the points satisfying the relation, $a^3/27 + b^2/4 = 0$ for $a < 0$ ($T < T_C$).

Based on the cGMP hypothesis, we have analyzed two successive cascades in visual transduction by the chemical model and have estimated the magnitude orders of final values of the variables for $\alpha < 0$ in the latter cascade (7). From the assumption, $k_1[A] \simeq k_2[A^*]$ at $\alpha = 0$, a relation, $k_1 \lesssim k_2$ is obtained. This seems to be a "paradox," however, if k_2 decreases considerably through the dissipative process under the condition satisfying Eq. 4, $\kappa_2(=\gamma k_4/k_2)$ increases and the amplification factor reduces. The above relation, $k_1 \lesssim k_2$, suggests that a back reaction of the system (Eq. 1) takes place easily through interactions with the other systems. This is considered to be an important specific property of the self-organized system. The variable k_2 in such a system keeps the enzyme action (cascade) under control. In the system with a back reaction, $\dot{T}$ and $\dot{P}$ ($\dot{a}$ and $\dot{b}$) should be decided by involving this reaction.

Because many biological systems in living organizations are transferred into a new state by a small stimulus, they are in the local minimum of the thermodynamic potential around the transition point. Those reactions may be treated with a tri-molecule scheme (4), which is transformed to a state equation representing a cusp catastrophic jump (27) by suitable transformations of the variables (28). Such a tri-molecule reaction has rarely been found so far in many chemical and biological reactions, however.

The nonlinear Eq. 4 is derived from the di-molecule scheme (Eq. 1), which has many examples, and a phase transition of the self-organized system can be described by a set of Eq. 4 and the $\eta^3 + a\eta + b = 0$, which shows a jump of ΔG due to the changes in a and b. This dynamic theory is applicable to chemical and biological reactions changing in a sigmoid manner with self-organization by initiation of any stimulus.

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