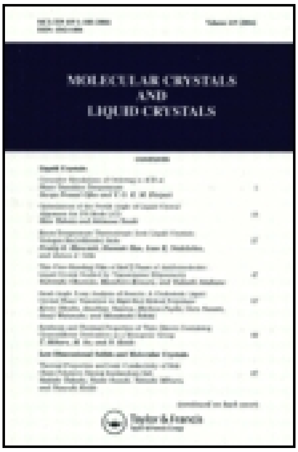

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S. Dante [a] , M. De Rosa [b] , E. Maccioni [a c] , A. Morana [b] , C. Nicolini [c] , F Rustichelli [a] , V. I. Troitsky [d e] & B. Yang [a]

[a] Istituto di Scienze Fisiche, University of Ancona, (Italy)

[b] Istituto di Biochimica delle Macromolecole, II University of Naples, (Italy)

[c] Istituto di Biofisica, University of Genoa, (Italy)

[d] Technobiochip, Marciana, Livorno, (Italy)

[e] Zelenograd Researh Institute of Physical Problems, Moscow, (Russia)

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THERMAL STABILITY OF BIPOLAR LIPID LANGMUIR BLODGETT FILMS BY X-RAY DIFFRACTION

S. Dante[1], M. De Rosa[2], E. Maccioni[1,3], A.Morana[2], C.Nicolini[3],
F Rustichelli[1], V.I.Troitsky[4*] and B. Yang[1]
[1]*Istituto di Scienze Fisiche, University of Ancona*
(Italy)
[2]*Istituto di Biochimica delle Macromolecole,*
II University of Naples (Italy)
[3]*Istituto di Biofisica, University of Genoa, (Italy)*
[4]*Technobiochip, Marciana, Livorno (Italy)*

Abstract The thermal stability and structure of two uniform Langmuir - Blodgett films made of bipolar lipids from archaea have been studied. X-ray diffraction measurements at various temperatures from 20°C to 100°C were performed on these films, during heating and cooling cycles. The Bragg peak position, the integrated intensity and the peak width have been recorded as a function of temperature. Both intensity of diffraction peaks and layer thickness diminish at high temperatures while the correlation length, normal to the multilayer plane , displays some significant changes during the heating cycle. The data suggest the occurrence of some phase transitions. As a final result, the thermal stability of these two bipolar lipids LB-films is quite different and very high for the Phospho 2 LB film, which is stable till 100°C and is very promising for technological applications.

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[*] Permanent address: Zelenograd Researh Institute of Physical Problems,Moscow,(Russia)

INTRODUCTION

Sulfolobus solfataricus is a thermoacidophilic archaeon whose natural habitat is at 85-90°C with a very low pH (about 3). The presence of unusual bipolar lipids in the plasma membrane of archaea raises questions regarding their organization [1]. Indirect evidence based on the absence of preferential fracture plane in the middle of the lipid layer suggests a monolayer organization of the plasma membrane; moreover, black films from one component of the membrane lipids assumed such a structure [2]. Another important characteristic of bipolar lipids is their polymorphism; in fact varying a limited number of parameters such as the water content in bulk phase and the chemical composition they may assume a wide number of conformations.The temperature as well plays an important role, particularly as physiological stabilizer of the cyclopentane rings similar to the one with double bonds and single bonds in usual lipids [3]. To clarify this point recent structural studies have been carried out by using X-ray diffraction [4, 5,]

An important contribution to the structural analysis of lipids and possible application of these stable molecules in bioelectronic is given by the multilayers production on solid substrates by Langmuir-Blodgett (LB) technique [6,7]. In this paper we will consider more specifically the thermal stability of artificial multilayers from bipolar lipids In differential scanning calorimetric studies the phase transitions of some bipolar lipids in the plasma membrane of archaea were reported [3]. In general, two phase transitions have been observed. In order to investigate the corresponding structural changes of such transitions, we produced thin films by using two mixtures of native bipolar lipids extracted from *Sulfobolus solfataricus*, synthetically named Glico 2 and Phospho 2.

In particular we have analyzed the structural changes occurring in the deposited LB films as a function of temperature. The experimental results reveal a different behaviour for different molecules and the existence of particular temperatures probably corresponding to phase transitions.

