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EVIDENCE FOR A CORRELATION BETWEEN SWIMMING VELOCITY AND MEMBRANE FLUIDITY OF *TETRAHYMENA* CELLS

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The influence of the physical state of the membrane on the swimming behaviour of *Tetrahymena pyriformis* was studied in cells with lipid-modified membranes. When the growth temperature of *Tetrahymena* cells was increased from 15°C to 34°C or decreased from 39°C to 15°C, their swimming velocity changed gradually in a similar to the adaptive change in membrane lipid composition. Therefore, such adaptive changes in swimming velocity were not observed during short exposures to a different environment. *Tetrahymena* cells adapted to 34°C swam at 570 μm/s. On incubation at 15°C these cells swam at 100 μm/s. When the temperature was increased to 34°C after a 90-min incubation at 15°C, the initial velocity was immediately recovered. On replacement of tetrahymanol with ergosterol, the swimming velocity of 34°C-grown cells decreased to 210 μm/s, and the cells ceased to move when the temperature was decreased to 15°C. To investigate the influence of the physical state of the membrane on the swimming velocity, total phospholipids were prepared from *Tetrahymena* cells grown under these different conditions. The fluidities of liposomes of these phospholipid were measured using stearate spin probe. The membrane fluidity of the cells cooled to 15°C increased gradually during incubation at 15°C. On the other hand, the fluidity of the heated cell decreased during incubation at 34°C. Replacement of tetrahymanol with ergosterol decreased the membrane fluidity markedly. Consequently, a good correlation was observed between swimming velocity and membrane fluidity; as the membrane fluidity increased, the swimming velocity increased linearly up to 600 μm/s. These results provide evidence for the regulation of the swimming behaviour by physical properties of the membrane.

Introduction

The environment to which living organisms are exposed varies from time to time. Living organisms certain abilities to survive in such a variable environment. Many cell functions are associated with cellular membranes, e.g. transport, energy transduction, cytosis and receptor coupling. Therefore, it is necessary for cells to maintain the physical state of their membranes within a certain range. Membrane functions are influenced by the interaction between lipids and proteins [1]. Indeed, alterations occurring in membrane lipids have been known to affect various membrane functions, for example membrane-bound enzymes [2,3], coupling of β-receptor and adenylate cyclase [4], and endocytosis [5]. Compositional changes in membrane lipids were widely observed in various cell types when the cells are exposed to a new environment. Temperature changes especially induce the alteration of lipid composition in *Escherichia coli* [6], *Bacillus stearothermophilus* [7], *Tetrahymena pyriformis* [8], *Acholeplasma laidlawii* [9], LM cells [10] and fish [11,12]. *Tetrahymena*, a unicellular eucaryote, is well suited to the study of adaptive modification of membrane lipids because of (1) its

bacteria-like rapid growth in defined medium and (2) the easy isolation of the different membrane fractions. Membrane lipids of *Tetrahymena* cells grown in various environments have been extensively investigated by biochemical and physicochemical techniques [13,14]. Also a native sterol-like compound in *Tetrahymena* surface membrane, tetrahymanol, can be replaced by the supplemented sterols; replacement with ergosterol modified both the phospholipid polar head and the fatty acid composition of *Tetrahymena* cells [15].

Swimming is a characteristic attribute of cells having cilia or flagella. Effects of growth temperature and lipid modification on swimming velocity were examined in *Salmonella typhimurium*, but no relationship was observed between lipid composition and swimming velocity [16].

In the experiment reported here, *Tetrahymena* cells were exposed to various environments to induce alterations in membrane lipids. The swimming velocities of *Tetrahymena* with lipids that were differently modified were measured together with the respective phospholipid fluidities. The results provided evidence for a close relationship between swimming velocity and a physical property of the membrane (membrane fluidity).

Materials and Methods

Cells

Tetrahymena, NT-1, a thermotolerant strain, was grown at various temperatures with constant shaking (90 strokes/min) in an enriched medium; 2% proteose-peptone (Difco), 0.5% glucose (Nakarai), 0.2% yeast extract (Difco) and 90 μM Fe^{2+}-EDTA complex [8]. Before changing the growth temperature, cells were grown up to about 10^5 cells/ml for a week at 15°C or for 24 h at a higher temperature (34°C or 39°C). In the cooling experiments (from 34°C (or 39°C) to 15°C) the rate of cooling was essentially linear (0.8 K/min), monitored by placing a sterile thermometer directly into the medium. In the heating experiments, the temperature was raised from 15°C to 34°C within 2 min.

