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An Approach to Molecular Artificial Life: Bacterial Intelligent Behavior and its Computer Model

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Abstract

We here describe an approach to "Molecular Artificial Life" which represents a digital life designed on the molecular basis of living systems. We choose a simple, free-living bacterium *Pseudomonas aeruginosa*, which is amenable to biochemical and genetic analysis, as a model organism for understanding real-life behaviors in molecular detail. We focus on the bacterial intelligent behavior called chemotaxis, because it is possibly the simplest real-life behavior that can be studied objectively and analyzed quantitatively. Biochemical and genetic analysis is carried out to characterize the molecular circuitry that is responsible for the chemotaxis of *P. aeruginosa*. On the basis of molecular evidence, we propose a computer model for bacterial chemotaxis. The model will be used as part of a digital life that simulates the whole bacterial system in molecular detail.

1. Introduction

The fact that life-like systems will play an important role in our future societies is becoming ever more widely accepted. Life-like systems are expected to actively monitor environmental conditions and to wisely respond to the changing conditions. Much work has been published on the phenomenological models for simulating the behavior of real-life systems [1]. However, these models are often superficial and unsatisfied from the biological viewpoint. The phenomenological models for instance give little insight into the fundamental properties of real-life systems, including flexibility and adaptability, at the molecular level. Short cuts and superficial attention to basic principles are likely to lead at best to poor performance and at worst to expensive failures.

Bacteria are small (typically less than 5 μm long), free-living organisms that are ubiquitous in a wide range of environments from soil and water to human host. They live in precarious environments where nutrient levels, temperature, humidity and other conditions can change rapidly and unexpectedly. Bacteria have evolved their intelligent skills to cope wisely with changing conditions. For example, bacteria can monitor many aspects of their surroundings by using various molecular sensors and actively respond to changing conditions by altering patterns of gene expression [2]. Most bacteria can also seek out favorable environments and escape away from unfavorable ones by changing their swimming direction in response to environmental stimuli [3]. These skills allow bacteria to communicate not only with the abiotic environment but also with each other in microbial communities.

Since bacteria are most amenable to biochemical and genetic analysis, they have assumed a special role in molecular and cellular biology. The bacterial system can also be treated as a model for studying the behavior of living systems in molecular detail. We here propose an approach to "Molecular Artificial Life" which represents a digital life designed on the molecular basis of free-living systems. We choose a monoflagellated, obligately aerobic bacterium *Pseudomonas aeruginosa* as a model organism. We focus on the bacterial intelligent behavior called chemotaxis [4], because it is possibly the simplest real-life behavior that can be studied objectively and analyzed quantitatively. The first section of this paper reviews our current understanding of the molecular chemotaxis machinery in *P. aeruginosa* [5,6,7,8,9]. The second section describes a simple computer model for bacterial chemotaxis which is designed on the basis of molecular evidence. The model can be used as part of a digital life that simulates the whole bacterial system in molecular detail.

2. Bacterial chemotaxis

Bacterial chemotaxis is the process by which bacterial cells migrate through concentration gradients of chemical attractants and repellents [4]. Chemotaxis can be viewed as an important prelude to metabolism, prey-predator relationships, symbiosis, and other ecological interactions in microbial communities [10]. In addition to its biological importance, chemotaxis has assumed a special role in giving insight into the signal transduction network of living systems [2]. Bacterial chemotaxis has also contrib-
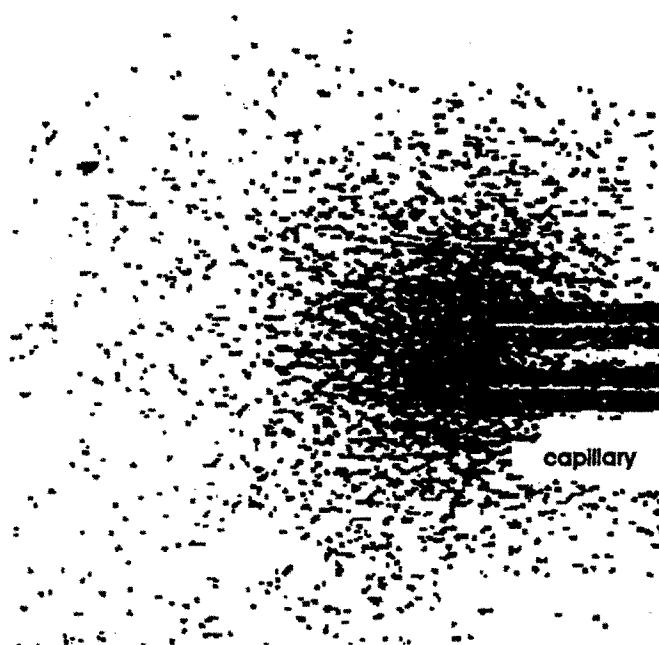
Fig. 1 Chemotactic response of *P. aeruginosa* toward inorganic phosphate (Pi). The capillary tube, with a diameter of approximately 10 μm, contained 10 mM Pi and 1% agarose. Bacterial cells, which are within the focus of microscope, can be seen as small dotts [11].

uted to the understanding of the mechanisms of sensory transduction in more complex organisms.

