Stochastic gating in diffusion-influenced ligand binding to proteins: Gated protein versus gated ligands

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(Received 13 May 1996)

The kinetics of the irreversible diffusion-influenced binding of a mobile ligand to a stationary protein is studied, when the ligand concentration is larger than that of proteins and the efficiency of binding is stochastically gated due to conformational fluctuations of one of the species. A general case of M conformational (gate) states is considered with the goal to better understand the difference between the cases of gated ligand and gated protein discovered for the two gate state model [Zhou and Szabo, J. Phys. Chem. **100**, 2597 (1996)]. It is shown that in the former case the binding goes faster than in the latter one. [S1063-651X(96)04610-7]

PACS number(s): 87.15.−v, 05.40.+j, 82.20.Mj

Consider a stationary protein molecule surrounded by mobile ligands. When the protein and a ligand come in contact ligand binding may occur. Efficiency of this process strongly depends on the conformational states of both partners [1]. Processes of such a type are often termed gated reactions associating formation of ''efficient'' conformations with the opening of a gate. After the binding the protein becomes inert. The quantity of the main interest to the kinetics is the survival probability of the protein without a bound ligand. Since the conformations are changing with time the changing rate is an important factor in the kinetics. After the study by Zhou and Szabo [2] it becomes clear that there is a significant difference between cases of gated ligands and the gated protein. Using the two-state model of gating they showed that the binding kinetics is faster when the gating is due to the ligands as compared to that in the case of the gated protein. The present work is devoted to a general analysis of this question.

It is worth noting that the phenomenon of gating is widely distributed and plays an important role in different biologically important processes. Among them are both intraprotein processes and the entrance of small ligands into the protein. Several recent studies [3–5] are devoted to the question of the gating influence on ligand motion inside the protein. A classical example of the processes of the second type is the entrance of oxygen into the heme pocket of myoglobin. Gating manifests itself here in blocking the entrance by the side chain of the protein. Studies of gating influence on such processes were initiated in [6,7]. It was initially assumed that it does not matter whether the gating is due to the ligands or the proteins, since the former diffuse independently. Recent studies [2] have shown that this assumption is not true in the general case. The goal of the present work is to shed some extra light on this question.

Assume that the protein and ligands are spheres, and that ligands independently move in the surrounding solvent in the diffusional manner. A ligand comes in contact with the protein when their centers approach at distance b which is referred to below as a contact radius. We estimate the survival probability of the protein $S(t)$ in two steps. First, we estimate the survival probability $S_\alpha(t)$ of the protein under given initial conditions denoted by α. Then $S_\alpha(t)$ is averaged over the initial conditions

$$S(t)=A\upsilon_\alpha\{S_\alpha(t)\}. \tag{1}$$

The initial conditions include an indication of initial positions of all ligands as well as initial states of all the gates. The difference between the cases when the gating is due to the protein conformational changes and those of the ligands manifests itself just here. Indeed, in the first case there is only one ''gate'' while in the second one there are N different gates, where N presents the number of ligands and $N\gg 1$. We assume that the gate has M possible states. So, there are M possible gate states in the case of the gated protein and M^N possible states in the case of gated ligands.

In the low concentration case, because of the independence of motion of different ligands the survival probability $S_\alpha(t)$ may be presented as a product of independent survival probabilities

$$S_\alpha(t)=\prod_{J=1}^{N} s_{j,\alpha}(t), \tag{2}$$

where $s_{j,\alpha}(t)$ is the survival probability of the protein with the presence of only one ligand, namely, the ligand $\#j$. So, to find $s_{j,\alpha}(t)$ one has to solve an isolated pair problem. What is important is that the solution of this problem is insensitive to the fact whether the protein or the ligand bears the gate [2].

The survival probability $S_\alpha(t)$ is expressed in terms of the M-component Green function

$$s_\alpha(t)=s(t|\vec{R},g)=\sum_{g'=1}^{M}\int G_{g'}(\vec{r},t|\vec{R},g)\,d\vec{r}, \tag{3}$$

where the component $G_{g'}(\vec{r},t|\vec{R},g)$ is the probability density to find at the time instant t, the ligand at the point $\vec{r}$, and the gate in the state g', under the condition that initially the ligand was at the point $\vec{R}$ and the gate was in the state g. The components satisfy a set of equations

$$\frac{\partial G_{g'}}{\partial t}=D\Delta G_{g'}-\sum_{\substack{g''\neq g'\\ g''=1}}^{M}(k_{g'\to g''}G_{g'}-k_{g''\to g'}G_{g''}), \quad (4)$$

where D is the diffusion coefficient and $k_{g'\to g''}$ is the jump rate from the gate state g' to the state g''. They also satisfy the initial conditions

$$G_{g'}(\vec{r},o|\vec{R},g)=\delta_{g,g'}\delta(\vec{R}-\vec{r}) \quad (5)$$

and the boundary conditions at the contact radius (we assume that the protein is located at the origin)

$$\frac{1}{b}(\vec{r},\mathrm{grad}_{\vec{r}}G_{g'}(\vec{r},t|\vec{R},g))\Big|_{|\vec{r}|=b}=\gamma_{g'}G_{g'}(\vec{r},t|\vec{R},g)\Big|_{|\vec{r}|=b}. \quad (6)$$

