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# Free Vertical Growth of Myelin Figures

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Myelin figures which grow from a floating egg-yolk lecithin lump on a surface of water/glycerin mixture have been found to grow only vertically towards the bottom of a glass cell and never to grow horizontally along the medium surface nor radially. The structure of the growing myelin figures is basically simple rod-like or a bundle of a few simple rods. Helixing, twisting or coiling myelin figures were never observed under the present experimental condition.

Taking the results obtained from the reported growth behaviour of myelin figures into consideration, it is proposed that one of the driving forces of helixing, coiling and twisting growth of myelin figures is thought to be due to a chemical potential difference caused by a concentration gradient along the radial direction perpendicular to the long axis of a myelin figure. The hydrocarbon chains in the fully hydrated stacked bilayer of myelin figures are proposed to be in a more ordered state than reported so far. The arrangement of hydrocarbon chains, both in myelin figures and in an egg-yolk lecithin/water complex, and the density of them also will be discussed.

*Keywords:* myelin figures; vertical growth; egg-yolk lecithin; density; X-ray diffraction; morphology

## INTRODUCTION

In most reports on morphology and growth behaviour of myelin figures, they were grown horizontally from the lecithin/water interface in a thin gap filled with water sandwiched between slide and cover glasses for optical microscopic observation. Ethyleneglycohole, glycerin and a water/glycerin mixture, were also used as the dispersive medium for the growth of myelin figures (e.g., 1, 2). This preparation restricts growing myelin figures in the narrow two-dimensional gap. The basic structure of these myelin figures is a simple rod-like one in which stacked

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lecithin bilayers, alternating with the medium layers, are concentrically wrapped around a rod-like core axis of the medium (2). The complicated structure of myelin figures is also observed in the experimental setup, which is a double helix structure consisting of two simple myelin rods with the same diameter, a twisting or coiling structure consisting of a coiling simple myelin rod and so on (2). In another growth system of myelin figures for observation of their cross sections by a cryo-scanning electron microscope(SEM), a drop of water was put on a small lecithin lump placed upon an aluminum block. Myelin figures grew upwards from the interface between the lecithin lump and the covering water layer. Simple rod-like, double helixing and coiling structures were also observed in the growth system (3).

In the course of examining the density of myelin figures, we found a vertical growth of myelin figures of egg-yolk lecithin. The growing behaviour was different from that of the above horizontal growth.

## EXPERIMENTAL PROCEDURES

A square pillar-like glass cell with 5 cm × 5 cm inner sides and with a depth of 13 cm was filled with a water/glycerin mixture with a density of 1.02 g/cm$^3$ as a dispersive medium. Egg-yolk lecithin was obtained from Sigma Chem. Co. A small lump of the lecithin was put on the surface of the medium at 25 °C. The lump stayed stationary floating on the surface of the medium. After about half an hour, myelin figures started to grow from the lump/medium interface vertically towards the bottom of the cell as shown in Fig. 1. The structures of growing myelin figures were observed by a polarizing microscope.

## RESULTS AND DISCUSSION

The structure of growing myelin figures was simple rod-like or a bundle of a few simple rods (Figs. 1 and 2) and that of the floating lump of the lecithin was an assembly of liposome-like structures(Fig. 2), which were observed by a polarizing microscope. These myelin figures successfully dipped up out of the medium of the glass cell by a pipette placed on a slide glass for microscopic observation. The observed simple rods were 20 μm ~ 70 μm in diameter. This growth behaviour shows that the density of myelin figures are larger than 1.02 g/cm$^3$ of the medium and also larger than the condensed phase of fully hydrated egg-yolk lecithin, i.e. the lump floating on the surface of the medium in the present work.

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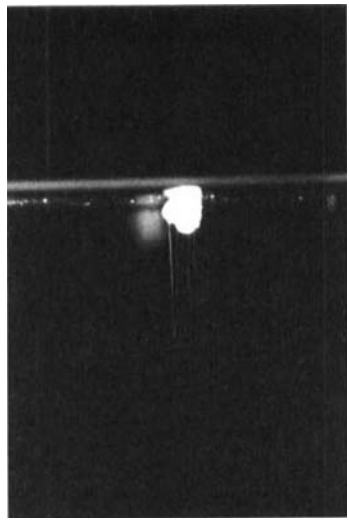

FIGURE 1 Vertically growing myelin figures. The horizontal size of the floating lump is about 4 mm in diameter (See Color Plate II at the back of this issue)

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When pure water was used as a medium, a lump of egg-yolk lecithin which was put on the surface of the medium gradually went down to the bottom of the cell, and vertical myelin figures were not observed.