EXPERIMENTAL DETAILS

Purification of archaeal lipids

The bipolar membrane lipids of *Sulfolobus solfataricus* are based on two series of tetraether core lipids, named caldarchaeol (Figure 1 a-c and 2 A-C) and nonitolcaldarchaeol (Figure 1 a'-c' and 2 A'-C')). The investigated mixtures contains: PHOSPHO 2 - 90% of the phospho-*myo*-inositol-β-D-glucopyranosyl derivative of nonitolcaldarchaeol and 10% of the phospho-*myo*-inositol-β-D-galactopyranosyl-β-D-glucopyranosyl derivative of caldarchaeol (Fig.1); GLICO 2 - 95% of glucopyranosyl derivative of nonitol caldarcheol and of 5% of b-D-galactopyranosyl-β-D-gluco-pyranosyl derivative of caldarchaeol (Fig.2).

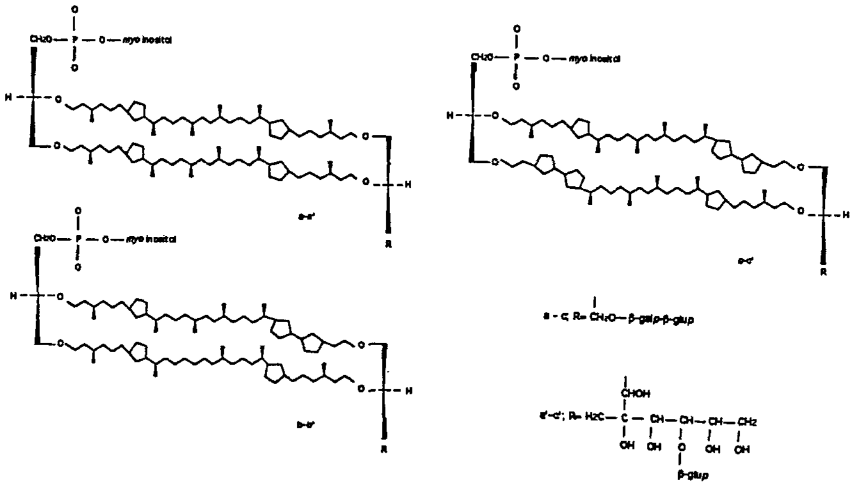

FIGURE 1 Molecular formula of Phospho 2 which is a mixture of lipids with different cyclization degree in isoprenoidic chains

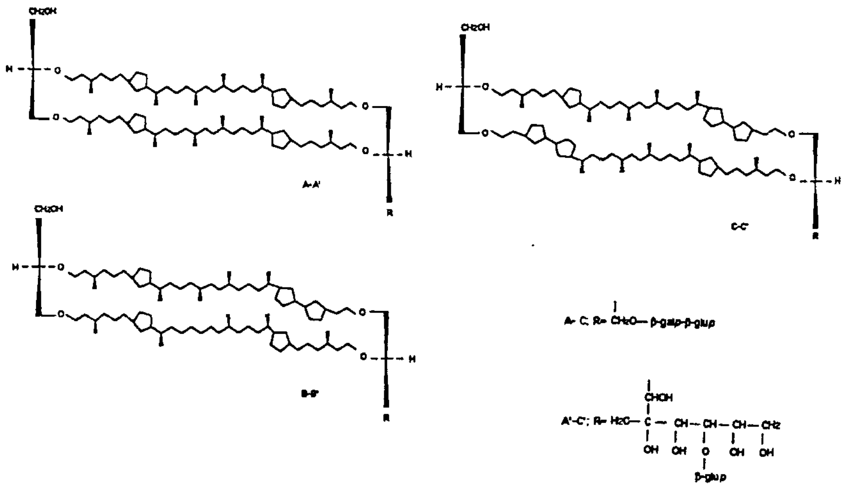

FIGURE 2 Molecular formula of Glico 2 which is a mixture of lipids with different cyclization degree in isoprenoidic chains

S. solfactaricus (DSM 1617) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig). The microorganism was grown at 87°C in a 100 l fermenter containing 90 l of medium as described by De Rosa *et al* [2]. Cells were harvested in the late stationary growth phase of incubation (48h) by centrifugation. The dried cells were extracted continuously with a Soxhlet apparatus for 12 h with chloroform/ methanol (1:1 v/v) and then with methanol/water (1:1 v/v). The total lipid extract was purified on silica gel (Merck Kieselgel 230-400 mesh) column, eluted under pressure using eluents of increasing polarities: chloroform, chloroform/methanol (85:15 v/v) chloroform/methanol (65:25 v/v) chloroform/ methanol/water (65:25:4 v/v/v).