2.1. Bacterial chemotactic behavior

Bacterial chemotaxis is a quick process that occurs within one second after cells are subjected to a chemical stimulus. The computer-assisted capillary technique has been developed for bacterial chemotaxis assays [11]. In this method, digital image processing is used to count the number of bacteria accumulating around the mouth of a capillary that contains a chemical compound plus agarose gel (Fig. 1). The bacterial response toward a strong attractant is so quick that a cloud of bacteria, as can be seen in Fig. 1, starts to form soon (typically less than 10 s) after the start of microscopic observation. The range of chemical stimuli that elicit behavioral responses varies from bacterial species to species. *P. aeruginosa* exhibits chemotactic responses to a wide range of chemical stimuli including L-amino acids, sugars and organic acids and inorganic phosphate (Pi) [5]. This organism is also known to be repelled by thiocyanic and isothiocyanic esters [12]. Several lines of evidence suggest the presence of regulatory mechanisms underlying the chemotaxis by *P. aeruginosa*. For example, *P. aeruginosa* taxis toward several L-amino acids is subject to control by nitrogen availability

in a manner similar to the control of various enzymes of nitrogen metabolism [13]. The strength of chemotactic responses to glucose and citrate is also dependent on prior growth of the cells on those carbon sources [14]. In addition, *P. aeruginosa* shows Pi taxis, only when the cells are starved for Pi [5]. However, these regulatory mechanisms are still poorly understood at the molecular level.

2.2. Mechanism for bacterial chemotaxis

The basic mechanism of bacterial chemotaxis has been intensively studied with enteric bacteria *Escherichia coli* and *Salmonella typhimurium*, and it is suggested that similar mechanisms work among a variety of bacterial species including *P. aeruginosa* [4]. *P. aeruginosa* is capable of swimming motility by rotating a polar flagellum that extends up to several cell lengths from its surface. The external filament is helical in shape and works against the medium. *P. aeruginosa* moves in a three-dimensional random walk. When cells swim toward higher concentrations of attractants, the random walk is biased to achieve net migration by reducing the probability of random reorientation. This is performed by modulating the direction of flagellar rotation. Bacteria can detect spatial gradients of chemicals by monitoring their concentration changes over time as they swim from one place to another. Since bacteria are small and are subject to the effects of Brownian motion, they have to compare concentrations over distances substantially greater than their own length.

To exhibit chemotactic responses, bacteria should have information processing machinery consisting of elements at least: (i) a measure of the present concentration of a chemoeffector; (ii) a memory of the chemoeffector concentration at the recent past; (iii) a comparator for measuring the difference between the present and recent past concentrations; and (iv) a switch that influences motor reversal according to the input from the comparator (Fig. 2). Bacteria also adapt to the continued presence of a stimulus. The immediate changes in swimming behavior that result when cells first are exposed to chemotactic stimuli diminish over time. Cells subsequently return to prestimulus behaviors although they remain in the presence of the chemoeffector. Adaptation is necessary for detecting new stimuli. For further information on chemotaxis, a number of reviews [2,4,15,16] are available.

2.3. Molecular chemotaxis machinery

The signal-transduction network that mediates bacterial chemotaxis allows cells to modulate their swimming behavior in response to changes in chemical stimuli. First, sensors at the cell surface receive environmental stimuli (Fig. 2). Signals are then converted to internal signals by chemotactic transducers. Transducers can also directly sense a variety of chemical stimuli [18]. After being
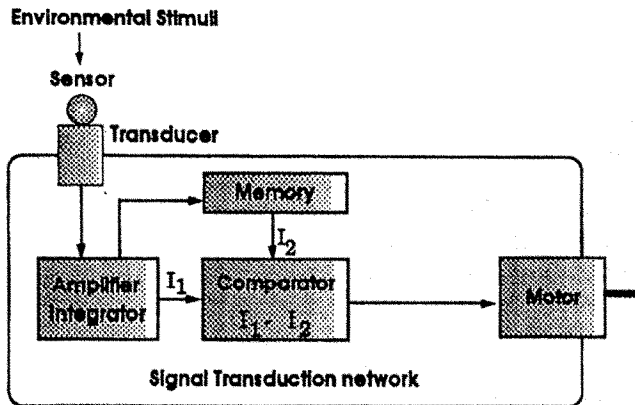
Fig. 2 Bacterial chemotaxis machinery. I_1 and I_2 are chemotaxis signals detected at present and recent past, respectively. Arrows indicate signal flow through the elements of chemotaxis machinery.