Based on the present experimental results, driving forces of helixing, coiling and twisting of growing myelin figures, the density and arrangement of hydrocarbon chains in myelin figures of the egg-yolk lecithin/water complex, and the growth rate of growing myelin figures will be discussed below.

## (i) Driving forces of helixing, coiling and twisting of growing myelin figures

Helical, coiling and twisting myelin structures, which were observed on the myelin figures grown horizontally in the medium between slide glass and cover glass(2) and also observed on those grown upwards into water covering the surface of the lump of lecithin(3), never appeared under the present experimental system. Several proposals to explain the mechanism of the helixing, twisting and coiling of the myelin figures have been reported. For example, it was reported that the coiling of myelin figures was caused by binding of $Ca^{2+}$ ions to the polar head group of lipid in the medium(5), which would change the surface energy in favour of inducing curvature to myelin figures.

In horizontal growth, the concentration gradient of the arranged lecithin molecules appeared not only along the long axis of myelin figures(4) but also radially, i.e. in the direction perpendicular to the long axis of the myelin figures, which explains the curvature of the rods(3). The density in the upper part of the myelin rod lying horizontally was estimated to be a little smaller than that of the lower part. It can be thought that simple rods of myelin figures grown vertically may not have a concentration gradient around its long axis. Therefore, it is proposed that one of the driving forces of helixing, coiling and twisting growth of myelin figures is a result of the concentration gradient along the radial direction perpendicular to the long axis of a myelin figure, which causes a chemical potential difference.

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## (ii) Density and arrangement of hydrocarbon chains in myelin figures

Myelin figures of egg-yolk lecithin are one of the lecithin/water complexes consisting of stacked bilayers of lipid with water layers. The water content is estimated as over 45 %. A concentration gradient of the arranged lecithin molecules in the stacked bilayers along the long axis of the myelin figure exists and the concentration is larger near the root part than in the top part of a myelin figure (4). On the basis of X-ray diffraction studies on fully hydrated egg-yolk lecithin consisting of stacked bilayers with excess water, the density was evaluated as between 1 g/cm$^3$ (6) and 1.04 g/cm$^3$ (7). These values were obtained from analyses of X-ray diffraction results of lecithin, in which the total thickness of the bilayer with water layer, $d$, and the area occupied by one lecithin molecule on the surface of the bilayer, $S$, were increased but the thickness of the lecithin bilayer part, $d_l$, and the thickness of the hydrocarbon chain part, $d_h$, were decreased with the increase of the water content of the bilayer system in the region of water content between 20 ~ 45 % as shown in Fig. 3 (8). Many reported X-ray diffraction results of egg-yolk lecithin are nearly the same as shown in Fig. 3. When the water content in the egg-yolk lecithin exceeded 45 %, $d$ was kept constant at 63 Å as shown in Fig. 3. In this region, it was obtained that $S$, $d_l$ and $d_h$ were about 70 Å$^2$, about 38 Å and about 28 Å, respectively(8). With use of these values, the density of the system is calculated as 1.04 g/cm$^3$ and that of the hydrocarbon chain part of the bilayer in this region is calculated as 0.75 g/cm$^3$, assuming that one of the hydrocarbon chains of the egg-yolk lecithin molecule was that of palmitic acid and the other one was that of oleic acid (9). The lump with fully hydrated stacked bilayers of lecithin had been floating on the surface of the medium of 1.02 g/cm$^3$ in density even for a day in the present study. This shows that the density of the lump was smaller than 1.02 g/cm$^3$ of the medium. Moreo-

ver, the calculated density of the hydrocarbon chain part is smaller than that of normal hexadecane in liquid state at room temperature, i.e. 0.773 g/cm$^3$. It is thought to be unreasonable that the two hydrocarbon chains which are connected to the phospholylcholine group of the lecithin molecule, are in a more disordered state than that of liquid normal hexadecane with both ends free.

On the other hand, in a study on magneto-orientation of myelin figures of egg-yolk lecithin, the myelin figures in the magnetic field were bent with the long axes parallel to the magnetic field. The hydrocarbon chains in the myelin figure arranged nearly parallel with each other on average, therefore, could give the enough magnetic anisotropy to the myelin figures to align them with the long axes of hydrocarbon chains perpendicular to the magnetic field (10). This observation shows that the arrangement of the hydrocarbon chains is in a more ordered state than in the amorphous state as in liquid normal hexadecane.

Myelin figures of the lecithin/water system, when observed by a polarizing microscope, show a distinct optical anisotropy with the principal optical axis being perpendicular to the long axes of the myelin figures. This implies also that the long axes of hydrocarbon chains are arranged parallel and in an ordered state on average (2).

