

Langmuir–Blodgett films of bipolar lipids from thermophilic archaea

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Abstract

The Langmuir–Blodgett technique is applied for depositing thin films of two lipids extracted from the thermophilic archaeon *Sulfolobus solfataricus* as well as of a derivative produced by hydrolysis of total lipid extract. These are monosubstituted glycerol–dialkyl–nonitol tetraether with a β -D-glucopyranosyl group (GL-GDNT), bisubstituted glycerol–dialkyl–nonitol tetraether with β -D-glucopyranosyl and phosphomyoinositol groups (GL-GDNT-PH) and asymmetric non-substituted glycerol–dialkyl–nonitol tetraether (GDNT). Films with satisfactory morphology are deposited by both horizontal and vertical lift techniques, but the results are not reproducible. To improve the quality of deposition and the uniformity of the film, mixtures of the lipid GL-GDNT and arachidic acid are used. After dissolution of arachidic acid from the multilayer by hexane highly uniform films are obtained. Studies of the monolayers at the air–water interface and of the deposited films show that probably the molecules in the monolayer are in a form of a U with close packing of isopranyl chains.

Keywords: Air–water interface; Glycerol–dialkyl–nonitol tetraether; Arachidic acid; Isopranyl chain; Archaeon lipid

1. Introduction

Archaea, a phylogenetically coherent separate group of micro-organisms, which differs from Eubacteria and Eukaria, comprises a variety of extremophilic bacteria living in extreme conditions such as high temperature, acidic or alkaline pH and saturated solutions [1]. The archaea membrane lipids have an unusual structure [2] based on isoprenoid chains of different lengths with ether linkage to glycerol or to more complex polyols. In particular, membrane lipids of thermophilic archaea, such as *Sulfolobus solfataricus* (optimal growth conditions are 87 °C and pH 3), possess bipolar architecture [3–5] characterized by the presence of two polar heads and a hydrophobic isoprenoid moiety of practically double average length with respect to that of classical ester lipids. These lipids play a key role in stabilization of the membrane under extreme conditions.

Lipids extracted from archaea and their chemically modified products have been the subjects of studies in Langmuir monolayers at the air–water interface [6–9]. Trials of the

deposition of Langmuir–Blodgett (LB) films have been undertaken in Ref. [9]. Intense X-ray scattering study of the lipids from *S. solfataricus* have been carried out by Gulik et al. [10]. A variety of phases have been observed when the temperature or water content in the sample are changed. In this respect, it is interesting to compare the structure of artificial multilayer systems created from these bipolar lipids with that of conventional amphiphilic molecules.

This work is the first part of a series of papers with a general goal of obtaining thin films containing valinomycin molecules and selectively sensitive to the potassium ions in the solutions [11]. In accordance with this goal films of archaeal lipids could be the matrices for valinomycin dissolution. The use of these lipids may result in increased thermal stability of sensing elements. At the first stage of the work deposition and study of thin films of native lipids was necessary to understand possible ways to create sensing elements.

We applied the LB technique [12,13] for depositing thin films of two native lipids extracted from the archaeon *S. solfataricus* as well as of a derivative produced by hydrolysis of total lipid extract (TLE) (Fig. 1), i.e. monosubstituted glycerol–dialkyl–nonitol tetraether with β -D-glucopyranosyl group (GL-GDNT), bisubstituted glycerol–dialkyl–nonitol

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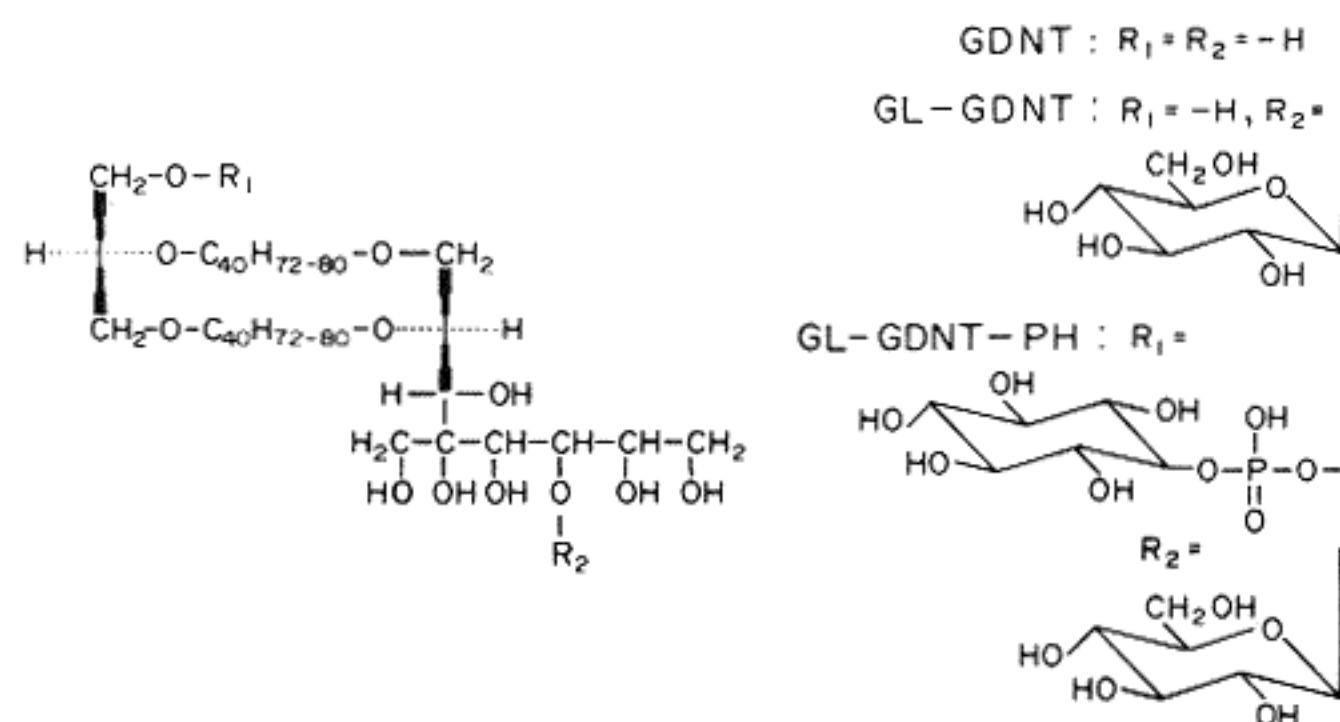


Fig. 1. Chemical formulae of the lipids extracted from *S. solfataricus* (GL-GDNT, GL-GDNT-PH) and of their hydrolytic derivative (GDNT). Iso-pranyl chains $C_{40}H_{72-80}$ contain from 0 to 4 cyclopentane rings [3].

tetraether with β -D-glucopyranosyl and phosphomyoinositol groups (GL-GDNT-PH) and nonsubstituted glycerol-dialkyl-nonitol tetraether (GDNT). LB films can be deposited under some conditions, but the morphology of the surface appears to be worse than that of usual multilayers. To obtain highly uniform films, a mixture of the GL-GDNT lipid with arachidic acid was first deposited onto the substrates. Then arachidic acid was removed from the samples by treatment in hexane. No deterioration of film morphology was observed. Thus, this method enabled us to obtain GL-GDNT films with considerably improved morphology.