amplified and integrated, the internal signals are transmitted through the intracellular signal transduction pathway to the flagellar motors. The components of the intracellular signal transduction pathway are chemotaxis proteins which function to regulate phosphorylation and dephosphorylation of a response regulator (CheY) that interacts with the flagellar motor switch complex to control swimming behavior [16]. Protein methylation and demethylation of transducer proteins is also caused to adjust the level of excitation to the chemical stimuli.

Molecular analysis of chemotaxis genes in P. aeruginosa was achieved only recently. We selected P. aeruginosa chemotaxis mutants by the swarm plate method after NTG mutagenesis [9]. These mutants were fully motile but incapable of swarming, suggesting that they had a defect in the intracellular signalling pathway. P. aeruginosa chemotaxis genes were then cloned by phenotypic complementation of these mutants. We have currently cloned a chemotaxis gene cluster which contains at least the cheY, cheZ, cheA, cheB and cheJ genes (Fig. 3). The predicted products of the first four genes shared high similarity with the enteric Che proteins [16]. However, the cheJ gene product had no significant homology with any known protein species. Insertional inactivation of the chromosomal cheJ gene rendered P. aeruginosa defective in chemotaxis. Since the chromosomal cheJ mutant was fully motile, the CheJ protein is not a component of flagellar apparatus. CheJ may play an unexpected role in the chemotactic signal-transduction pathway in P. aeruginosa.

Biochemical evidence suggested that P. aeruginosa possesses approximately 73 kDa proteins that are covalently modified by dynamic methylation and demethylation reactions in response to L-amino acids [17]. To clone the chemotactic transducer genes in P. aeruginosa, we selected another mutant that is defective in taxis toward L-serine

but normal to peptone. A P. aeruginosa chemotactic transducer gene, designated pctA, was cloned by complementing this mutant. The PctA protein had the typical structural features of methyl-accepting chemotactic transducers (MCPs) in enteric bacteria [4]. The strongest homology with the enteric MCPs was found in the "highly conserved domain" (HCD) [8]. The chromosomal pctA mutant was defective in taxis toward glycine, L-serine, L-threonine and L-valine, indicating that PctA serves as the chemosensor for these L-amino acids. Further sequence analysis of the pctA region revealed the presence of at least two additional genes encoding MCP-like proteins. Our current understanding of the chemotaxis machinery in P. aeruginosa is summarized in Fig. 3.

3. Model for bacterial chemotaxis

To understand how bacterial intelligence arises from the simple signal transduction network, a mathematical model for bacterial chemotaxis was developed (Figs. 4 and 5). The model presented here is designed on the molecular basis of E. coli chemotaxis which has been most intensively investigated [15,18].

The transducer protein is likely to be involved in the processes for measurement of current concentration, a record of recent past concentration and a means for comparison [18]. Events through to occur at the transducer can be presented as a model for a cycle of excitation and adaptation (the upper part of Fig. 4). In the unstimulated state [I], the ligand-binding sites are unoccupied. Occupancy of the ligand-binding site is a measure of current concentration. Binding results in a shift in the state of the transducer from [I] to [II] which generates a positive internal signal for a chemotactic response. This signal activates the dephosphorylation of CheA. Ligand binding activates the methyl-accepting sites for a net increase in
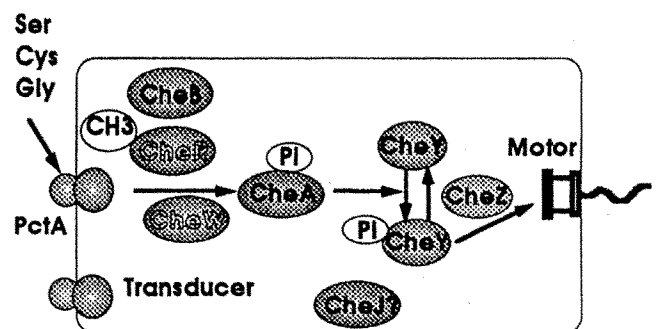