Preparation of ergosterol-replaced cells

Tetrahymena cells were grown on enriched medium containing 10 μg/ml of ergosterol (Sigma) [15]. The mean generation time was prolonged about 3-fold in an ergosterol-containing medium. The completeness of ergosterol replacement was confirmed on Silica gel H thin-layer plates developed with petroleum ether/diethyl ether/acetic acid (70:30:1, v/v).

Measurement of swimming velocity

In order to measure the swimming velocity of *Tetrahymena* cells, a device for particle microelectrophoresis (Rank Brothers Co.) was employed with some modifications. A flat glass cell composed of a 1 mm × 10 mm × 20 mm rectangular prism space and glass tubes at both ends was adopted for the measurement of swimming velocity. The glass cell was dipped in water whose temperature was controlled by a Coolnit CL-19 device (Toyo Co.). The *Tetrahymena* cell suspension (4 ml) was poured into the flat glass cell and the behaviour of the cells was observed using a TV camera equipped with an objective lens. *Tetrahymena* cells swam quickly and at random just after being poured into the glass cell, and gradually they came to swim straight at a certain speed. To avoid artificial effects, the measurements were started after 10 min incubation. Swimming velocity was calculated from the time (measured by a 1/100 s stopwatch) which *Tetrahymena* cells took to swim horizontally from one line to the next (180 μm distance) on the scale of the TV screen. This time was measured for more than 40 cells. During this measuring period, only a few *Tetrahymena* cells swam backward or along a helical trajectory; these cells were excluded from the measurements.

ESR spectroscopy

The stearic acid spin probe, *N*-oxyl-4',4'-dimethyloxazolidine derivative of 5-ketostearic acid, was purchased from Syva Associates (Palo Alto, CA). For preparation of spin-labeled liposomes, the phospholipids were mixed with 1 mol% of the spin probe in benzene and the solvent was evaporated first under a nitrogen stream and then under reduced pressure. To this lipid mixture, 0.1 ml of Tris-buffered saline (150 mM NaCl/50 mM Tris-HCl, pH 7.5) was added, and the mixture sonicated for 30 s with a microtip in a Branson sonifier (B-12) set at level 1.

The spin-labeled liposomes thus prepared were taken into a glass capillary and ESR spectra were measured at various temperatures using a commercial X-band spectrometer (JEOL-FE 1X) equipped with a temperature controller. The parallel ($T'_{\parallel}$) and perpendicular ($T'_{\perp}$) principal values of the hyperfine tensor of an axially symmetrical spin Hamiltonian were estimated from the ESR spectra, and the order parameter, S, was calculated using the following relation:

$$S = \frac{(T'_{\parallel} - T'_{\perp})}{T_{zz} - \left((T_{xx} + T_{yy})/2\right)} \cdot \frac{a}{a'}$$

where $a = (T_{xx} + T_{yy} + T_{zz})/3 = 14.9$ G, $a' = (T'_{\parallel} + 2T'_{\perp})/3$, $T_{xx} = T_{yy} = 5.9$ G and $T_{zz} = 32.9$ G are the hyperfine principal values of the nitroxide radical.

Preparation of total phospholipids from Tetrahymena cells

Lipid was extracted from *Tetrahymena* cells according to the method of Bligh and Dyer [17], and phospholipids were separated from neutral lipids by silicic acid column chromatography [14].

Results

Effect of cell density on swimming velocity

The effect of cell density on swimming velocity was examined in *Tetrahymena* cells grown at 39°C isothermally. The swimming velocity, measured at 39°C, gradually increased as the cell suspension was diluted with cell-free medium obtained from the same growth stage of culture. The mean values were 420, 440, 477 and 530 μm/s at $6 \cdot 10^5$, $3 \cdot 10^5$, $1.5 \cdot 10^5$ and $1 \cdot 10^5$ cells/ml, respectively (the deviations of the measured velocities were within 75 μm/s). The density dependence of the swimming velocity was rather small; a 6-fold dilution caused only a 25% increase in swimming velocity. When the cell suspension was diluted with unused medium, the swimming velocity increased as much as by dilution using the cell-free used medium. In the present experiment, $1 \cdot 10^5$ cells/ml was adopted as a cell density for measurement of swimming velocity.