The films have been studied by optical microscopy, electron microscopy, X-ray small-angle scattering and electron diffraction techniques.

2. Experimental details

2.1. Production of lipids

The micro-organism *S. solfataricus* was grown at 87 °C and pH 3.5 [14]. The GL-GDNT and GL-GDNT-PH native lipids were purified from TLE and characterized as reported by De Rosa et al. [3]. To produce the GDNT lipid, TLE was hydrolysed with methanolic HCl under reflux, and the hydrolytic mixture was passed through a silica gel chromatograph column [4].

2.2. Conditions of monolayer formation and film deposition

Studies of the monolayers at the air–water interface and deposition of the films were carried out in a clean room of Class 100 by using an LB-MDT System with additional internal air filtration. Temperature in the room was always 23 °C. The temperature of water in the trough under the monolayer was 18 °C. The LB System used enables one to measure surface pressure and monolayer area with accuracies of 0.05 mN m⁻¹ and 1 mm² respectively. The area of the trough was 310 cm² approximately. Precision of deposition pressure control in a feedback mode depends on the viscosity of the

monolayer and on the conditions of deposition. In our experiments it was not worse than 0.2 mN m⁻¹.

The search for optimal conditions of LB film deposition for every compound included the selection of the solvent composition of the spreading solution, checking the possibility of monolayer transfer by the LB and horizontal lifting techniques, change of substrate pretreatment and composition of water subphase, and variation of deposition parameters such as surface pressure, dipping speed and pause duration for film drying in the upper position. Silicon and sapphire substrates were used at this stage of the work. To obtain hydrophilic surfaces the substrates were treated with sulphuric acid or by air glow discharge. To make silicon substrates hydrophobic, they were treated by dimethyldichlorosilane or by hydrofluoric acid. Attempts at deposition were done from the surface of the water subphase at several pH values in the range 3–9. Required buffer solutions were prepared by dilution of commercial Merck standard buffers and provided constant pH values of the subphases in the trough within an accuracy of about 0.1 for 3–4 h at least (neutral media). Water subphases containing 10⁻⁴ M of barium or cadmium ions were also used. Water used for the preparation of the solutions was distilled first with an AQUA-TRON A4S distillator and then deionized with an UHQ-II ELGASTAT System. Resistivity of water after deionization was 10¹⁸ MΩ cm. Organic solvents were supplied by Merck and Fluka; arachidic acid was obtained from Sigma.

For deposition of the LB films monolayers were compressed up to 2.5 mN m⁻¹ with a constant speed of 0.5 cm min⁻¹, which correspond to 0.031, 0.028 and 0.025 nm² molecule⁻¹ min⁻¹ for GL-GDNT-PH, GL-GDNT and GDNT respectively. Then, in the range from $\pi_i = 2.5$ mN m⁻¹ up to deposition pressure π_d compression speed v was gradually decreased from $v_i = 0.5$ cm min⁻¹ up to $v_d = 0.1$ cm min⁻¹ according to the formula $v(\pi) = v_i - (\pi - \pi_i)(v_i - v_d)/(\pi_d - \pi_i)$, where π is the current value of surface pressure. Finally, we always left an interval of 5 min before the deposition while surface pressure was equal to π_d . Surface pressure–area isotherms were recorded at a constant speed of 0.25 cm min⁻¹. Other conditions which were finally used for forming the monolayers at the air–water interface and transferring them onto the substrates to achieve the best possible quality of deposition were the following.

2.3. Optimal conditions of deposition

The GDNT lipid was spread at the surface of pure water using solution of the compound (0.33 mg ml⁻¹) in a mixture of chloroform, methanol, ethanol and hexane (2:1:1:2 by vol.). Deposition was carried out by both LB and horizontal lifting techniques after compression of the monolayer up to a surface pressure of 25 mN m⁻¹. For application of the horizontal lifting technique the surface of the monolayer was separated with a grid onto sections with dimensions approximately equal to those of the substrates. After each touching

of the surface the drop of water was removed from the substrate with a stream of compressed air. Dipping speed in the case of the LB technique was 0.5 cm min^{-1} . The substrates were dried in the upper position for 2 min.

The films of the GL-GDNT lipid with the best morphology were obtained when the initial concentrated solution of the compound (10 mg ml^{-1}) in a mixture of methanol and chloroform (1:1 v/v) was diluted with ethanol down to a concentration of 0.5 mg ml^{-1} and spread on the surface of the water subphase at pH 5.0. Deposition was carried out by the LB technique at a surface pressure of 27 mN m^{-1} with a speed of 0.5 cm min^{-1} . Pause duration in the upper position was equal to 3 min. The GL-GDNT mixtures with arachidic acid in the molar compositions of 1:10 and 1:5 were deposited under the same conditions, but at a pH value of 7.0, which was selected empirically to obtain the best morphology of the film. GL-GDNT films were deposited also with the horizontal lifting technique in the same manner as for the GDNT lipid.

To prepare the films of GL-GDNT-PH lipid a solution of the compound (0.33 mg ml^{-1}) in a mixture of chloroform, methanol and water (65:25:4 by vol.) was spread on the surface of pure water. Deposition was carried out by the horizontal lifting technique after compression of the monolayer up to 27 mN m^{-1} . Although deposition of GL-GDNT-PH by the LB technique was not successful, transfer ratios have been measured when this technique was applied. To do that the process was carried out at 27 mN m^{-1} from the surface of pure water.