Fig. 3 Chemotactic signal transduction network in P. aeruginosa. The components of the intracellular signal transduction pathway are chemotaxis (Che) proteins. CheR and CheW, which are found to be involved in the E. coli chemotaxis system, have not been identified in P. aeruginosa. The function of CheJ is unknown.

methylation, thus setting the state of adaptation [III]. The modification reactions are slow on the time scale of changes in occupancy, which is likely to provide a memory of recent past concentration. The balancing of ligand occupancy and modification is likely to be a comparison of current and past concentrations. Loss of ligand creates a negative stimulus [IV]. In this state, demethylation is activated, and the transducer is restored to the null signalling state [I]. CheR and CheB proteins are the enzymes catalyzing methylation and demethylation, respectively.

Four cytoplasmic proteins, CheA, CheW, CheY and CheZ, are involved in the intracellular signal transduction (the lower part of Fig. 4). CheA, which is an auto-phosphorylating kinase, is the central component of the signal transduction network [16]. The phosphorylated CheA transfers Pi to CheY, and the phosphorylated CheY in turn biases the flagellar motor toward clockwise (CW), resulting in a change of swimming direction [4]. CheZ accelerates hydrolysis of Pi from CheY. The phosphorylated CheA also transfers Pi to CheB, increasing the methylesterase activity. CheW is thought to couple a primary signal received by the transducer to CheA.

Our model equations are simply the conservation

equations for the components in the signal transduction network (Fig. 5). On the basis of molecular evidence, the first four differential equations (Fig. 5(a)) represent the fraction of transducer in each of four states as a function of time. The concentration changes in chemotaxis proteins as a function of time are given by the last six equations (Fig.5(b)). The frequency of reversal of swimming direction is assumed to be dependent on the value of the ratio C_y/C_{yp}. When the ratio C_y/C_{yp} is lower than 1.0, the flagellar motor is biased toward clockwise, resulting in change of swimming direction. Parameters, v_{12}, v_{12}, v_{23}, v_{34}, v_{43}, v_{41}, v_b, v_{bp}, v_y, v_{yp} and v_{yz} are constant, while v_{41}, v_{ap} and v_a are functions of C_{bp}, C_4 and C_y respectively (see the legend to Fig. 5).

