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## Monolayer properties of membrane lipids of the extreme halophile *Halobacterium cutirubrum*, at the air / water interface

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Compression isotherms relating surface pressure with mean molecular area of the two major membrane lipids of *Halobacterium cutirubrum* have been recorded at the air / water interface. The lipid constituents studied were diphytanyl ether analogues of ammonium salts of phosphatidylglycerol phosphate and glycolipid sulphate. The influence of subphase pH and NaCl concentration on surface characteristics such as limiting molecular area, collapse pressure, collapse area, surface compressional modulus were investigated. The effect of pH (0 to 5.6) and electrolyte concentration (0.01 to 1 M NaCl) on these parameters is not significant in the case of phosphatidylglycerol phosphate in contrast with glycolipid sulphate where pH and particularly electrolytes cause major changes in film characteristics. There is a marked increase of molecular area and decrease in both collapse pressure and surface compressional modulus of glycolipid sulphate monolayers with increasing subphase NaCl concentration. Theoretical cross-sectional areas of the phytanyl chains and of the polar groups have been calculated for different conformations and these have been used to interpret the experimental data. It is concluded that the polar group of phosphatidylglycerol phosphate is readily accommodated beneath the phytanyl chains and both limiting molecular area and area occupied per molecule at collapse pressure are determined by the chains rather than the polar head group. The collapse area of glycolipid sulphate, however, is determined by its polar head group, which penetrates deeply into the aqueous subphase at low electrolyte concentrations, but at high NaCl concentrations it is oriented up into the interface, presumably, due to a salting out effect. These characteristics are discussed in terms of the properties of the lipids in aqueous dispersions and in purple membrane of *Halobacterium cutirubrum*.

### Introduction

The extreme halophiles are characterised by growth conditions that demand saturated or al-

most saturated salt solutions [1,2]. The lipid constituents of the cell membrane of these organisms are distinguished from those of other organisms by the presence of two saturated phytanyl groups linked by ether bonds to a glycerol backbone at carbons 2 and 3. It is believed that this molecular configuration serves to stabilise the membranes by resisting peroxidative damage, being ether-linked alkyl groups they are stable to chemical hydrolysis

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and because of the position of attachment to the glycerol, they are afforded protection against attack by phospholipases of other organisms [3].

The major lipids of both the red and purple membrane of *Halobacterium cutirubrum* are analogues of 2,3-di-*O*-phytanil-*sn*-glycerol. The two dominant lipid classes contain polar groups linked to the carbon 1 of the glycerol backbone and consist of 3'-phospho-*sn*-glycerol-1'-phosphate (phosphatidylglycerol phosphate) [4] and 1-*O*-[galactosyl-3'-sulphate( $\beta 1' \rightarrow 6'$ )mannosyl( $\alpha 1' \rightarrow 2'$ )glucosyl( $\alpha 1' \rightarrow 1$ )]-*sn*-glycerol (glycolipid sulphate) [5]. Details of the chemistry and biochemistry of these membrane lipids have been described elsewhere [6,7].

Dispersions of total polar lipid extracts from *H. cutirubrum* in water form large liposomes consisting of concentric lamellar arrangements which act as ideal osmometers in KCl and NaCl solutions providing the concentration does not exceed 0.2 M. At higher salt concentrations the arrangement of the lipid changes and the structures become osmotically inactive [8]. It has been suggested that charge shielding at high ionic strength leads to a decrease in the surface area occupied by the polar groups as charge repulsion between neighbouring molecules is reduced. Since the area occupied by the phytanyl chains is unaffected by charge, the chains are said to interact strongly by cohesive dispersion forces and prevent the formation of stable bilayer structures. Thus interactions of the charged lipids with membrane proteins and other constituents is believed to be required to preserve stability of the membrane at high salt concentrations.

A previous freeze-fracture study [9] performed on ammonium salts of lipids from *H. cutirubrum* compared structures formed when they were dispersed in water or 5 M NaCl. It was found that phosphatidylglycerol phosphate forms small liposomal-like structures in water which consist of relatively few layers. The glycolipid sulphate, on the other hand, exists as large multilamellar liposomes. Dispersion in 5 M NaCl solutions causes a dramatic change in the structure of the lipids and both lipids are characterised by a heterogeneous phase behaviour. In the glycolipid sulphate dispersions a lamellar structure tends to dominate the phase but non-bilayer arrangements are also ob-

served. Phosphatidylglycerol phosphate prefers a non-lamellar arrangement in high salt concentrations particularly at temperatures of 70°C or greater. These studies also showed that, in water, phosphatidylglycerol phosphate is more hydrated than glycolipid sulphate at the same water content. Presumably, this is due to electrostatic effects, since phosphatidylglycerol phosphate has two charged phosphate groups and glycolipid sulphate has only a single sulphate group. The general conclusions from the freeze-fracture studies were that glycolipid sulphate tends to form multibilayer structures in salt concentrations and temperatures akin to the growth conditions of the bacterium, whereas phosphatidylglycerol phosphate tends to form a mixed lamellar and non-lamellar phase.