2.4. Checking of monolayer stability

To choose proper values of surface pressure of deposition the stability of the monolayers was checked in the ranges from 10 to 32 mN m^{-1} for GDNT and GL-GDNT lipids and from 10 to 38 mN m^{-1} for GL-GDNT-PH. For this purpose, the dependences of displacement of the barriers on time were recorded at four or five constant values of surface pressure. Compression was carried out as described above for the deposition process. It appears that at a surface pressure finally chosen for deposition the area of the monolayer for each of the three compounds diminishes only by 2–4% per hour.

2.5. Optical microscopy study

Observations of the films deposited under different conditions onto silicon substrates with an AXIOSCOP Carl Zeiss optical microscope were carried out at magnifications of up to 1000 to reach the best possible morphology of the film. When the thickness of the film is equal to about 50–100 nm variations of interference colour in the defective places are very strong, which immediately show the result caused by the particular change of the preparation conditions. Non-uniformity of thickness of 3–5 nm can be easily detected. Practically every prepared sample was observed with the optical microscope.

2.6. Electron microscopy and electron diffraction

More detailed information on the morphology was obtained by transmission electron microscopy. The films of archaea lipids were deposited onto copper grids covered by collodion films 10–20 nm thick. The films of 15 and 29 monolayers of GDNT, 9, 15 and 29 monolayers of GL-GDNT, 9 and 15 monolayers of GL-GDNT-PH and 25 monolayers of GL-GDNT mixture with arachidic acid deposited under optimal conditions were studied. Besides, 15 monolayers of GDNT and GL-GDNT deposited using spreading solution of chloroform and methanol mixture (1:1 by vol.), as well as 9 and 29 monolayers of GL-GDNT deposited at pH 7.0 were observed. Other conditions of deposition were not varied. Electron micrographs and electron diffraction patterns were obtained with a CM 12 Philips electron microscope at an accelerating voltage of 80 kV.

2.7. X-ray small-angle diffraction

X-ray small-angle diffraction experiments for structural characterization of the films were carried out with a rotating anode RV 300 Rigaku Denki generator (40 kV accelerating voltage, 200 mA, Ni-filtered Cu K α radiation, $\lambda = 0.154 \text{ nm}$). A powder diffractometer with Θ – 2Θ geometry was used. From 19 to 94 monolayers were deposited onto silicon substrates for the measurements. Most of the samples were deposited under optimal conditions, but similar to the case of electron microscopy the films were also prepared under variation of pH or spreading solution composition.

3. Results and discussion

Thermal stability and stability of biological membranes of lipids from *S. solfataricus* in acid media have been the aim for creating artificial multilayer systems from native compounds. We expect that these properties will be useful for the general purpose of the work (see Introduction).

3.1. Monolayer stability limit measurements

At first, to characterize the monolayers at the air–water interface in respect of the principal possibility of the LB film deposition, the values of surface pressure corresponding to the monolayer stability limit (MSL), i.e. to equilibrium between the monolayer and freshly collapsed material, [15] were measured. For the usual surfactants the deposition of LB films is possible at a surface pressure higher than both MSL and equilibrium spreading pressure (ESP). It is hardly probable that ESP can be used as a characteristic for the possibility of film deposition in the particular case of archaeal lipids. Indeed, the monolayer at the surface of water seems to be composed of molecules in a U-shape [6,8], which should be preserved in a successful deposition of LB film. In contrast to this case, the extended shape of the molecules is