It is best to determine the rate constants experimentally. However, in vitro kinetic experiments with purified proteins are time-consuming, and no systematic study has been reported on determining the rate constants. In this preliminary work, the rate constants in the model equations were adjusted to represent the general features that have been observed in studies of sensory response and adaptation. The general features considered are: (i) the response of a bacterial cell to a chemical attractant is typically observed within 1 s after being subjected to the stimulus; (ii) the time required for intracellular signal transduction is approximately 200 ms; (iii) adaptation occurs less than 1 min after being subjected to stimuli; (iv) the dephosphorylation of CheB is so fast that at most 10-20% of CheB remains phosphorylated; and (v) the half-life of phosphorylated CheY is to the extent of 6-15 s in the absence of CheZ. We also assumed that the ratio of C_y/C_{yp} is 1.0 under the unstimulated conditions. Fig. 6 shows the temporal change in the fraction of transducer in each of four states, which occurs upon addition of a saturating stimulus. The initial conditions and rate constants used in this simulation are given in the legend to Fig. 6. Upon
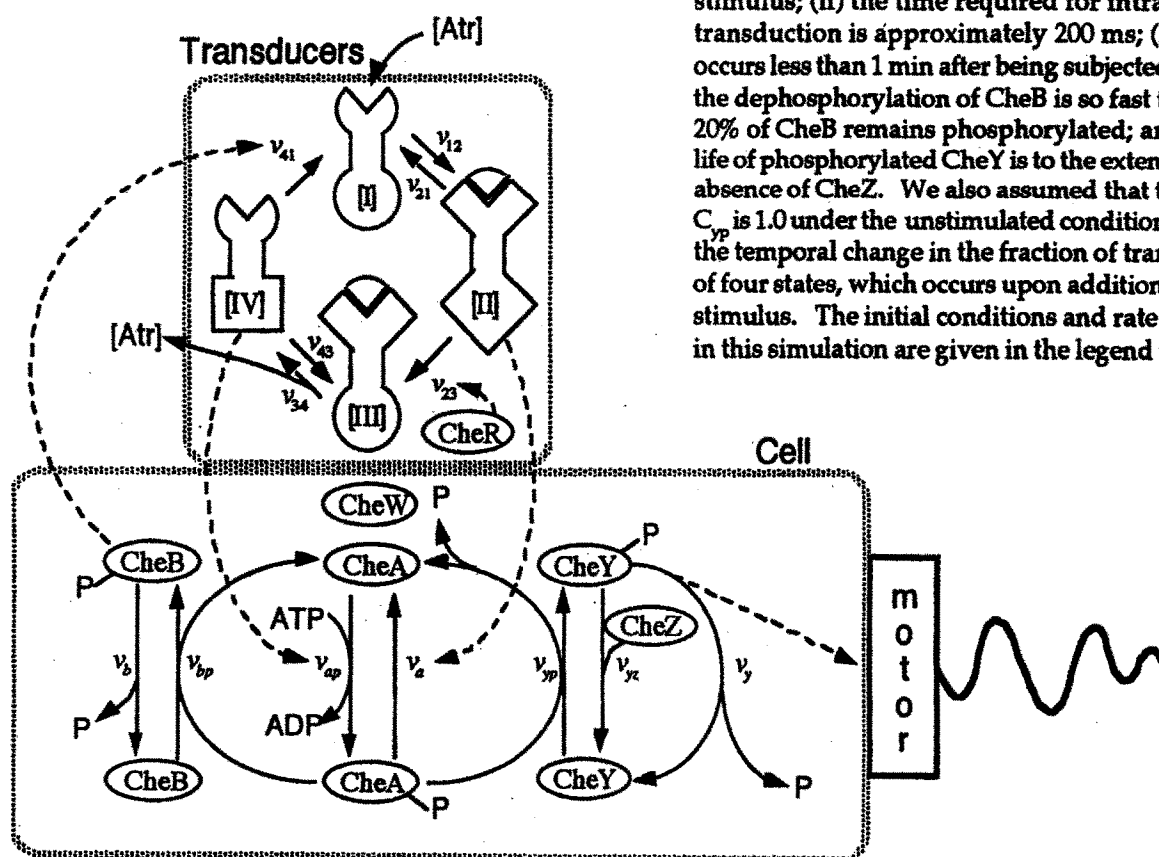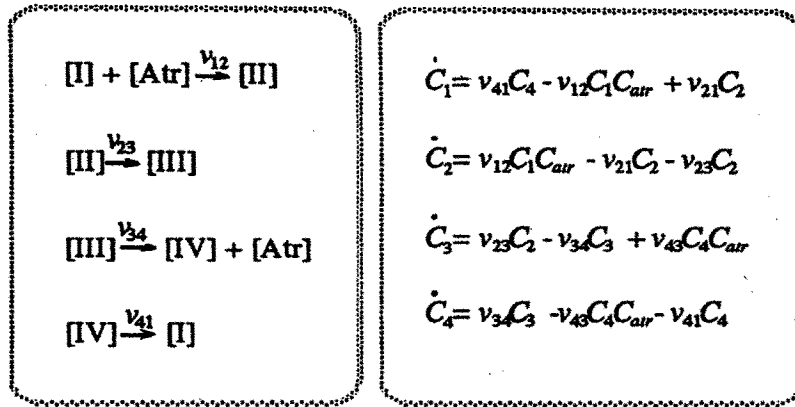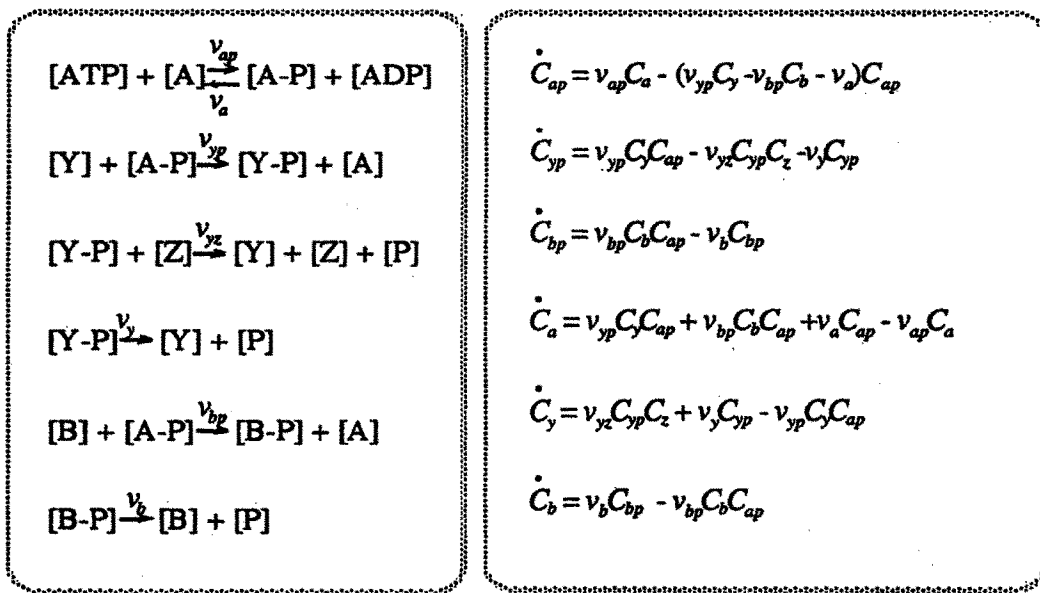