From the above two experimental results, it is thought that the arrangement of hydrocarbon chains in the stacked bilayer of the lecithin/water complex with excess water is kept in an ordered state to some extent and is not in such a disordered state as suggested from large $S$ and short $d_h$ from analyses of X-ray diffraction results. Therefore, it is proposed that the hydrocarbon chains of stacked lecithin bilayers in the myelin figures are in an ordered state with a smaller area, $S$ and longer $d_h$ and a larger density than those evaluated from X-ray diffraction studies of fully hydrated lecithin/water complexes.

The discrepancies in both the arrangement of hydrocarbon chains and the density between myelin figures and fully hydrated lecithin/water complexes would be caused by the assumption in X-ray data analysis that the structure of the lecithin/water complex is entirely uniform. X-ray reflections are obtained as lines or spots which come from only regions with ordered molecules. The existence of disordered regions and/or defects in stacked bilayer systems is neglected in the conventional analysis of X-ray diffraction studies. If it is assumed that water regions exist not only between stacking lipid bilayers but also in a lipid bilayer of myelin figures as patches, smaller $S$, longer $d_h$ and consequently an ordered arrangement of hydrocarbon chains with a larger density might be obtained. Although the existence of disordered regions and/or defects in stacked bilayer systems is still not substantiated, large scale fluctuations of the stacked bilayer system (13) might be one of possible candidates to induce them into the system.

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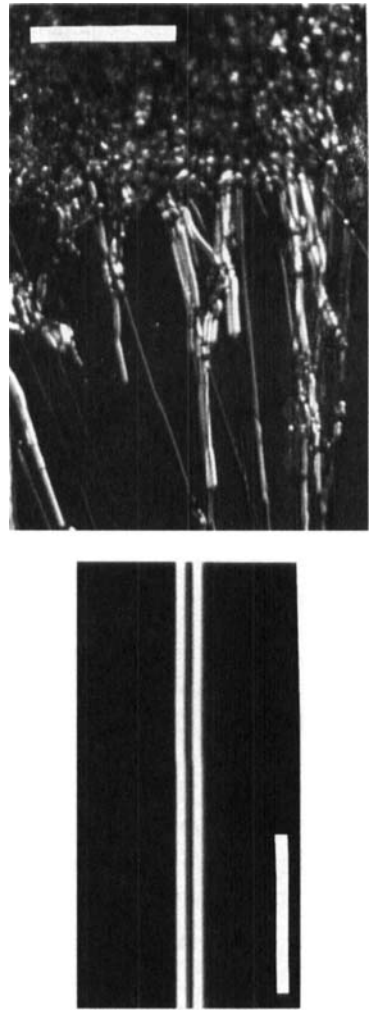

FIGURE 2 Polarizing microscope photos of the myelin figures grown vertically in the present growing method. They were not always successfully dipped up out of the medium of the glass cell by a pipette. After having been removed from the medium, they were put on the slide glass for microscopic observation. They were partly damaged and deformed. (a) The floating lump and vertically growing myelin figures (b) A simple myelin figure. Bars show 200 μm in (a) and 50 μm in (b) (See Color Plate III at the back of this issue)

At least the density of the myelin figures in the present study is larger than the lump part floating on the surface of water which are in the condensed phase, Lα, with excess water. The details for a proposed molecular packing in the lecithin/water system will be reported in the near future.

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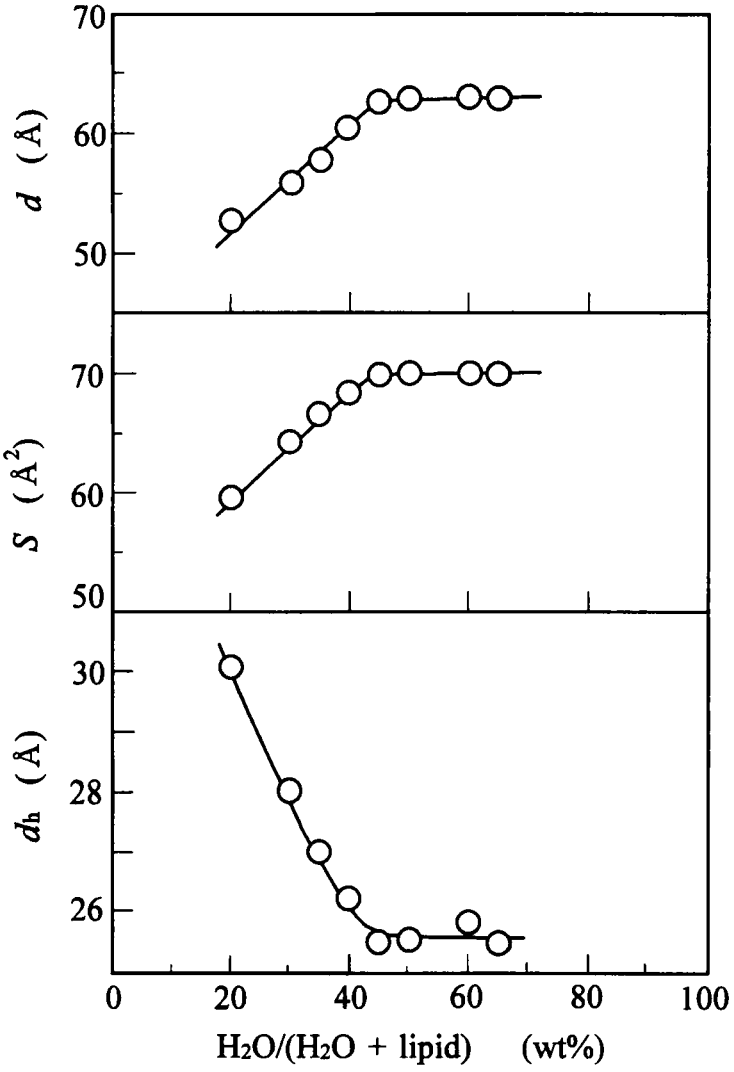


