A COHERENT KINETIC MODEL OF SENSING AND RESPONSE IN HALOBACTERIUM SALINARIUM PHOTOTAXIS BASED ON THE MECHANISM OF FLAGELLAR MOTOR SWITCHING

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Keywords: Halobacterium, taxis, motor switching, mathematical model, simulation

Summary

Halobacterium salinarium shows a qualitatively different swimming behavior than E. coli, what demands a different mechanism of flagellar motor switching. In this study we postulate general properties of the switching mechanism in Halobacteria, derived from experimental findings and present a detailed model that quantitatively reproduces various different experimental results with the same set of parameters. Even seemingly paradox findings are accomplished by the presented model.

Introduction

Like E. coli, Halobacteria can perform tactic movements by modulating the probability of switching the rotational sense of their flagellar motors. But the swimming behavior is different in both organisms: E. coli performs runs and tumbles, while Halobacterium swims back and forth by rotating its flagella counter-clock-wise (CCW) or clock-wise (CW), respectively. The different swimming behavior demands a different switching behavior of the flagellar motors, though the switching signal CheY-P that binds to the switching complex is orthologous in both bacteria. CheY phosphorylation is regulated by the receptor complexes in a stimulus-dependent manner. In response to a repellent stimulus, the intracellular concentration of CheY-P is increased, which in turn stabilizes the tumbly state in E. coli, while in Halobacteria the current swimming phase is terminated early (see Fig. 1). Additionally Halobacteria are able to sense light of different wavelengths apart from various chemo-effectors, what allows working with complex experimental stimulation programs, even at singlecell level.

Model

The proposed model of the switching complex is shown in Fig. 2. The exact mathematical formulation and parameters can be seen in: http://wwwa.mpi-magdeburg.mpg.de/people/torsten/switchmodel.html. The functional principle is described in the next section.

Fig. 1. General switching behavior of E. coli and H. salinarium. The model for E. coli is in accordance to the four-state model presented by (Kuo, Koshland, 1989) and the model for Halobacteria is based on (McCain et al., 1987).
Results

We postulate the following qualitative properties of the switching mechanism of *Halobacterium salinarium* (cf. Fig. 1): a) effect of stimulus is symmetric in respect of rotational sense; b) mechanism must comprise a refractory, a responsive and a reversing period; c) switching process is an energy consuming, irreversible cycle process; d) after several stimulus-dependent switching steps there seems to be a number of stimulus independent steps.

The most apparent property of the switching mechanism is its symmetric behavior in respect to stimuli applied at either swimming direction. This symmetry property is reflected by the symmetric design of the Model (Fig. 2).

From experiments of (Krohs, 1994) it can be concluded that the switching mechanism proceeds through a refractory period directly after the last reversal before it enters the responsive period. Cells respond differently during refractory period than during responsive period. While given during the responsive period a repellent stimulus induces a quick reversal of the swimming direction with a distinct frequency distribution of the response time, the same stimulus applied during refractory period results in a delayed response with a broad response time distribution. This property is represented in our model by different transition probabilities to and from the responsive period that are assumed to depend on the occupancy level of the switching complex. In refractory period the affinity of CheY-P to the switch is assumed to be low, resulting in a net dissociation of CheY-P and increasing the probability to the responsive period. In the responsive period, binding of CheY-P is tighter again leading to net association of CheY-P and switch complex and increasing the probability to switch to the rotational sense.

Another qualitative property can be derived from the same experimental result In *Halobacteria* the switching process must undergo an irreversible cycle process from refractory CW to responsive CW to refractory CCW to responsive CCW and again to refractory CW. This process must be virtually irreversible because 100 % of the cell population reacts in a delayed way to a repellent signal directly after a spontaneous reversal, what is characteristic for the refractory period, while after some seconds all cells react in the responsive prompt way, i.e. they are in the responsive...
Thus the underlying process must be irreversible, because there is no equilibration between the different states. This is represented in the model by irreversible transitions from refractory to responsive and irreversible reversing transitions.