Measurement of swimming velocity was also carried out at various stages of growth. As the stage proceeded from the middle logarithmic ($6 \cdot 10^5$ cells/ml) to the stationary ($1.2 \cdot 10^6$ cells/ml) phase, the swimming velocity was reduced from 550 μm/s to 450 μm/s at the same cell density ($1 \cdot 10^5$ cells/ml). Therefore, cells in the middle-logarithmic phase were used throughout the following experiments.

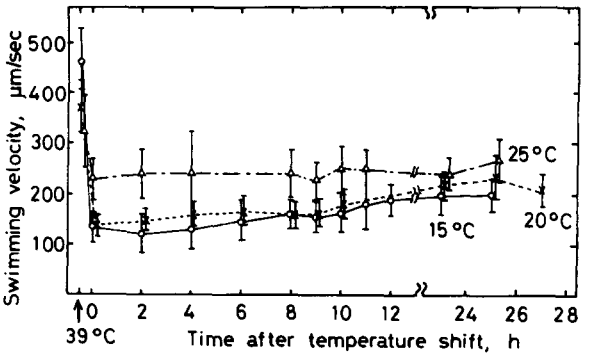

Fig. 1. Time-course of the swimming velocity of *Tetrahymena* cells after cooling to 15°C, 20°C and 25°C. *Tetrahymena* cells were grown at 39°C for 24 h, then cooled to each growth temperature at a rate of 0.8 K/min. Values presented are averages and S.D. values of 40–70 measurements.

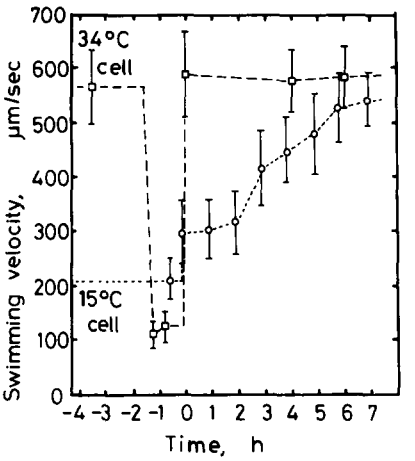

Fig. 2. Effect of raising of the growth temperature on the cells acclimated or non-acclimated to 15°C. O— — —O, *Tetrahymena* cells were grown at 15°C for a week then heated to 34°C within 2 min. □— — —□, *Tetrahymena* cells were grown at 34°C for 24 h, then cooled to 15°C at a rate of 0.8 K/min. After incubation at 15°C for 90 min, *Tetrahymena* cells were heated to 34°C within 2 min. In order to keep the cell density constant, the culture was diluted with equi-volume of fresh medium every 3 h after the shift-up. Values presented are averages and S.D. values of 40 measurements.

Changes in swimming velocity during lowering and raising of the growth temperature

Tetrahymena cells were grown at 39°C for 24 h, then cooled to 25°C, 20°C and 15°C at a rate of 0.8 K/min. The swimming velocity, ranging from 400 to 500 μm/s at 39°C, was reduced in a temperature-dependent fashion to 230, 130 and 120 μm/s at 25°C, 20°C and 15°C, respectively. To investigate whether adaptation of cells influences the swimming velocity, incubation at the lowered temperatures was continued for 24 h after the shift. No significant increase in swimming velocity was observed during the first 9 h. In the following period the swimming velocities of 15°C- and 20°C-incubated cells gradually increased to 2-fold at 24 h, whereas 25°C-incubated cells showed little increase in velocity (Fig. 1).

When *Tetrahymena* cells were grown at 15°C for a week, they swam at around 200 μm/s which was compatible with the velocity of cells adapted to 15°C after cooling from 39°C. Then the growth temperature was raised to 34°C, above which the 15°C-adapted cells were no longer able to survive. The culture was diluted with fresh medium every three hours in order to maintain a constant cell density, i.e. constant growth stage. As shown in Fig. 2. *Tetrahymena* cells grown at 15°C increased their swimming velocity gradually when warmed to 34°C, and could swim nearly as fast as the cells isothermally grown at 34°C after a 7 h-adaptation period.

To examine in more detail the relationship between temperature acclimation and swimming velocity, 34°C-grown cells were cooled to 15°C at a rate of 0.8 K/min, and then heated to 34°C after 90-min incubation at 15°C. As demonstrated in Fig. 2, the swimming velocity decreased from 570 μm/s to 100 μm/s by lowering of the growth temperature, but the initial velocity was instantaneously recovered upon heating to 34°C after incubation at 15°C for 90 min.