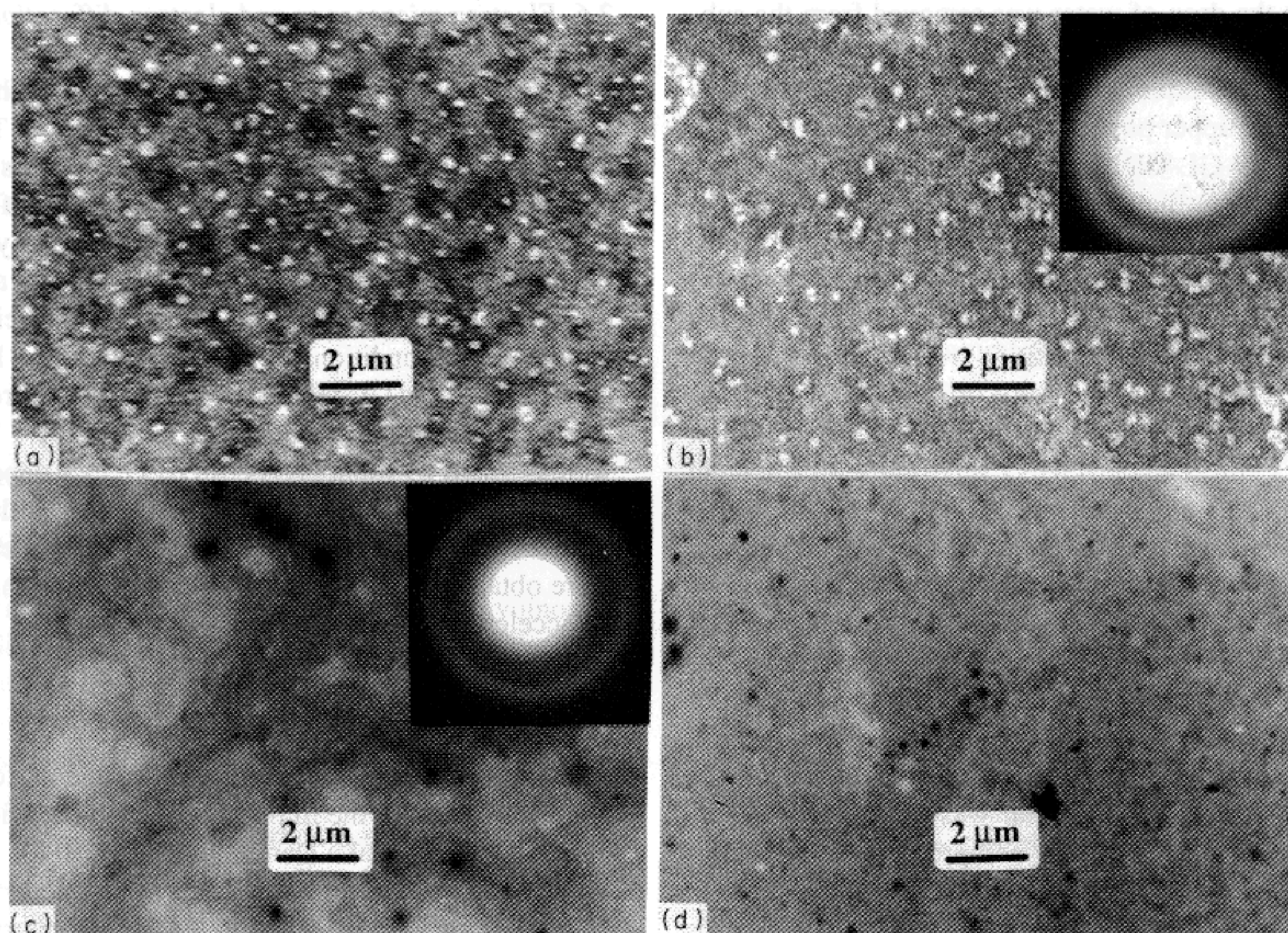


Fig. 2. Transmission electron micrographs of multilayers deposited onto collodion films by horizontal lifting (hl) or LB techniques: (a) 15 monolayers of the GL-GDNT-PH transferred from the surface of pure water at 27 mN m^{-1} (hl); (b) 29 monolayers of the GL-GDNT deposited at a surface pressure of 27 mN m^{-1} (LB) and pH 5.0; (c) 15 monolayers of the GDNT deposited from the surface of pure water at 24 mN m^{-1} (hl); (d) 25 monolayers of the GL-GDNT mixture with arachidic acid (1:10 mol:mol) before treatment in hexane deposited at 27 mN m^{-1} (LB) and pH 7.0.

typical for numerous phases which can exist in the bulk sample [10]. Thus, from the point of view of equilibrium with the monolayer at the air–water interface the LB film and bulk material may display quite different behaviour. The structure of collapsed material seems to be like that of an LB film, but due to the ability of archaeol lipids to form easily the phases containing a considerable amount of water [10], an essential difference between LB film and collapsed material may also exist. At least the content of water in the LB film should be much less because the film is dried during the pulling out of the substrate from water as well as during the pause in the upper position.

MSL values for GL-GDNT-PH, GL-GDNT and GDNT at a temperature of pure water under the monolayer of 18°C are equal to $28.8 \pm 0.3 \text{ mN m}^{-1}$, $30.6 \pm 0.2 \text{ mN m}^{-1}$ and $31.0 \pm 0.2 \text{ mN m}^{-1}$ respectively. Thus, the possibility of deposition with the LB technique was expected first only for GL-GDNT-PH. There is also a narrow interval between MSL and collapse pressure for GL-GDNT and GDNT, but at these values of surface pressure monolayers are unstable and unsuitable for deposition. For this reason, we tried to carry out the deposition with the LB technique at lower surface pressure. It appeared unexpectedly that GL-GDNT and GDNT may be deposited with this technique while the films of GL-GDNT-PH cannot be obtained. Possible reasons for such behaviour are discussed below.

3.2. Deposition of GL-GDNT-PH multilayers

Nevertheless, films of the GL-GDNT-PH of satisfactory uniformity can be deposited by the horizontal lifting technique, although the morphology shown in Fig. 2(a) is worse than that of usual LB films of fatty acid salts. Application of the LB technique for GL-GDNT-PH is usually unsuccessful in spite of variations of the conditions of film formation. In reality, the process of deposition occurs as follows. The transfer of the monolayer takes place first if a hydrophobic surface is used. However, then the film is transferred sometimes from the substrate back onto the water subphase during dipping, so that an occasional considerable decrease of transfer ratio may take place when odd monolayers are deposited. As a result, macroscopic non-uniform areas arise. There is also a tendency for the average transfer ratio to decrease when the number of deposited monolayers increases. Average transfer ratios for the first 20 monolayers recorded during two typical processes of GL-GDNT-PH deposition are shown in Table 1. Accuracy of transfer ratio determination is equal to approximately 0.05. Regions of the deposition curves corresponding to meniscus reorientation and monolayer transfer near the upper and bottom boundaries of the film have been excluded when calculating average transfer ratios. On the other hand, in the places where the film remains its morphology is practically the same as that in Fig. 2(a). Using the optical micro-

scope one can observe the areas of the film on silicon substrate, especially near the upper boundary, with the same interference colour as that for the equal number of monolayers deposited by the horizontal lifting technique, which means they are equal in thickness. Thus, really the process of deposition takes place in the normal way, layer by layer, and the structure of Y-type is expected. Strong interaction of hydrophilic groups of the molecules with water owing to formation of hydrogen bondings may be a reason to transfer some pieces of film back onto the water surface. Indeed, this interaction determines the kinetics of water penetration inside the film. In the case of GL-GDNT-PH this process appears to be fast and deposition becomes impossible in accordance with the MSL value presented above.

3.3. Electron microscopy, X-ray and electron diffraction studies of GL-GDNT-PH films

The electron diffraction pattern shows a practically amorphous film in the layer plane. The origin of the smallest precipitates in Fig. 2(a) has not been determined because they do not give reflections in the diffraction patterns and their appearance is not influenced by the variation of the conditions of deposition or by filtering the initial solution. Probably they arise during spreading of the monolayer. Unfortunately, to vary the composition of spreading mixture for GL-GDNT-PH is extremely difficult because even if the changes of the composition are not considerable the solutions become slightly turbid as a rule.

Table 1

Transfer ratios for typical processes of LB film deposition of lipids from archaeobacteria at optimal conditions (process 1, the best deposition; process 2, bad deposition)

Number of monolayer	Lipid					
	GL-GDNT-PH		GL-GDNT		GDNT	
	process 1	process 2	process 1	process 2	process 1	process 2
1	0.82	0.76	0.90	0.92	0.95	0.97
2	1.10	0.99	1.05	1.00	0.96	0.99
3	0.31	−0.05	0.92	0.90	0.92	0.96
4	0.92	0.90	0.95	0.92	0.93	0.92
5	0.20	−0.40	0.94	0.90	0.85	0.84
6	0.90	0.90	0.96	0.94	0.88	0.94
7	0.22	−0.54	0.90	0.88	0.84	0.56
8	0.90	0.84	0.92	0.90	0.87	0.90
9	0.20	−0.21	0.90	0.88	0.81	0.21
10	0.85	0.80	0.91	0.90	0.86	0.92
11	−0.43	−0.14	0.90	0.65	0.76	0.10
12	0.86	0.78	0.92	0.92	0.84	0.90
13	−0.28	0.23	0.88	0.43	0.76	0.12
14	0.83	0.76	0.92	0.88	0.82	0.86
15	−0.10	−0.72	0.86	0.22	0.78	0.10
16	0.78	0.64	0.90	0.90	0.80	0.86
17	0.12	−0.54	0.72	0.24	0.76	0.27
18	0.67	0.60	0.91	0.88	0.82	0.90
19	−0.72	−0.42	0.86	0.18	0.76	0.35
20	0.68	0.60	0.90	0.80	0.80	0.92