The third property mentioned above implies that **several rate limiting reaction steps** are necessary for the switching process. This conclusion is apparent from the frequency distribution of swimming interval length. The frequency (probability density) of a spontaneous switching event is very low for short interval lengths, but then increases steeply and finally decays exponentially again for long interval lengths (Hildebrand, Schimz, 1985) and (Marwan, Oesterhelt, 1987). If the underlying switching process would be mediated by only one reaction step, the frequency distribution would be exclusively exponential. If it would consist of two reaction steps it would increase linearly and then fall exponentially again what still doesn’t reflect the experimental results. The more rate limiting reaction steps are necessary for a particular process the lower the probability for short durations of the process (first passage time) and the steeper and distinct the distribution becomes.

The underlying reaction steps of the process can be further divided into some **stimulus dependent steps** followed by a number of **stimulus independent switching steps**. Experiments of (Marwan, Oesterhelt, 1987) with single and double blue light pulses of varying intensity and duration have shown that the mean reversal time of the cells, after refractory period, is: $t_R = t_{\min} + b/\tau + D\tau/\tau$. Where $t_{\min}$ is a constant time that is not influenced by the stimulus. The reversal time $t_R$ is proportional to the total light pulse $I_{bl}\tau$ ($b$ is a constant) and is delayed by the dark period $D$ weighted by the ratio of the second pulse $\tau_2$ and the total pulse $\tau$. The main statement of this formula is that the second blue light pulse still has the same effectiveness (proportional to its duration) in inducing the reversal event as the first one, only that its impact is delayed by the dark period $D$. Thus the minimal time $t_{\min}$ can not result from nonlinear saturation effects, but a separate stimulus independent process has to be considered (Nutsch et al., 2003). This process must consist of numerous necessary
reaction steps, because the frequency distribution of the response time measured after applying a strong repellent light stimulus shows a very narrow peak (Hildebrand, Schimz, 1985).

The **Simulation Results** are shown in Fig. 3. Not only the mean reversal time, but also the frequency distribution of interval lengths is described by the model in good agreement with the experimental findings for spontaneous, repellent and attractant stimuli. Also the transition from refractory period to responsive period (Fig. 3C) is well made by our model while keeping the set of parameters. For simulating the response to attractant stimuli we had to introduce an adaptational mechanism into the kinetic model of the excitation pathway, because the reversal time upon to attractant stimulation is in the same range as the adaptation time. Details of the adaptation mechanism are not given here, although the time-course of CheY-P concentration in the repellent and attractant stimulated case is shown in Fig. 3B.

In addition, seemingly paradox experimental results that were published in (McCain *et al*., 1987) are described correctly reproduced by our model (simulations not shown): An orange light pulse of 1s normally induces an attractant response, i.e. the suppression of reversals. But the same pulse induces reversals when it is applied 2 to 6s after a repellent stimulus. The following mechanism is proposed by our model: The CheY-P concentration is increased by the repellent stimulus at the beginning of the experiment, what prolongs the refractory period to approximately 8s. A short attractant light pulse during this time transiently reduces the CheY-P-concentration, what induces the transition to responsive period. After the attractant light pulse the CheY-P concentration rises again and initiates the next reversal.

**Discussion**

The switching behavior of *Halobacterium salinarium* as measured in various types of stimulation experiments is described for the first time by a new model for the switching mechanism. The same set of parameters could be used throughout and no complex and mechanistically questionable time-courses of CheY-P concentration (Naber, 1997) had to be assumed. In addition, the experimental results that have led to the assumption of an oscillator-driven switching mechanism (Schimz, Hildebrand, 1985) can be reproduced by our model (simulations not shown).

The kinetic scheme in Fig. 1 can be regarded as a parent model (McCain *et al*., 1987), which qualitatively explains the above-mentioned experimental results while the of the detailed model, containing more kinetic states (Fig. 2) quantitatively reproduces all experimental observations analyzed in this respect until today. Hence we propose the first coherent kinetic model of sensing and response in prokaryotic signaling.

**References**