Fig. 4 Schematic illustration of chemotactic signal transduction in *E. coli* [15,18]. In this model, chemical attractants are directly sensed by chemotactic transducer proteins. Atr, attractant; P, inorganic phosphate (Pi).

(a) Transducers

$$[I] + [Atr] \xrightleftharpoons{v_{12}} [II]$$

$$[II] \xrightarrow{v_{23}} [III]$$

$$[III] \xrightarrow{v_{34}} [IV] + [Atr]$$

$$[IV] \xrightarrow{v_{41}} [I]$$

$$\dot{C_1} = v_{41}C_4 - v_{12}C_1C_{atr} + v_{21}C_2$$

$$\dot{C_2} = v_{12}C_1C_{atr} - v_{21}C_2 - v_{23}C_2$$

$$\dot{C_3} = v_{23}C_2 - v_{34}C_3 + v_{43}C_4C_{atr}$$

$$\dot{C_4} = v_{34}C_3 - v_{43}C_4C_{atr} - v_{41}C_4$$

(b) Chemotaxis proteins

$$[ATP] + [A] \xrightleftharpoons[v_a]{v_{ap}} [A\text{-}P] + [ADP]$$

$$[Y] + [A\text{-}P] \xrightarrow{v_{yp}} [Y\text{-}P] + [A]$$

$$[Y\text{-}P] + [Z] \xrightarrow{v_{yz}} [Y] + [Z] + [P]$$

$$[Y\text{-}P] \xrightarrow{v_y} [Y] + [P]$$

$$[B] + [A\text{-}P] \xrightarrow{v_{bp}} [B\text{-}P] + [A]$$

$$[B\text{-}P] \xrightarrow{v_b} [B] + [P]$$

$$\dot{C}_{ap} = v_{ap}C_a - (v_{yp}C_y - v_{bp}C_b - v_a)C_{ap}$$

$$\dot{C}_{yp} = v_{yp}C_yC_{ap} - v_{yz}C_{yp}C_z - v_yC_{yp}$$

$$\dot{C}_{bp} = v_{bp}C_bC_{ap} - v_bC_{bp}$$

$$\dot{C}_a = v_{yp}C_yC_{ap} + v_{bp}C_bC_{ap} + v_aC_{ap} - v_{ap}C_a$$

$$\dot{C}_y = v_{yz}C_{yp}C_z + v_yC_{yp} - v_{yp}C_yC_{ap}$$

$$\dot{C}_b = v_bC_{bp} - v_{bp}C_bC_{ap}$$

Fig. 5 Mathematical model for bacterial chemotaxis. The model consists of the conservation equations for transducers (a) and chemotaxis proteins (b). Parameters, v_{12}, v_{12}, v_{23}, v_{34}, v_{43}, v_{41}, v_y, v_{bp}, v_y, v_{yp} and v_{yz} are assumed to be constant. $v_{41} = f_{41}(C_{bp} - C_{bp}(o)) + k_{41}$, $v_{ap} = f_a C_4 + k_{ap}$, $v_a = f_a C_2 + k_a$, where f_{41}, k_{41}, f_{ap}, k_{ap}, f_a and k_a are constant. $C_{bp}(o)$ is the initial concentration of C_{bp}.

addition of an attractant, there is a rapid shift in the state of transducers from [I] to [II]. The subsequent increase of C_3 indicates the adaptation of transducers due to methylation. The time course of concentrations of chemotaxis proteins, as well as the ratio C_y/C_{yp}, is illustrated in Fig. 7. The concentration of phosphorylated CheY drastically increased approximately 200 ms after the addition of an attractant. Then the dephosphorylation of CheY proceeded, showing the adaptation to the stimulus. These preliminary results encourage us to use the present model for analyzing the dynamic behavior of bacterial chemotaxis machinery.

