Biochimica et Biophysica Acta 815 (1985) 149–152
Elsevier

BBA 70219

BBA Report

Concentration gradient along the long axis of myelin figures of phosphatidylcholine

I. Sakurai

Institute of Physical and Chemical Research, Hirosawa 2-1, Wako, Saitama 351 (Japan)

(Received January 2nd, 1985)

Key words: Myelin figure; Concentration gradient; Phosphatidylcholine; Growth; Diffusion

The existence of a concentration gradient along the long axis of, i.e. along the growth direction of, growing myelin figures was found by measurements of transmitted-light intensity through the myelin figures at different places along the axis during growth. The observed transmitted-light intensities under crossed-Nicols were stronger at a micro-area near the root of the growing myelin figures than near the top of it. The difference of the intensities between the two micro-areas decreased with the growth time. Since the intensity measured is nearly proportional to the number of the ordered phosphatidylcholine molecules in the selected area, the present results should prove our recent report that the initial growth process of myelin figures is diffusion-limited (Sakurai, I. and Kawamura, Y. (1984) Biochim. Biophys. Acta 777, 347–351).

At the interface between a lump of phosphatidylcholine in the L_α phase and an aqueous liquid medium, e.g. water, an elongated cylindrical rod-like structure called the myelin figure grows. The myelin figure is composed of many lamellae of phosphatidylcholine bilayers stacked coaxially around the rod-axis with the liquid medium between every other bilayer [2–5].

Recently, from measurements of the growth rate of myelin figures it is suggested that the initial growth process of myelin figures of egg-yolk phosphatidylcholine is limited by a diffusion process, in which two mechanisms work; one is a diffusion of a mass of phosphatidylcholine molecules with a diffusion constant D_1 of approx. 10^{-6} cm^2/s, which is related to the medium viscosity by Einstein-Stokes type relation, and the other is a lateral diffusion type with a diffusion constant D_2 of the order of approx. 10^{-7} cm^2/s [1]. Since phosphatidylcholine molecules are insoluble molecularly into the aqueous liquid medium, the growth mechanism of myelin figures is not expected to be similar to the usual growth mechanism found for a solution-grown crystal. Thus the myelin figures grow from the surface of a lump of phosphatidylcholine into the surrounding aqueous medium through the cooperative two diffusion processes represented by D_1 and D_2. In the process D_1, the phosphatidylcholine molecules in a less hydrated phosphatidylcholine lump may be transported to the roots of more hydrated myelin figures by swelling of the surrounding aqueous medium and rapidly rearranged and incorporated into their bilayers and translated toward the top of myelin figures. During that process the process D_2, a lateral diffusion type process, should work together with D_1 translating the lecithin molecules toward the top of myelin figures [1].

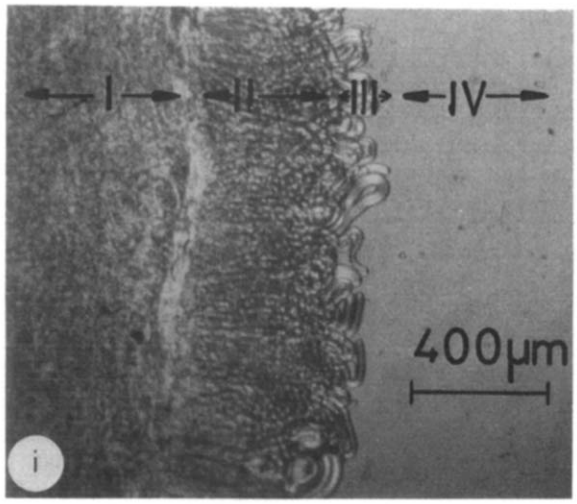
But this growth mechanism would be difficult to be readily accepted, because the mechanism is considerably different from the conventional growth mechanism of a crystalline structure from a solution. To make the situation more clear, we feel that we should show the existence of a con-

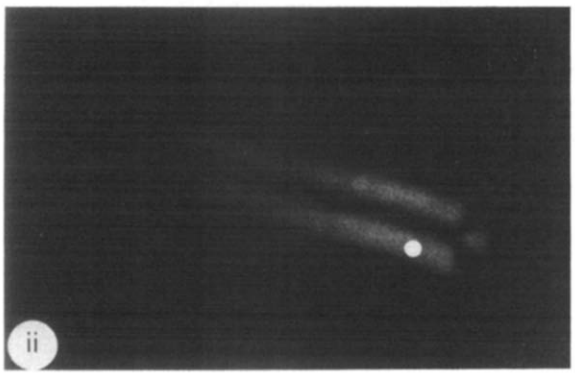
centration gradient along the growing axis of the myelin figure, which is just the driving force of the diffusion process.