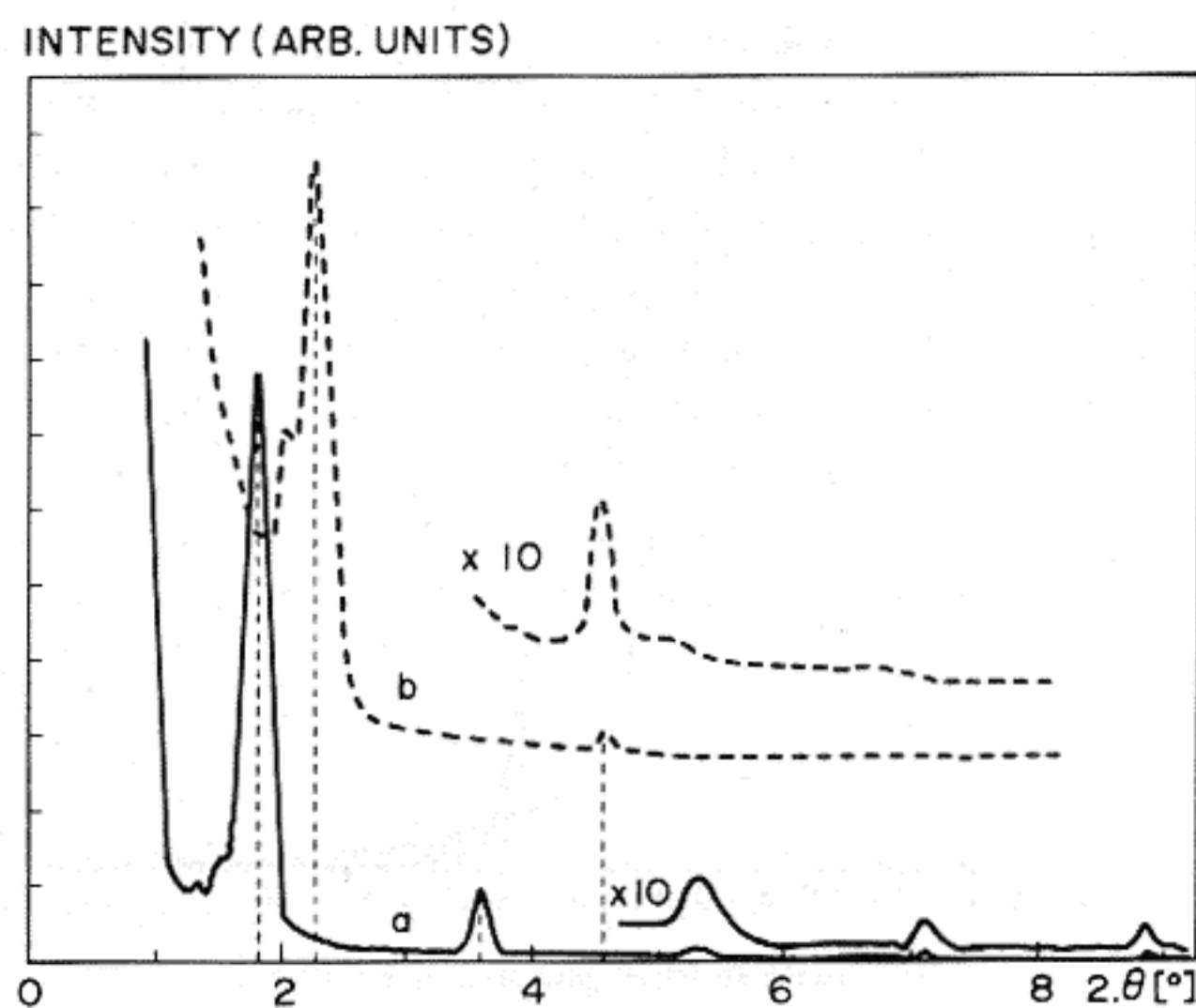


Fig. 3. Small-angle X-ray diffraction curves of 50 monolayers of the GL-GDNT-PH (a) and 60 monolayers of GDNT (b) deposited by the horizontal lifting technique from the surface of pure water onto hydrophobic silicon substrates. Calculated periods are equal to 5.02 and 4.00 nm for the curves (a) and (b) respectively.

The GL-GDNT-PH films are structurally well organized in the direction normal to the substrate plane. The X-ray small-angle diffraction curve (Fig. 3) shows five Bragg reflections, which is rather surprising because in reality the compound is a mixture of several components with a different number of cyclopentane rings in the $C_{40}H_{72-80}$ isoprenoid chains. Thus, although molecules do not form crystallites the thickness of the monolayer is fixed with high precision. The