Film deposition

LB films were deposited on hydrophobic silicon substrates. In order to obtain high quality films, the deposition of samples of each bipolar lipid was performed under different experimental conditions (varying surface pressure of deposition, deposition speed, values of pH etc.)

Monolayers were formed on a Langmuir trough (LB-MDT-4000). Twice distilled water was used for the subphase preparation. Organic solvents were supplied by Sigma.

Phospho 2 was dissolved in a mixture of chloroform, methanol and water in the ratio of 65:25:4 (v/v/v). The concentration of the solution was equal to 2 mg/ml. The deposition was carried out by vertical lift technique after compression of the monolayer up to 25 mN/m on hydriphobic silicon treated by dimethyldichlorosylane.

Glico 2 was dissolved in a mixture of methanol and chloroform (10mg/ml, 1:1) and was then diluted by ethanol up to 0.5 mg/ml. The best deposition was carried out on silicon hydrophobic substrates treated by hydrofluoric acid by vertical lift technique with a speed of 0.5 cm/min at the surface pressure of 27 mN/m and pH 5.0. Pause duration in up position was equal to 3 minutes to allow the evaporation of the attached water.

X-ray measurements

X-ray diffraction experiments were performed by a rotating anode generator "RIGAKU DENKI RV300" (40KV and 200mA), Ni-filtered Cu-Kα radiation (λ=1.54Å) was used. X-ray diffraction patterns were recorded by using a coventional powder diffractometer; θ-2θ reflection geometry was employed.

LB samples were placed on a home made, dedicated hot-stage containing two electrical resistors, whose temperature was controlled with a precision of ±0.1°C up to 200°C by a BT 300/301 control system supplied by SMC (Grenoble, France). Before each measurement the sample was allowed to equilibrate for about five minutes at new temperature.

RESULTS AND DISCUSSION

Informations about the structure of LB film can be obtained by the analysis of their X-ray diffraction patterns. In this paper we analyze in particular three parameters:

1) the Bragg peak position which provides the layer thickness;

2) the absolute integrated peak intensity (measured as the area of the first order layer reflection after subtraction of the local background)

3) the correlation length of the layers, evaluated from the Full Width at Half Maximum (FWHM) of the first order layer reflection.

These quantities for each sample at different temperatures, could be indicative of the order of LB-film. For instance, if the film is partially or totally melted at higher temperature, the peak intensity decreases or becomes zero.

The correlation length L is calculated according to the Scherrer equation:

$$L = K\lambda/(\beta\cos\theta)$$

where K is a constant approximately equal to 0.9, β is the intrinsic width of the X-ray diffraction peak, calculated by applying the Warren's formula:

$$B^2 = \beta^2 + b^2$$

The parameter B is the observed width of the peak (FWHM) whilst b is the broadening effect due to the diffractometer (in our case b=0.082°).

LB-films of Phospho 2 and Glico 2 were investigated during heating and cooling cycles.

Figures 3 presents the low-angle X-ray diffraction pattern and the layer thickness of Phospho 2 film as a function of temperature in the heating cycle. It appears that the first order

Bragg peak remains till 100°C, indicating that still at this temperature a periodic organization exists, in spite of a reduced order indicated by a lowering of the peak intensity.

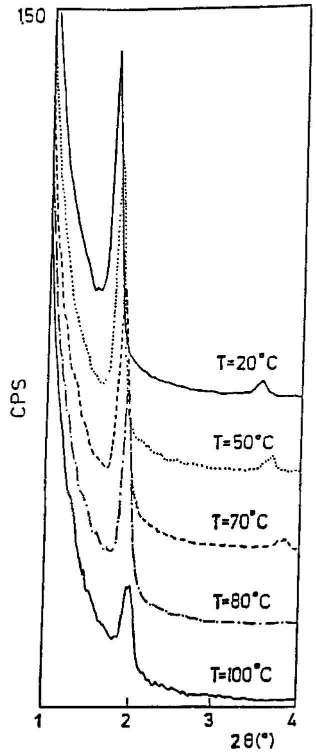

FIGURE 3 X-ray diffraction profiles of Phospho 2 LB film during the heating process recorded at the temperature of T=20°C, T=50°C, T=70°C, T=80°C, T=100°C.