The myelin figures were obtained as follows. After placing small lumps of egg-yolk phosphatidylcholine on a slide glass, a cover glass was put on the lumps and pressed slightly. A drop of ethylene glycol, the aqueous liquid medium used in the present experiments, was brought into contact with the edge of the cover glass so that the liquid medium spread over into the gap. As soon as the edge of a lump of phosphatidylcholine was made to come in contact with the medium, myelin figures of a simple rod-like form were observed all together to start growing (see Refs. 1 and 6). In this initial growth stage, the observation of an individual myelin figure was difficult because myelin figures showed a rapid growth rate and grew all together in crowds. After about 10 min from the start of growth, however, top regions of simple rod-like myelin figures isolated from the others became observable at the growth front of many myelin figures under the microscope as shown in the region III of Fig. 1(i). The myelin figure has a strong optical anisotropy with the optical axis being perpendicular to the long axis, which results from an ordered arrangement of hydrocarbon chains in the stacked bilayers constructing the myelin figure.

By use of a microscopic spectrophotometer system (MMSP, OLYMPUS) with crossed-Nicols, the intensity of transmitted-light in diagonal position with respect to the optical axis of myelin figures was measured through selected micro-areas of 8 μm in diameter for myelin figures of about 20–40 μm in diameter. In every measurement, the core of myelin figure, which was seen as an optically isotropic region implying to be a medium core, was arranged parallel to a bisector of the Nicols so as to be in diagonal position. The center of the micro-bean-spot of 8 μm in diameter was put just on the center between the medium core and contour of the myelin figure as shown in Fig. 1 (iii) in order to avoid the change of observed intensity with position along the radius of the myelin figure. The observed transmitted-light intensity is nearly proportional to the number of ordered phosphatidylcholine molecules in a selected area of the myelin figure, since the number of stacking bilayers wound in the myelin figure is uniform all along it, on the average, as judging from observations that not only the radius of it but also the radius of its medium core are nearly the same all along its long axis, even in a very long myelin figure of a simple form. And also the orientational ordering of the hydrocarbon chains of molecules in the stacking bilayers in L_α phase should be uniform everywhere in a myelin figure, since it is difficult to imagine that the conformation of molecules in bilayers might be different from place to place. In addition, the X-ray diffraction pattern from myelin figures consists of low-ordered sharp lines from layer stacking in small angle region and a very diffuse halo from disordered hydrocarbon chains in wide angle region with spacing of about 4.6 Å, implying that the system is in the L_α phase. Thus the measurements of the transmitted-light intensity by changing the measuring position along the long axis of a myelin figure will give the concentration change of phosphatidylcholine along the long axis. The measurements were made at several places of a myelin figure during the growth process of it. The results obtained were as follows.

Firstly, the transmitted-light intensities through an isolated part of myelin figures measured for several selected micro-areas along the long axis tended to become slightly weaker as the selected micro-areas approached to the top of myelin figure. Secondly, for two myelin figures shown in Fig. 1(iii) a and b, the transmitted-light intensities for the two micro-areas on the same myelin figure, one of which is located near the top part of each myelin figure as shown in Fig. 1(ii) and the other is located 130 μm or 300 μm apart from the top part of a or b towards the root part, respectively, were measured during the growth process. Here the selected micro-area at top part was taken in the cylindrical part just adjacent to the cap-like top part of a myelin figure as shown in Fig. 1(ii). Since the measurements were done between about 10 to 30 min from the beginning of growth, the increase in the length of myelin figure of this stage of growth occurred mainly at the root of it, i.e. near the border between the region I and II in Fig. 1(i), at the rate of within about 10 μm/min. An additional slight increase also occurred at the top in this stage, by which no more than a few microns of elongation per minute along the long axis was





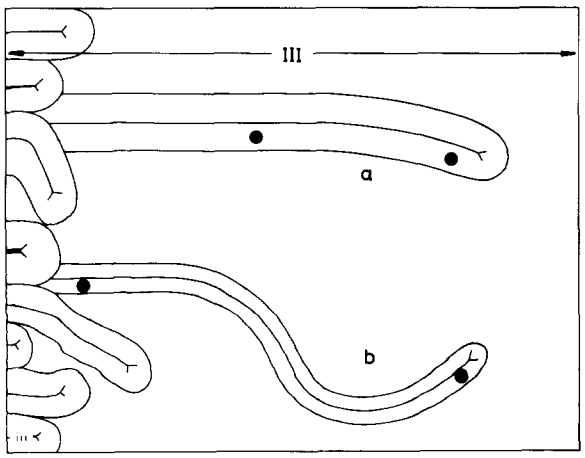


Fig. 1. (i) A photograph of the myelin figures in egg-yolk phosphatidylcholine/ethylene glycol system grown in the gap between a slide glass and a cover glass, taken after 15 min from the beginning of growth by use of a polarizing microscope. Region I is the part of lump of phosphatidylcholine, region II the part of myelin figures grown all together in an aggregated state, region III the top-part of the aggregate where top-parts of simple rod-like myelin figures without contact to others are observed, and region IV the part of aqueous medium. (ii) A photograph of a simple rod-like form of a myelin figure in region III of (i) observed by a microscopic spectro-photometer system with crossed-Nicols. A bright spot in the top part of the myelin figure, whose contrast is artificially emphasized in this photograph, is 8 μm in diameter. It shows a micro-area where a measurement of transmitted-light intensity has been done. The measurements were made at several positions along the long axis of each myelin figure. The wavelength of the measuring light is 540 nm. (iii) Schematic drawing of myelin figures in region III of (i), where the micro-spots for the measurements of transmitted-light intensities are indicated to show the location of each spot on each myelin figure. The distances between each pair of spots are about 130 μm for a and 300 μm for b, respectively; the diameters of the myelin figures, on which the spots are depicted, are 40 μm and 20 μm, respectively.