This represents radiation boundary conditions with the gate state dependent rate constant $\gamma_{g'}$. The case of $\gamma_{g'}=\infty$ corresponds to the absorbing boundary (contact) while the case of $\gamma_{g'}=0$ corresponds to the reflecting one. When $D=0$ (the case of frozen diffusion) Eq. (4) describes transitions between the states of the gate. When all $k_{g'\to g''}=0$ (the case of frozen gate states) different components of the Green function do not mix conserving the initial gate state. The component $G_{g'}(\vec{r},t|\vec{R},g)$ gives the solution of the isolated pair problem when the efficiency of the contact is characterized by the rate constant γ_g.

To find $S(t)$ we have to average $S_\alpha(t)$, Eq. (2), over the initial conditions. We will do this for both cases under consideration starting with the case of the gated protein (GP). We assume that initially ligands are uniformly distributed in space and the probability to find the gate in the state g is equal to ν_g, $\sum_{g=1}^{M}\nu_g=1$. Introduce an auxiliary large volume Ω containing $N=c\Omega$ ligands, where c is the ligand concentration. Assuming that all initial positions of a ligand inside Ω, with the exception of the sphere of radius b around the origin, are equally probable we can write down the averaging over initial conditions as

$$\begin{aligned}S_{\mathrm{GP}}(t)=A\nu_\alpha\{S_\alpha(t)\}&=\sum_{g=1}^{M}\nu_g\lim_{\Omega\to\infty}\frac{1}{(\Omega-\upsilon_b)^N}\int_{\Omega-\upsilon_b}\cdots\\ &\times\int_{\Omega-\upsilon_b}\prod_{j=1}^{N}s(t|\vec{R}_j,g)d\vec{R}_j\\ &=\sum_{g=1}^{M}\nu_g\lim_{\Omega\to\infty}\left[\frac{1}{\Omega-\upsilon_b}\int_{\Omega-\upsilon_b}s(t|\vec{R},g)d\vec{R}\right]^N\\ &=\sum_{g=1}^{M}\nu_g\exp\left\{-c\int_{|\vec{R}|>b}q(t|\vec{R},g)d\vec{R}\right\}, \quad (7)\end{aligned}$$

where υ_b is the volume of a sphere of radius b and

$$q(t|\vec{R},g)=1-s(t|\vec{R},g) \quad (8)$$

is the binding probability for time t of a single ligand under the condition that initially the ligand was at the point $\vec{R}$ and the gate of the protein was in the state g. In the case of gated ligands (GL) the survival probability Eq. (1) takes the form

$$\begin{aligned}S_{\mathrm{GL}}(t)=A\nu_\alpha\{S_\alpha(t)\}&=\lim_{\Omega\to\infty}\frac{1}{(\Omega-\upsilon_b)^N}\\ &\times\int_{\Omega-\upsilon_b}\cdots\int_{\Omega-\upsilon_b}\prod_{j=1}^{N}\left\{\sum_{g_j=1}^{M}\nu_{g_j}s(t|\vec{R}_j,g)d\vec{R}_j\right\}\\ &=\lim_{\Omega\to\infty}\left[\frac{1}{\Omega-\upsilon_b}\int_{\Omega-\upsilon_b}\left\{\sum_{g=1}^{M}\nu_g s(t|\vec{R},g)\right\}d\vec{R}\right]^N\\ &=\exp\left\{-c\sum_{g=1}^{N}\nu_g\int_{|\vec{R}|>b}q(t|\vec{R},g)d\vec{R}\right\}. \quad (9)\end{aligned}$$

Using angular brackets as a notation for the averaging over initial gate states we can rewrite Eqs. (7) and (9), which are one of the main results of this work, as

$$S_{\mathrm{GP}}(t)=\left\langle\exp\left\{-c\int_{|\vec{R}|>b}q(t|\vec{R},g)d\vec{R}\right\}\right\rangle_g, \quad (10)$$

and

$$S_{\mathrm{GL}}(t)=\exp\left\{-c\int_{|\vec{R}|>b}\langle q(t|\vec{R},g)\rangle_g d\vec{R}\right\}, \quad (11)$$

respectively. These expressions show that the survival probability $S_{\mathrm{GL}}(t)$ may be interpreted as a mean field approximation of the survival probability $S_{\mathrm{GP}}(t)$. It should be noted that due to the use of Eq. (2), Eqs. (7) and (10) are only an approximation result.