As described above, these two profiles of plots in Fig. 2 were completely different after heating to 34°C. At the time the growth temperature was raised to 34°C, *Tetrahymena* cells of these two batches should be in quite different stages of temperature acclimation since one batch was exposed to 15°C for only 90 min while another batch was kept for a week. An adaptational change in *Tetrahymena* cells can be seen clearly in their lipid composition [8,13,14]. Therefore, the difference patterns of swimming behaviours in Fig. 2 may be due to the different extent of lipid modifications.

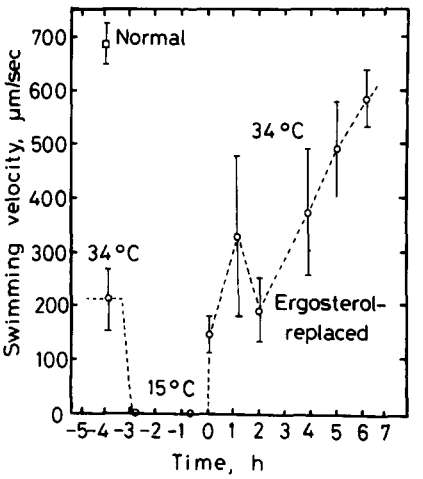

Fig. 3. Changes in swimming velocity of ergosterol-replaced *Tetrahymena* cells during lowering and raising of the growth temperature. *Tetrahymena* cells were grown at 34°C for 24 h in the presence of 10 μg/ml of ergosterol. Lowering and raising of the growth temperature was carried out as described in the legend of Fig. 2. The culture was diluted with equi-volume of fresh medium containing 10 μg/ml of ergosterol every 3 h. Values presented are averages and S.D. values of 40 measurements.

Effect of ergosterol replacement on the swimming velocity of Tetrahymena

To prove the assumption that lipid change in composition may affect swimming behaviour, another type of lipid modification, the ergosterol replacement, was performed. When *Tetrahymena* cells are grown in medium containing ergosterol (10 μg/ml), synthesis of its native sterol, tetrahymanol, is inhibited, consequently tetrahymanol is completely replaced by ergosterol. The replacement has been observed to alter the membrane lipid composition [15] and reduce membrane fluidity [18] in *Tetrahymena*. Fig. 3 shows changes in the swimming velocity of ergosterol-replaced cells by lowering and raising of the growth temperature. Ergosterol-replacement decreased the swimming velocity markedly; the velocity was only 210 μm/s even at 34°C, and cells ceased to move when cooled to 15°C. After the 270-min incubation at 15°C, the growth temperature was raised to 34°C.

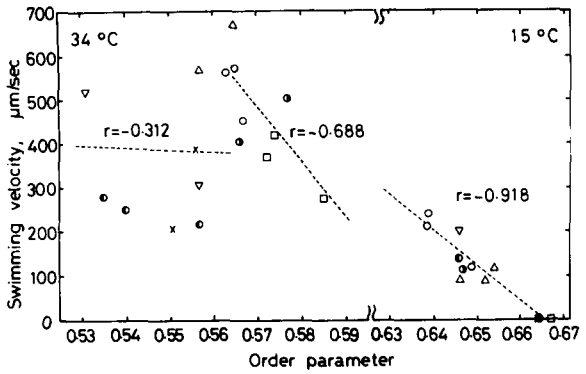

Fig. 4. Correlation between swimming velocity and order parameter of total phospholipids in *Tetrahymena* cells under various conditions; grown at 34°C, grown at 15°C, different times after the change of temperature, and normal and ergosterol-replaced cells. After the measurement of swimming velocity, total phospholipids were prepared from the cells. The order parameter was calculated from ESR spectrum of phospholipid liposomes containing 1 mol% stearic acid spin probe. Dotted lines were obtained by the linear regression of data. Correlation ratios (r) are shown in the figure.

Consequently, cells began to swim again at 180 μm/s, and the velocity was further progressively increased, while the culture was maintained at 34°C. From the comparison of swimming velocity between the normal and the ergosterol-replaced cells grown at 34°C, it may be implied that swimming velocity was affected principally by their lipid composition without any change in growth temperature, i.e. at constant temperature.