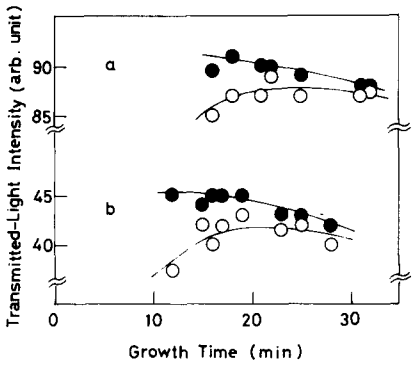


Fig. 2. Transmitted-light intensities through selected micro-areas on the two myelin figures represented in Fig. 1(iii) a and b plotted against the growth time. Filled circles are for those near the root parts and open circles for those near the top parts.

observed. Therefore, we will neglect this slight elongation in what follows.

Fig. 2 is a plot of the transmitted-light intensities for the two selected micro-areas, one near the top part and the other near the root part, against growth time for two myelin figures shown in Fig. 1(iii) a and b. From Fig. 2, two distinctive features are recognized. Firstly, I_r, the transmitted-light intensity through the selected micro-area near the root of myelin figure, appears to be stronger than I_t, the intensity through the micro-area near the top, during all the growth period. Secondly, the difference between I_r and I_t becomes gradually small as a result of the slight decrease in I_r and slight increase in I_t with growth time. The first

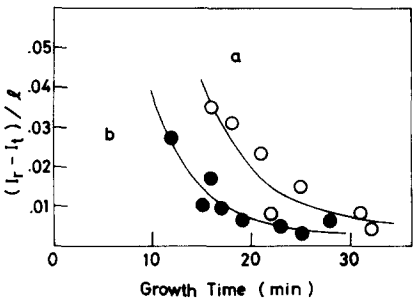


Fig. 3. Concentration gradient plotted against the growth time. The concentration gradient is estimated from the transmitted-light intensities for the pair of micro-spots on the same myelin figures, a and b in Fig. 1(iii), by use of data plotted in Fig. 2.

observation implies the existence of a concentration gradient along the long axis of myelin figure and the second observation implies a tendency of reducing the concentration gradient through the diffusion of phosphatidylcholine molecules along the long axis towards the top of myelin figure.

In order to show the behaviour more clearly, the data is replotted by $(I_r - I_t)/l$ versus growth time in Fig. 3, where l is the distance between two selected micro-areas, which locate near the root part and near the top part, along the long axis of myelin figure.

Neglecting the difference in diameter of myelin figures, and assuming slow spatial variation, we may write the concentration gradient ∇C of the phosphatidylcholine molecules as $\nabla C \backsim (C_r - C_t)/l \propto (I_r - I_t)/l$. Here C_r and C_t express concentrations of phosphatidylcholine molecules at the positions of two micro-areas where I_r and I_t are measured. The value of $(I_r - I_t)/l$ (arbitrary unit), i.e. ∇C, decreases rapidly with growth time in the initial approx. 20 min, and then the decrease becomes very slow as shown in Fig. 3. But the value remains finite within the present growth time region, i.e. within about an hour from the start of growth in the egg-yolk phosphatidylcholine/ethylene glycol system at room temperature, implying a continuing growth of myelin figures.

In conclusion, both the existence of the concentration gradient along the long axis which coincides with the growth direction of the myelin figure and the reduction of the concentration gradient with growth time are observed, which would prove that the initial growth process of myelin figure is limited by a diffusion process.

The author is grateful to Dr. T. Seto of Tokyo Metropolitan University and also to Drs. Y. Kawamura, A. Ikegami, T. Sakurai and S. Iwayanagi of The Institute of Physical and Chemical Research for their valuable discussions. This work was supported in part by the Special Coordination Funds for Promoting Sciences and Technology from the Science and Technology Agency of Japan.

References

1 Sakurai, I. and Kawamura, Y. (1984) Biochim. Biophys. Acta 777, 347–351
2 See for example, Bouligand, Y. (1978) in Solid State Physics, Suppl. 14 (Liebert, L., ed.), pp. 259, Academic Press, New York
3 Luzzati, V. and Tardiu, A. (1974) Annu. Rev. Phys. Chem. 25, 79
4 Chapman, D. and Fluck, D.J. (1966) J. Cell Biol. 30, 1
5 De Gennes, P.G. (1974) The Physics of Liquid Crystals, Oxford University Press, London
6 Sakurai, I. and Kawamura, Y. (1983) Biochim. Biophys. Acta 735, 189