FIGURE 3 The total thickness of bilayer $d$, thickness of hydrocarbon chain region $d_h$, and surface area occupied by one lecithin molecule $S$, obtained from X-ray diffraction results of an egg-yolk lecithin-water complex are plotted against water content. If the bilayer is covered with $q$ mol of water molecules per egg-yolk lecithin molecule, $S$ and $d_h$ are derived by the following equations. $S = (2/d)(v_l + qv_w)$ and $d_h = v_h\,(2/S)$, where $v_l$ and $v_w$ are the volumes per lecithin molecule and water molecule, and $v_h$ is the volume of the two hydrocarbon chain portions of a lecithin molecule. The molecular weight was estimated as 763 assuming the egg-yolk lecithin to be 1-palmitoyl-2-oleyl-phosphatidylcholine. Using the molecular weight and the partial specific volume of egg-yolk lecithin in Lα phase to be 0.986 at 23 °C (12), we estimated the volume of an egg-yolk lecithin molecule, $v_l$, from the partial specific volume and the molecular weight to be 1250 $A^3$. $v_w$ was 30 $A^3$ (8)

### (iii) Growth rate

For the horizontal growth of myelin figures in the lecithin between slide glass and cover glass, it was reported that the initial growth process of myelin figures was suggested to be diffusion-limited with an apparent diffusion coefficient $D$ of approx. $10^{-6}$ $cm^2/s$. $D$ can be expressed by the sum of two processes. One is considered to describe the diffusion of an aggregate of lecithin molecules within bilayers and the other mainly to describe a so-called lateral diffusion of molecules in the bilayer membranes which constitute myelin figures. In this case, the length of the myelin figures of egg-yolk lecithin in water amounted to about $6 \times 10^{-1}$cm on average after 400 sec from the start of growth from the interface of the lecithin lump/water at 25 °C (1). In the present vertically growing myelin figures, the length of myelin figures in the initial growth stage along the long axes of growing myelin figures became 5 ~ 7 cm within about half a minute from the start of growing in a vertical direction and it was much faster than in the case of the above-mentioned horizontal growth rate(1). This faster growing rate in the vertical growth, being observed under the present system of egg-yolk lecithin and the medium of water/glycerin mixture with the specific gravity of 1.02 $g/cm^3$, may result from a free growth without contact with glass surfaces and also from an additional contribution of vertical forces, e.g. gravity, for arranging lipid molecules whose specific gravity is larger than floating aggregated lipid molecules.

## CONCLUDING REMARKS

Myelin figures which grow from a floating egg-yolk lecithin lump on a surface of a water/glycerin mixture have been found to grow vertically and straight towards the bottom of a glass cell. The structure of the growing myelin figures is basically that of a straight simple rod-like one and not of a helixing, twisting or coiling one. On the basis of the observation of growth behaviour, it is proposed that one of the driving forces of helixing, coiling and twisting growth of myelin figures is thought to be due to a chemical potential difference caused by a concentration gradient along the radial direction perpendicular to the long axis of a myelin figure.

The faster growing rate in the vertical growth may result from the free growth without contact with glass surfaces and also a contribution of vertical forces in addition to that limited by diffusion along the long axes of myelin figures. The density of the myelin figures is larger than that obtained from X-ray diffraction results for a lecithin/water mixture. Therefore, the reported molecular packing of

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lecithin in the fully hydrated stacked bilayer, especially that of the hydrocarbon chain part, is in need of re-examination.

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