Relationship between membrane fluidity and swimming velocity

Immediately after the measurement of swimming velocity, lipids were extracted from *Tetrahymena* cells grown under the above different conditions and phospholipids were separated from neutral lipids by column chromatography as described previously [14]. Then order parameters were measured for these phospholipid liposomes at the temperatures where cells had been grown. In Fig. 4, swimming velocity vs order parameter at 34°C were plotted in the left half of figure and those at 15°C in the right. The order parameter is determined theoretically by two factors; the time-averaged orientation and the rate of motion of spin probes. Although each factor affects the order parameter to a different extent under various lipid membrane systems [19], the order parameter is used as a measure of membrane 'fluidity' in this paper. At 15°C, there was a good correlation between swimming velocity and fluidity (order parameter) of the membrane lipid. Swimming velocity was linearly increased as fluidity was increased (as order parameter was decreased). And the correlation ratio, r, was -0.918 in the plots at 15°C. Correlation was also seen at 34°C within the order parameter range from 0.57 to 0.59; the correlation ratio was -0.688. However, below order parameter 0.57, i.e. in the region of higher fluidity, the relationship was hardly observed; the correlation ratio was -0.312.

Discussion

Relationship between membrane function and membrane structure has been studied in different systems using various techniques. In the present study, we intended to show that the physical state of the membrane may affect the swimming behaviour of *Tetrahymena*. Swimming behaviour of this cell is attained by a beating motion of cilia which are composed of microtubules surrounded by the plasma membrane (ciliary membrane). This membrane is electrophysiologically specialized with an exclusive locus of calcium channels [20,21]. Therefore, swimming velocity of *Tetrahymena* may be affected by alteration of the membrane fluidity. Indeed, a good correlation between membrane fluidity and swimming velocity was observed in the present experiment. As we have shown in previous papers [8,13,14], *Tetrahymena* cells adapt to a new environment by the alteration of membrane lipids. When the growth temperature of *Tetrahymena* cells was lowered, the contents of unsaturated fatty acids and 2-aminoethylphosphonolipids were increased, with concomitant decreases in saturated fatty acids and phosphatidylethanolamine. Consequently, the reduced membrane fluidity recovered almost to the initial level. Although the adaptive modification of membrane lipid plays an important role in temperature acclimation, temperature changes influence various activities other than membrane lipids, such as many enzymes in metabolic pathways in the cell. Enzyme activity itself, in general, depends on temperature. Therefore, the composition of membrane

lipids was modified by the addition of ergosterol under the condition of constant growth temperature. Ergosterol replacement caused decreases in both membrane fluidity and swimming velocity, which was independent of temperature change. Another type of experiment was carried out to investigate the relationship between membrane fluidity and swimming velocity. When *Tetrahymena* cells were returned to the initial growth temperature (34°C) after exposure to 15°C for 1.5 h, swimming velocity recovered immediately to the initial level. This finding suggests that lipid composition (membrane fluidity) influences swimming velocity since the change in lipid composition is very small during such a short incubation at 15°C [14,22]. Therefore, the lipid composition of 34°C-grown cells was maintained in these temperature-shifted cells. When the growth temperature of 15°C-isothermal culture was shifted up, a rapid increase in swimming velocity was not observed despite the increased fluidity. These results imply that swimming velocity of *Tetrahymena* cells is rather low in a supernormal membrane fluid state. There may be an optimum fluidity for proper swimming behaviour. Indeed, in the order parameter range of 0.53 to 0.56, i.e. in higher fluidity, the swimming velocity showed lower values than those at 0.57.

In *Salmonella typhimurium,* swimming velocity was not dependent on the physical state of membrane [6]. This is not compatible with the present results obtained with *Tetrahymena*. The discrepancy may be due to difference in cell type. *Tetrahymena* is an eucaryote and its cillium has a membrane structure with calcium channels, while *S. typhimurium* is a procaryote and its flagellum consists of polymers of flagellins and the flagellum is rotated at its basal body for swimming. Therefore the swimming velocity of *S. typhirium* seems to be unaffected by lipid composition and membrane fluidity.

There must be several factors influencing the swimming velocity of cells. Synthesis of ATP and activity of contractile proteins appear to be major factors which depend on temperature. Indeed, the swimming velocity of *S. typhimurium* increased linearly with raising temperature [16]. In *Tetrahymena*, correlation between swimming velocity and membrane fluidity was not so clear at 34°C, which might suggest that the metabolism of the cells was related to the swimming velocity. At 15°C, however, the principal regulating factor to modulate swimming velocity is not the intercellular metabolism but a physical property of the membrane, since a good correlation between swimming velocity and fluidity is observed. To our knowledge, this is the first report that the swimming velocity of the ciliate *Tetrahymena* is influenced by the lipid composition of its membrane.

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