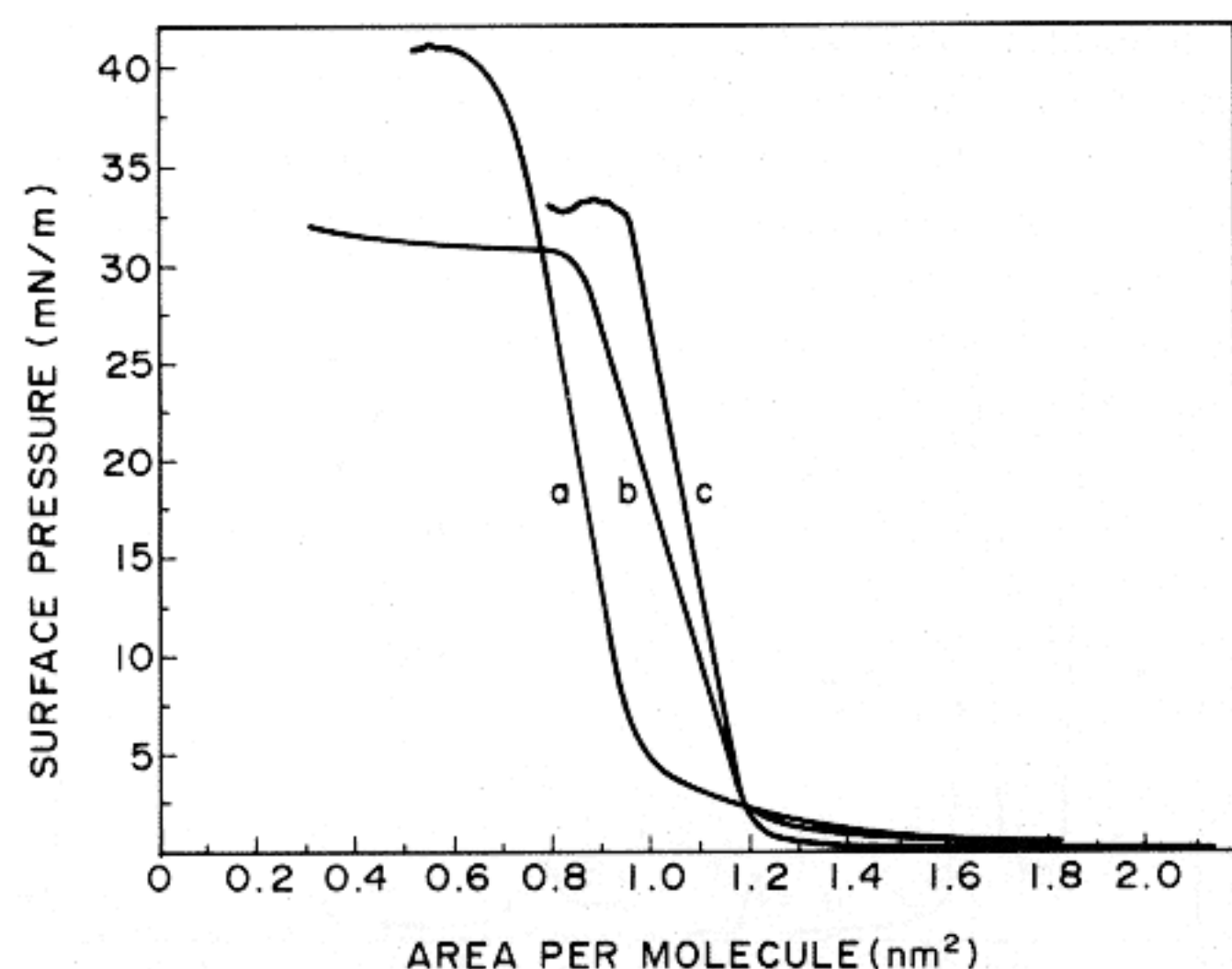


Fig. 4. Surface pressure–area isotherms of GL-GDNT-PH (a), GL-GDNT (b) and GDNT (c) monolayers spread at the surface of pure water. The initial solution of the GL-GDNT (10 mg ml^{-1}) in methanol–chloroform (1:1, v/v) was diluted by ethanol up to a concentration of 0.5 mg ml^{-1} . A solution of GL-GDNT-PH (0.33 mg ml^{-1}) in chloroform–methanol–water (65:25:4 by vol.) was used. GDNT was dissolved in chloroform–methanol–ethanol–hexane (2:1:1:2 by vol., 0.33 mg ml^{-1}). Areas per molecule extrapolated to zero surface pressure are equal to 1.00 , 1.18 and 1.20 nm^2 for the curves (a), (b) and (c) respectively.

structure does not depend on the deposition technique. Period in the normal direction is equal to 5.02 nm . Under the condition of Y-type deposition the thickness of the monolayer equals 2.51 nm . The surface pressure–area isotherm (Fig. 4, curve a) shows the area per molecule extrapolated to zero surface pressure of $1.00 \pm 0.03 \text{ nm}^2$. Extrapolated area per molecule for a condensed monolayer at the air–water interface usually corresponds with satisfactory accuracy to the area occupied by the molecule after transferring the monolayer onto the solid substrate. The morphology of the film in Fig. 2(a) proves rather satisfactory spreading of the monolayer at the air–water interface if one takes into account that a selection of good spreading solvent for native archaeol lipids is a difficult problem in principle.

3.4. Structure of monolayers and films of GL-GDNT-PH

Under such conditions the U-shape of the molecule with approximately vertical arrangement of the tails is preferred because the minimum possible area per chain is equal to approximately 0.27 nm^2 [10], and the total cross-section of four closely packed chains equals 1.08 nm^2 . A small decrease in the experimental value of the area per molecule with respect to the expected value may be explained by formation of the precipitates mentioned above if one supposes that they arise during spreading process. Evaluations show that the thickness of the monolayer in the case of vertical arrangement of the chains is in agreement with the value of the period (5.02 nm). Indeed, the length of the isopranyl chain is about 4 nm [10] and the minimum dimension of two hydrophilic groups in the direction normal to the layer plane cannot be

less than 1.0 – 1.2 nm . The last value is roughly equated as a ratio of the calculated volume of the groups [10,16] and the area per molecule. Strictly speaking, a situation with molecules in a U-shape cannot be distinguished from that with highly tilted molecules in the extended form if only the area per molecule and the period in normal direction are used for analysis. But the extended structure of the molecule is unlikely. First of all, both hydrophilic groups interact strongly with water and there are not sufficient reasons for one of them to be pushed out from the aqueous subphase. Then, proceeding from the area per molecule of 1.00 nm^2 and cross-section of two chains normal to the axes of 0.54 nm^2 one can evaluate the tilt angle of the molecule to be equal to approximately 57° . That is quite unusual for the monolayer with a sharp surface pressure–area isotherm and high value of collapse pressure. Lastly, before compression of the monolayer the molecule in an extended form must occupy an area at the air–water interface of about 5.00 nm^2 , which can be calculated from the data on molecule dimensions presented above. Approximately at this value of area per molecule the surface pressure should increase because the molecules begin to interact with each other, but really the surface pressure is equal to zero up to 1.8 nm^2 and begins to increase sharply at approximately 1.1 nm^2 .