Moreover a progressive shift of the angular position of the peak towards higher scattering angle is observed, as far as the temperature increased, indicating a progressive reduction of the layer thickness, as it will be shown below in a quantitative way.

Fig.4 reports the low angle diffraction patterns during the cooling process of the same Phospho 2 LB film. In this case no appreciable variation is observed in the diffraction pattern, in particular in the peak intensity and angular position. This implies that no recrystallization process takes place during the cooling, and that some irreversible structure changes occur during the heating process.

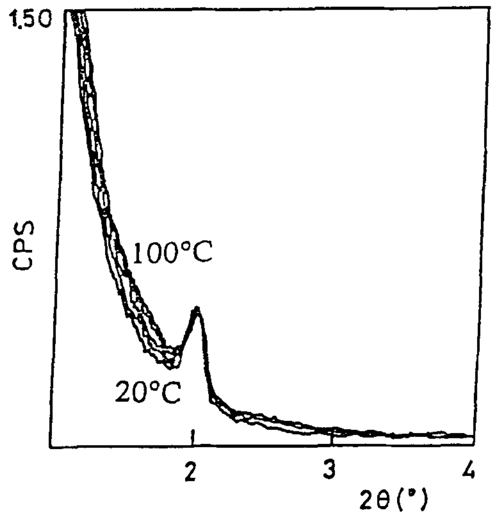

FIGURE 4 Diffraction peaks profiles of Phospho 2 LB film during the cooling process at different temperatures

Fig. 5 shows the temperature dependence of the layer thickness both during the heating and the cooling processes. A progressive reduction is observed of the repeat period from about 50 Å to 45 Å as the temperature increases from room temperature till 70°C. Then a plateau is observed till 90°C and finally a slight decrease to 44Å.

During the cooling process the layer thickness remains almost constant, showing an hysteresis effect.

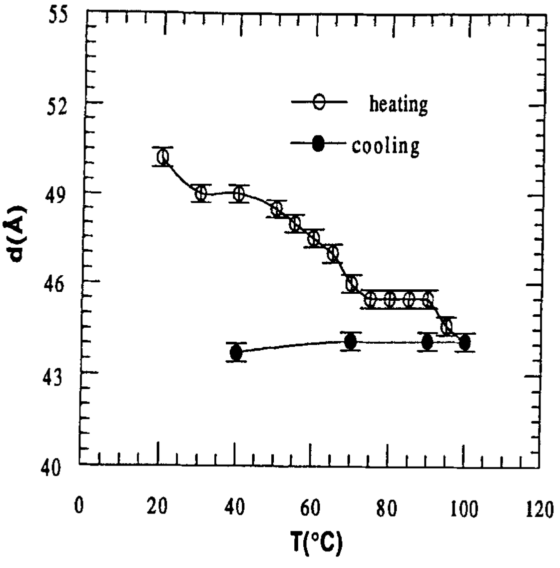

FIGURE 5 The changes of layer thickness d of Phospho 2 LB film with the temperature during the heating (white circles) and the cooling (black circles) processes.

Fig.6 reports the temperature dependence of the integrated intensity of first order layer Bragg reflection of the same LB film. It appears that the intensity remains almost constant till 70°C and then decreases almost linearly till a value of 30% at 100°C. During the cooling process the intensity remains constant. This fact may indicate that some melting process starts in the film at 70°C or that another kind of phase transition occurs involving a redistribution of atoms in the lattice cell, with a consequent modification of the structure factor.