In real-life systems, the rate constants are determined by the biochemical properties of chemotaxis proteins which are the products of the chemotaxis genes on the bacterial chromosome. Therefore, there exist relationships between the rate constants and the chemotaxis genes as shown in Fig. 8. Taking into account these relationships, we are now analyzing how chemotaxis proteins act in concert to generate bacterial intelligence by means of "Ge-
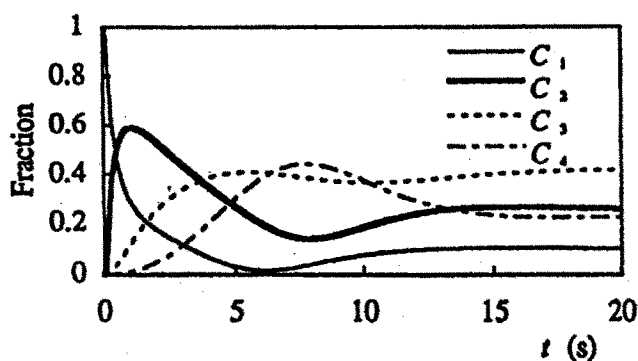
Fig. 6 Temporal change in the fraction of transducer in each of four states, which occurs upon addition of a saturating stimulus. The initial conditions are: $C_1(0)=1.0$, $C_2(0) =C_3(0)=C_4(0) = 0$, $C_a(0) = C_{atr}(0) =C_s(0) =1.0$, $C_{ap}(0) =C_{yp}(0) =C_{bp}(0) =C_a(0) =C_y(0) =C_b(0) =0.5$. Rate constants are: $v_{12}=3.0$, $v_{21}=0.1$, $v_{23}=0.3$, $v_{34}=0.2$, $v_{43}= 0.1$, $f_{41}= 5.0$, $k_{41}=0.1$, $f_{41}= 5.0$, $k_{ap}= 20.0$, $k_a= 5.0$, $f_{ap}= 900.0$, $f_a=500.0$, $v_{bp}=0.1$, $v_b=0.05$, $v_{yp}=30.0$, $v_{yz}=15.0$, $v_y=1.0$.

netic" Algorithms (GA). "Genetic" operations, including crossover and mutation, are performed for the digital bacterial "chromosome" consisting of rate constants. The intelligence of digital-form bacteria is then evaluated by their chemotactic responsiveness which is predicted by computer simulation. Particular attention will be paid to the understanding of the mechanism by which a memory and a comparator function. We also expect in the simulation study to seek out digital-form bacteria that may exhibit unexpected intelligent behaviors. Evolution of digital-form bacteria is another topic in our future simulation study.

4. Concluding remarks

Our interest is not to simulate simple, well-characterized biological events, but to address in molecular detail real biological problems which are too complex to solve by means of biochemical and genetic techniques. In the present work, particular attention was directed to the use of bacterial system as a model for studying the behavior of living systems. Unlike viruses which can reproduce
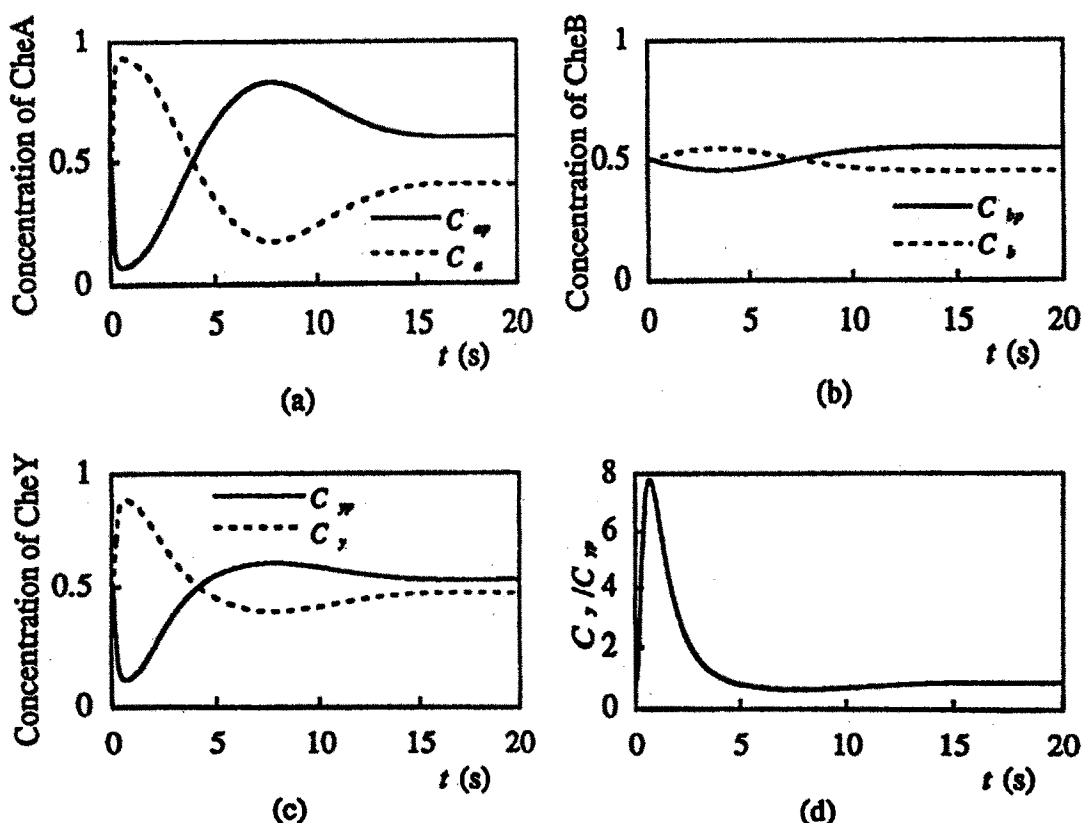