3.5. Deposition and study of LB films of GL-GDNT

The GL-GDNT multilayer can be deposited by LB and horizontal lifting techniques. Unexpected deposition of GL-GDNT with the LB technique although the MSL value is higher than the surface pressure of deposition may be explained by the slow rate of water penetration into the film, which was dried before every dipping down. In this case the system does not reach equilibrium. Deposition is possible only onto hydrophobic substrates. It appears that the morphology of GL-GDNT film depends slightly on the pH value of the water subphase although the reasons for such behaviour are not clear enough. An example of rather satisfactory deposition is presented in the electron micrograph in Fig. 2(b). However, the reproducibility of the deposition quality is bad and the results are unpredictable. That is demonstrated by the transfer ratios for two processes of depositions (first 20 monolayers) in Table 1. Surface pressure–area isotherms (Fig. 4) show an area per molecule of about 1.20 nm^2 . The deposition of Y-type takes place. The period in the normal direction calculated from the X-ray diffraction pattern (Fig. 5) equals 4.70 nm , which can be explained again by the model of the molecule in a U-shape. The electron diffraction pattern in Fig. 2(b) shows a practically amorphous film in the layer plane.

3.6. Deposition and study of GDNT films

The GDNT produced from TLE can be considered as the result of hydrolysis of the GL-GDNT and GL-GDNT-PH with acid. Its deposition occurs approximately in the same

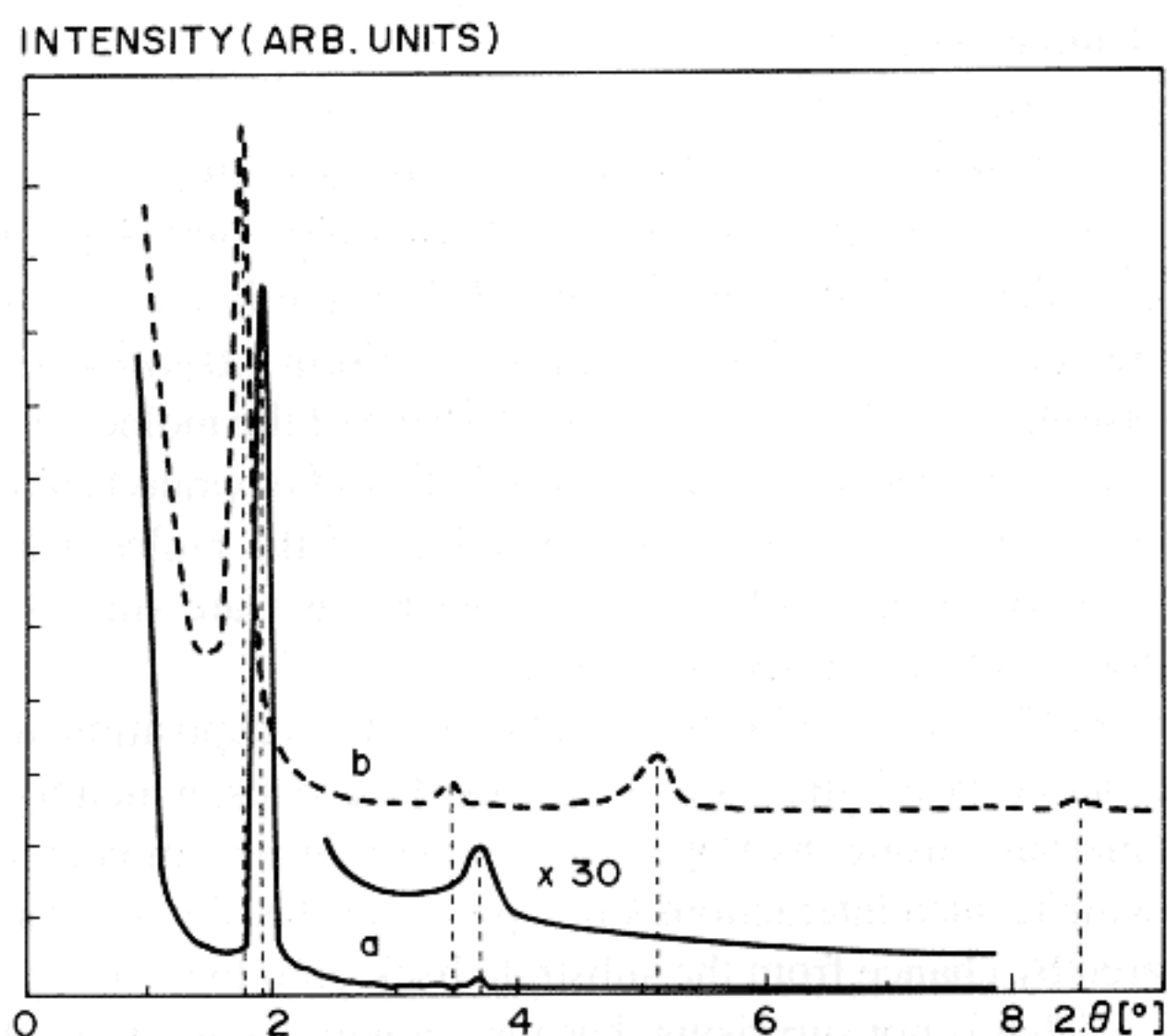


Fig. 5. Small-angle X-ray diffraction curves of 54 monolayers of GL-GDNT (period of 4.70 nm) (a) and of 94 monolayers of GL-GDNT mixture with arachidic acid at a molar ratio of 1:10 (period of 5.07 nm) (b) deposited onto hydrophobic silicon substrates. GL-GDNT films were deposited by the horizontal lifting technique from the surface of pure water. The mixed monolayers were transferred by the LB technique at pH 7.0.

manner as the deposition of the GL-GDNT (Table 1). The possibility of successful LB technique application can be explained also by slow kinetics of water penetration inside the film. The area per molecule is equal to approximately 1.20 nm² (Fig. 4) and the period diminishes with respect to that of GL-GDNT films. It is equal to 4.00 nm (Fig. 3). The electron diffraction pattern in Fig. 2(c) shows only weak broad rings. Thus, changes of the main feature of molecular packing with respect to that of GL-GDNT-PH and GL-GDNT, i.e. packing of the molecules in a U-shape in the monolayer, are not expected. Morphology of the deposited film can be considered satisfactory, which is confirmed by optical and electron microscopy studies. The electron micrograph is shown in Fig. 2(c).

3.7. Peculiarity of molecular structure of archaeol lipids

On the basis of these experimental data we may also expect that the bend in the isopranyl chains somewhere in the middle position happens in such a manner that the four portions of U-bent chains of each molecule appear to be pushed out from water and closely packed with each other initially after spreading of the monolayer. Otherwise the sharp increase of the surface pressure under compression of the monolayer only at the area per molecule close to the cross-section of four chains is not understandable. To a certain extent the archaeol molecule with such a configuration is similar to the usual surfactant molecule at the air–water interface.