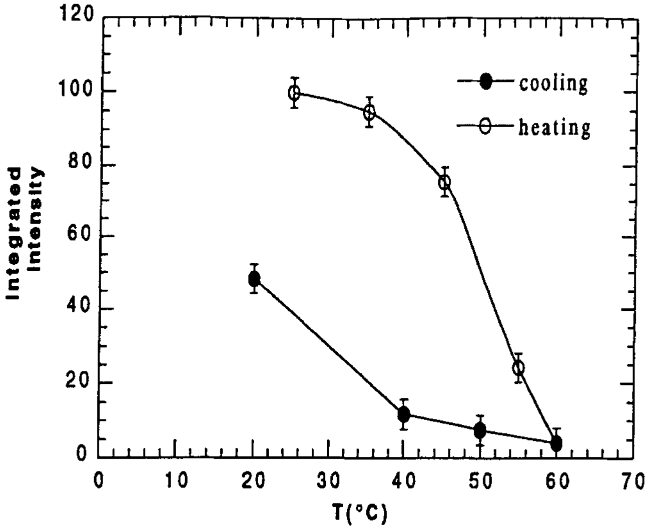

FIGURE 6 Integrated intensity of the first order layer reflections of Phospho 2 LB film as a function of temperature during the heating (white circles) and the cooling (black circles) cycles

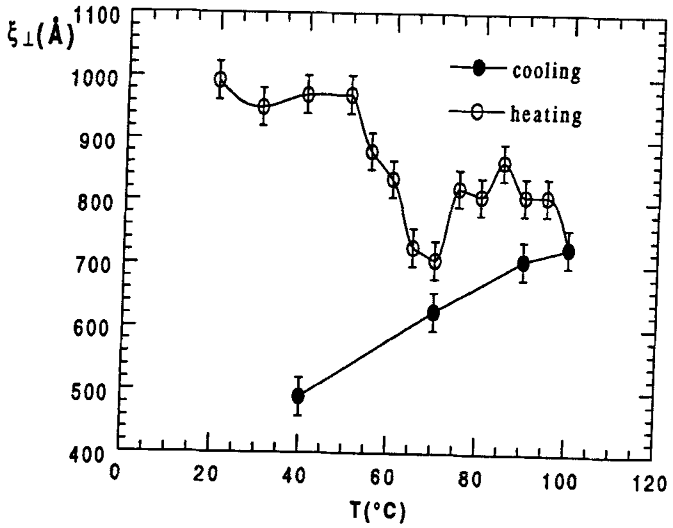

FIGURE 7 Correlation length $\xi_{\perp}$ of Phospho 2 film along the direction normal to the layers as a function temperature during the heating and the cooling processes

Fig.7 reports the temperature dependence of the correlation length $\xi_\perp$ of the same LB film along the direction normal to the layers. It appears that $\xi_\perp$ remains almost constant till 50°C, then rapidly decreases almost linearly till 70°C and rises again at higher temperatures. This behaviour is again characteristic of a phase transition at 70°C. As a general remark it should be emphasized that at 70°C significant changes occur in all the considered physical quantities, namely layer thickness, intensity and correlation length, supporting the hypothesis either of a phase transition or of important changes in the molecular conformation.

Fig.8 reports the low angle diffraction patterns from Glico 2 LB film during the heating process. Qualitatively the same trend is observed as for the Phospho 2 LB film, but quantitatively an important difference is observed in the thermal stability. In fact in this case already at 60°C an almost vanishing Bragg peak is observed.

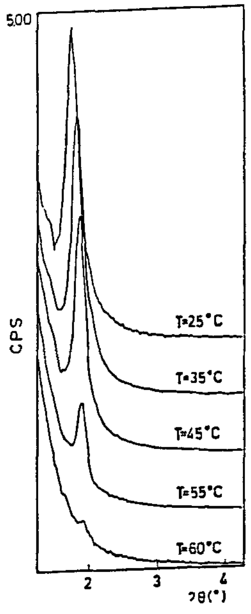

FIGURE 8 Diffraction patterns obtained from a Glico 2 LB film at different temperatures

Fig. 9 reports the low angle diffraction patterns from Glico 2 LB film during the cooling process. A different behaviour is observed, also from a qualitative point of view, as compared to the Phospho 2 LB film. In fact in this the peak intensity increases at lower temperatures, probably as a consequence of a molecular recrystallization on the substrate. However the intensity does not reach a value as high as the one at the beginning because of some irreversible modifications in structure which occur during the heating process.