There is a general Jensen's inequality, which gives the relationship between the mean value of a convex function $f(x)$ of a random variable x and the value of this function when its argument equals the mean value of the random variable [8]. According to this inequality

$$\overline{f(x)}\geqslant f(\overline{x}), \quad (12)$$

where the bar is used as a notation of averaging. Application of this inequality to Eqs. (10) and (11) leads to an important conclusion that the binding goes faster when the gating is due to the ligands than that in the case of the gated protein

$$S_{\mathrm{GP}}(t)\geqslant S_{\mathrm{GL}}(t), \quad t>0. \quad (13)$$

The equality takes place only in the case of frozen gate states when, in addition, only one gate state is initially occupied. The conclusion is in complete agreement with the results obtained in [2] for the two gate state model.

The general analysis above is illustrated by the dependencies presented in Fig. 1. They show the time behavior of the survival probability for a simple two gate state model of binding, in which one state corresponds to absorbing boundary conditions whereas the second corresponds to the reflecting ones. The model describes the case of frozen gate states assuming that both states are initially equally populated. When the gating is due to the ligands the survival probability

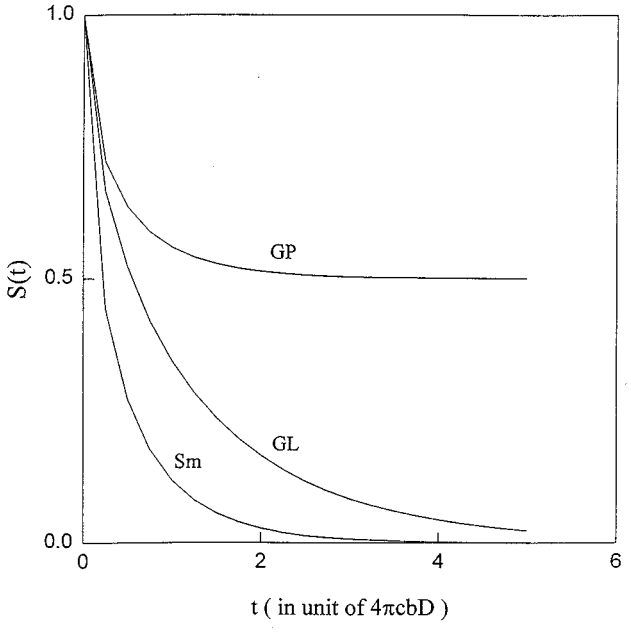

FIG. 1. Survival probability of the protein as a function of time. Curves GL and GP correspond to the cases of gated ligands, Eq. (14), and the gated protein Eq. (16), respectively. For comparison the Smoluchowski solution Eq. (15) is also shown (curve Sm).

of the protein is described by the Smoluchowski theory with the concentration equal to one half of the total concentration of the ligands. It occurs because only one half of the total number of the ligand molecules is potentially able to bind. So,

$$S_{\mathrm{GL}}(t) = S_{\mathrm{Sm}}(t|c/2), \tag{14}$$

where $S_{\mathrm{Sm}}(t|c)$ is the Smoluchkowski solution for the survival probability with a fixed concentration c of the ligands [9]

$$S_{\mathrm{Sm}}(t|c) = \exp\left\{ -c\left(4\sqrt{\pi} bDt + 8\sqrt{\pi} b^2 \sqrt{Dt} \right) \right\}. \tag{15}$$

At the same time, in the case of the gated protein, the protein molecule is able to bind only in one-half of the copies of our statistical ensemble. Therefore

$$S_{\mathrm{GP}}(t) = \tfrac{1}{2} + \tfrac{1}{2} S_{\mathrm{Sm}}(t|c). \tag{16}$$

Figure 1 clearly demonstrates the difference between the cases of gated ligands and the gated protein as well as the difference of both from the kinetics predicted by the traditional Smoluchowski theory Eq. (15).

This work was supported in part by Academia Sinica and in part by the NSC (Taiwan). One of us (A.M.B.) would like to express his thanks to Dr. A. Szabo, Dr. G. Weiss, and Professor H.-X. Zhou for numerous insightful discussions of different aspects of the gating phenomenon and to Dr. A. Szabo and Professor H.-X. Zhou as well as Dr. N. Eizenberg and Professor J. Klafter for the privilege of reading their papers prior to publication. A.M.B. is grateful to the Russian Foundation of Basic Researches for partial support of this work (Grant No. 95-03-09133a).

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