3.8. Mixed films of GL-GDNT and arachidic acid

After experiments with the GL-GDNT-PH, GL-GDNT and GDNT films it was concluded that to improve the deposition

reproducibility as well as the multilayer morphology other attempts to vary deposition procedure must be undertaken. The presented results show that in the case of deposition onto a hydrophobic substrate the main difficulties arise because the interaction between hydrophilic surfaces of two adjacent monolayers in the archaeol lipid film is not strong enough with respect to that between monolayer and water. This ratio can be changed if archaeol lipid is mixed with a surfactant compound which possesses a low ESP value and can be deposited without difficulties. Such an approach was used in many works to deposit the films containing some unusual molecules. Deposition of mixed films of valinomycin and arachidic acid, for example, have been reported in Ref. [17]. We have tried to produce highly uniform monocomponent GL-GDNT films using the deposition of mixed films as the first step. The GL-GDNT multilayer is insoluble in hexane. Thus, deposition of the GL-GDNT mixed film with some surfactant compound soluble in hexane can be carried out first. Then, the second component can be removed from the film by treatment in hexane. In our case arachidic acid was used as a second component of the mixture. Such a method has already been applied, for instance, to deposit uniform films of C₆₀, which is quite an unusual compound for the LB technique [18].

To achieve comparable values of area occupied by molecules of both types in the monolayer, mixtures with molar ratios of the GL-GDNT and arachidic acid of 1:5 and 1:10 were chosen for film deposition because the area per arachidic acid molecule is 0.20 nm² [13]. The transfer of such mixed monolayers onto the substrates appears to be reproducible and the uniformity of the film is better than that of the GL-GDNT. The morphology of the film is presented in Fig. 2(d). The X-ray diffraction curve (Fig. 5) shows reflections from the separate phase of arachidic acid only. The structure of the areas between the islands of arachidic acid cannot be determined from the experimental data. These areas may consist either of a disordered phase of GL-GDNT or of solid solution of GL-GDNT and arachidic acid. The presence of both phases is also possible. Electron microscopy, electron diffraction and X-ray small-angle diffraction studies show that arachidic acid phase consists of small aggregates of 20–30 nm in size which are distributed homogeneously in the film.

3.9. GL-GDNT films obtained by treatment of mixed multilayers in hexane

The solubility of the film of each type in hexane was checked by comparison with standards of thickness, i.e. with the films on silicon substrates consisting of different numbers of the monolayers. The standards were prepared for every compound. Variation of interference colour after immersion of the sample in the solvent enables one to detect a change of thickness of about 3–5 nm when a film 50–100 nm thick is used. Thus, the portion of the compound dissolved from the film can be determined with an accuracy of about 2–5%. The experiments on dissolution were carried out at room temper-

ature. LB film of pure arachidic acid consisting of 40–50 monolayers is removed completely in a few seconds from the substrate by hexane. No traces of the film can be detected with the optical microscope. The thickness of GL-GDNT film with the same number of monolayers does not change in 1 h at least. This result is understandable because GL-GDNT is a component of the polar lipid extract which is obtained from the total lipid extract after washing of the latter with hexane [3].

After immersing the sample with mixed multilayer in hexane the thickness of the film decreases approximately proportionally to the content of arachidic acid. That was checked by comparing the interference colour of non-dissolved film with the GL-GDNT standard of thickness because it was obvious beforehand that GL-GDNT would be the main component of the film after immersing the sample in hexane. It is remarkable that the morphology of the multilayer does not deteriorate under such treatment. The electron microscopy images remain practically the same as that in Fig. 2(d). Thus, after the dissolution of the arachidic acid phase GL-GDNT fills any arising cavities in some way, so that the film remains continuous and uniform.

However, the expected thicknesses of the films after removing arachidic acid are 25% less than experimental values for both molar ratios of GL-GDNT and arachidic acid in the mixture. X-ray diffraction patterns show the presence of a separate arachidic acid phase in the sample. Intensities of the peaks from the arachidic acid phase decrease approximately 5–6-fold with respect to the initial ones. The thickness of the film does not change any more after treatment of the sample in hexane for several hours. Probably, owing to the interaction with the GL-GDNT molecules, small aggregates of arachidic acid cannot be removed from the multilayer. The formation of some insoluble salt with impurities dissolved in water may be another reason for this result. Indeed, mixed films with the best morphology are deposited at pH 7.0, while the optimal range of film deposition for pure arachidic acid is between 4.7 and 5.0. Although the salt content depends on the nature of impurity ions and on their concentration, which are unknown, it is clear that at pH 7.0 the fraction of salt must be considerably higher. However, the first reason is more likely because spacings for the crystalline phase of salt should differ from those of the acid phase. The crystalline order of the GL-GDNT phase does not change under the treatment, i.e. film remains practically amorphous if we exclude small residuals of crystalline arachidic acid phase. It seems that no recrystallization of GL-GDNT takes place because of its absolute insolubility in hexane.

In principle, such a method is suitable for depositing uniform LB films of lipids from archaea because the small residuals of arachidic acid cannot influence strongly the properties of the films. However, fatty acids seem to be non-optimal media for dissolution of the lipids. Better results are expected if the matrix molecules do not form a crystalline phase and their properties do not depend on pH.

4. Conclusion

Lipids from archaea can be deposited onto solid substrates under some conditions by the LB technique although the molecules used possess an unusual structure in comparison with compounds which form highly uniform LB films. This possibility may be caused by the U-shape of the molecules at the air–water interface with close packing of isopranyl chains at the same time, so that the behaviour of the molecules at the interface and their interaction with water are similar to those for usual surfactant molecules.

Insufficient reproducibility of the results on deposition may be caused by the structure of hydrophilic groups, which form numerous strong hydrogen bonds with water molecules. Owing to such interactions small pieces of the film are transferred by chance from the substrate back onto the water surface. That is not surprising, because in native conditions the GL-GDNT and GL-GDNT-PH, in particular, exist in membranes surrounded by aqueous solution.

Application of the method of two-component mixed multilayer deposition may result in formation of highly uniform films of lipids from archaea. That can be achieved by the dissolution of one component from the sample with suitable solvent after the multilayer film is deposited. Practically no change of the film morphology is observed with optical and electron microscopes after such treatment if the phase of lipid from archaea is quite insoluble in the solvent.

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