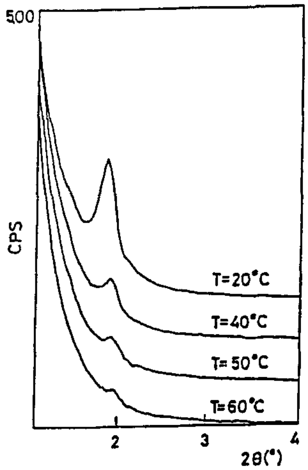

FIGURE 9 Diffraction patterns obtained from a Glico 2 LB film during the cooling process at diffrerent temperatures

Fig.10 reports the temperature dependence of the layer thickness in Glico 2 LB film. A regular decrease is observed during the heating process from 48 Å to 44Å, which is lower but of the same order of magnitude as in the case of Phospho 2 LB film. During the cooling process a partial recovery is observed also in this quantity.

Fig. 11 reports the temperature dependence of the integrated intensity of the first order Bragg reflection from Glico 2 LB film. The intensity drops down very fast when the temperature is over 45°C indicating that the film starts to melt. The partial recovery during the cooling process was already discussed qualitatively.

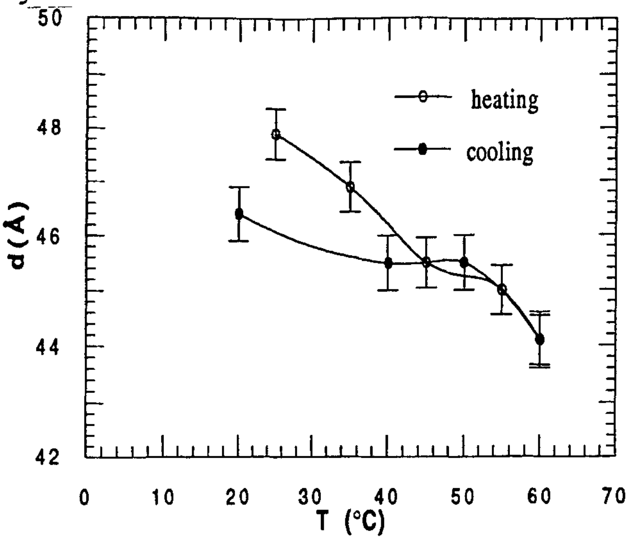

FIGURE 10 The changes of layer thickness d of Glico 2 LB film with the temperature during the heating and the cooling processes

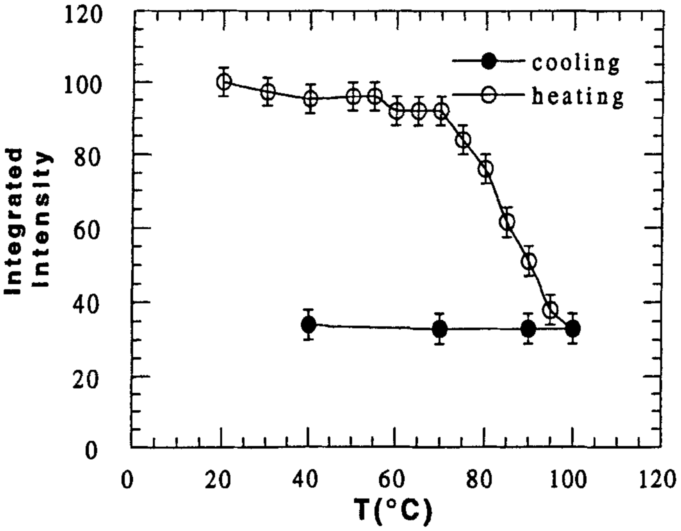

FIGURE 11 Integrated intensity of the first order layer reflection of LB film of Glico 2 as a function of temperature during the heating cycle (white circles) and the cooling cycle (black)

Fig.12 reports the temperature dependence of the correlation length $\xi_\perp$ of the Glico 2 LB film along the direction normal to the layers. In this case only minor changes are observed around an average value of $\xi_\perp = 500$ Å. As a general remark no evidence for some critical temperature is observed for this LB film, resembling to the one at 70°C of the other film.