(a)

(b)

(c)

(d)

Fig. 7 Time course of concentrations of chemotaxis proteins, as well as the ratio C_y/C_{yp} which occurs upon addition of a saturating stimulus. Concentrations are shown by a normalized scale. The initial conditions and rate constants are given in the legend to Fig. 6.
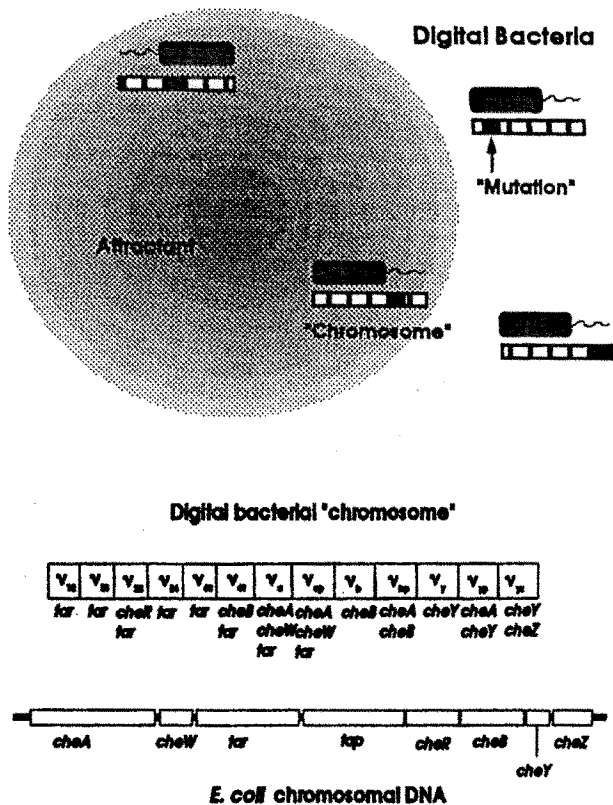
Digital Bacteria

"Mutation"

Digital bacterial "chromosome"

| Vₐ | Vₐ | Vₐ | Vₐ | Vₐ | Vₐ | Vₐ | Vₐ | Vₐ | Vₐ | Vₐ | Vₐ | Vₐ |

E. coli chromosomal DNA

Fig. 8 Evolution of digital bacteria. "Genetic" Algorithms (GA) is used to generate crossover and mutation in the digital "chromosome" consisting of a set of rate constants. The digital "chromosome" can be related to the chemotaxis genes on the E. coli chromosome. Related genes are shown below relevant rate constants.

only with the help of the cells they infect, bacteria are free-living organisms and are still susceptible to biochemical and genetic analysis. In free-living organisms, it remains unknown how gene products act in concert to regulate the whole system. The future of biology is in the analysis of complex systems [19], and the behavior of complex system can not be understood simply by analyzing the expression of individual genes. Therefore, the computer model presented here should have great utility for extrapolating biochemical and genetic analysis of real-life behaviors. As far as bacterial systems are concerned, the simulation results can be examined by biochemical and genetic analysis. Conversely, findings from biochemical and genetic analysis can provide ideas for improving the computer model. Both the approaches are necessary to perfect the understanding of real-life behaviors. We believe that the approach described here is also helpful in presenting a conceptual framework for gaining the information needed to sustain life.

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