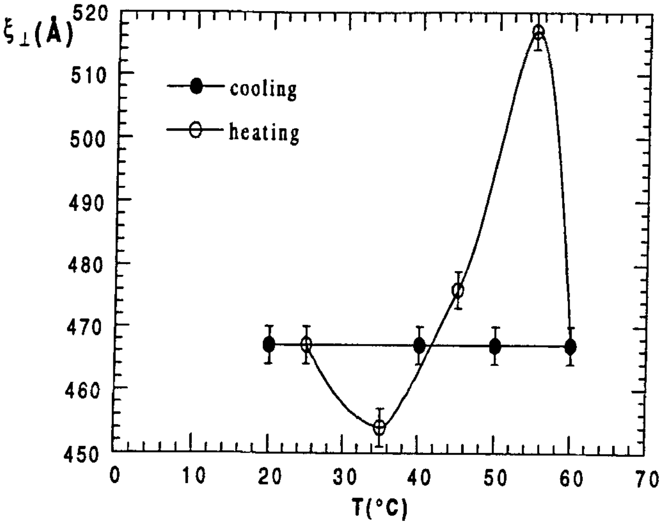

FIGURE 12 Correlation length $\xi_\perp$ of Glico 2 LB film along the direction normal to the layers as a function of temperature during the heating and the cooling processes

It is a remarkable fact that the melting points of Phospho 2 and Glico 2 are quite different. Films of Phospho 2 are more resistant at high temperature than the Glico 2 films. It can be supposed that differences in the way the film structure develops as a function of temperature for the two lipids, depend on their different molecular structural formula; in fact in the Glico 2 molecule, the glycerol headgroup is unsubstituted. Bipolar lipid polymorphism studies reports the evident tendence of this group always to segregate away from the other types of headgroups and, in presence of adequate content of water, to partition preferentially in the hydrocarbon matrix.

The periodical order within Langmuir-Blodgett films of Glico 2 disappears in correspondence of temperatures of about

The periodical order within Langmuir-Blodgett films of Glico 2 disappears in correspondence of temperatures of about 60°C, but cooling down to 20°C molecular rearrengements or recrystallization processes seem to be possible. This behaviour could be similar to the one of fatty acid salts films where the contraction of period D is due to the predominance of the rotation around the C—C axis and of rubberlike elasticity over the stretching and scissoring modes [3].

On the contrary in Phospho 2 LB films, the heating cycle causes a progressive and irreversible destruction of the structure organized in the z direction, yet it's necessary to reach higher temperatures (about 100°C), to observe a complete disappearing of any Bragg reflection.

Hence it can be supposed that submitting the films to a temperature enhancement, the glycerol headgroup of Glico 2 should disarrange the strictly packed structure troughout each layer, more strongly than the Phospho 2 polar head (glycerol+phosphomyoinositol). The recrystallization during the cooling process also could be attributed to glycerol headgroup mobility: this probably rearrange so that the film order is partially recovered.

In Phospho 2 films the process is slower, but irreversible: the molecule in fact is more rigid than the Glico 2 molecule and probably interactions between the polar heads are more consistent as well. This hypothesis is confirmed by the fact that it is necessary to raise the temperature to 100°C to definitively destroy the lamellar order. It is quite improbable that interaction strenghts between hydrophobic chains of close layers, generally weak London interactions, play an important role in thermal stability of bipolar lipid films.

Concerning the correlation length, it is possible to note a structure disorder at temperatures below the melting point. This kind of disordering change is reversible and it may be caused by melting of paraffinic chains in the molecules. The irreversible changes may occur as the temperature reaches the point in correspondence of which the head group "lattice" melts. In order to confirm this explanation further and more sophisticated axperiments should be performed.

CONCLUSIONS

The thermal stability of two uniform LB films made of bipolar lipids from archaea living at high temperature (87°C) and high acidity (pH=3) was investigated by X-ray diffraction. The main results was the observation that the Phospho 2 LB film is stable till very high temperature (100°C) and is suitable for incorporation of functional molecules in view of technological application. The Glico 2 LB film is stable only till 60°C. Strong hysteresis effect are observed in both samples (with a partial recovery for the case of Glico 2) in the layer thickness, integrated intensity and correlation length $\xi_\perp$ in the direction perpendicular to the layers. Finally the data suggest that some important structural changes occur in the Phospho 2 LB film at 70°C, namely a phase transition or appreciable molecular conformation modifications.

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