Some calorimetric [8] and  $^{31}\text{P}$ -NMR [10] studies of dispersed total polar lipids of *H. cutirubrum* or fractions thereof suggest that the phytanyl chains are in a disordered configuration and no phase transitions can be detected above 0°C. Spin-labelled data and specific volume measurements in the temperature range -11°C to 45°C [11], however, indicate that in the presence of 4 M NaCl or 0.1 M  $\text{MgCl}_2$  transitions take place in the polar head group region of the molecules. In the present paper a monolayer study of the ammonium salts of the major lipid constituents of *H. cutirubrum* is reported. Information on the physical state and conformation of the molecules in monolayers spread at the air/water interface have been derived and compared with theoretical conformational models. This approach has proved useful in helping to understand the behaviour of these lipids in aqueous dispersions and in cell membranes.

## Materials and Methods

The extraction, purification and preparation of lipids was the same as described elsewhere [9]. The chemical structures of phosphatidylglycerol phosphate and glycolipid sulphate are illustrated in Fig. 1.

The compression isotherms at the air/water interface relating surface pressure ( $\pi$ , mN/m) with mean molecular area ( $A$ , nm<sup>2</sup>/mol) were recorded at 17°C, using the Wilhelmy method. The lipids

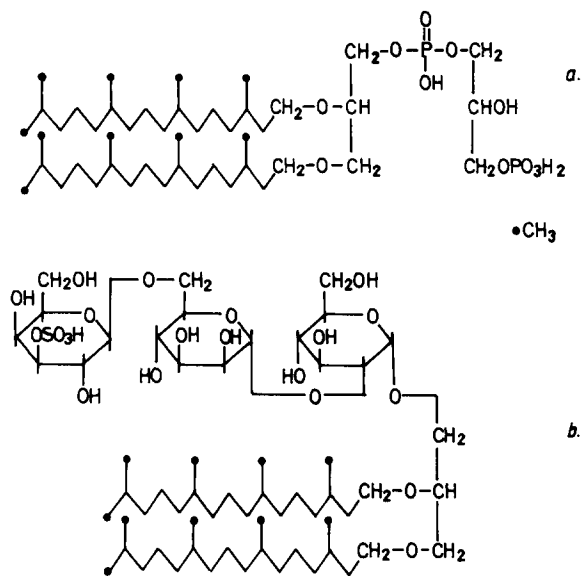


Fig. 1. Structure of the membrane lipids used in monolayer studies. a, phosphatidylglycerol phosphate; b, glycolipid sulphate.

were spread from a micropipette in a solvent mixture consisting of chloroform (Reactivul, Bucharest) and *n*-hexane (Merck) (1:9, by vol.). The solvents were used without further purification. The presence of surface-active impurities in the spreading solvents was excluded because deposition of these solvents on the aqueous subphases did not alter the interfacial tension. Water that was twice distilled (pH = 5.6) was used and NaCl was added to the aqueous subphase in amounts required to achieve concentrations of between 0.01 and 4 M. Where adjustment of the subphase pH was made, this was achieved using appropriate additions of HCl (pH 0–2) or potassium phosphate buffer (pH 8). All electrolytes were of Analar purity and used without further purification. They were reputed to contain no impurities with surface activity and this was confirmed by surface tension measurements recorded before spreading lipid monolayers.

Generally, before compressing monolayers an equilibration period of between 10 and 60 min was allowed during which time residual spreading solvent evaporated and internal equilibrium within the film was achieved. No effect of additional equilibration times of up to 120 min were ob-

served as judged from monolayer characteristics or the performance of compression isotherms on any of the subphases used. One consistent observation was that glycolipid sulphate did not spread readily over the surface of subphases containing NaCl concentrations greater than 1 M and it was necessary to reduce the concentration of the surfactant initially spread on such subphases.

A set of usually ten  $\pi$  versus  $A$  curves were recorded for each monolayer. The equilibration between the lipid film and the various subphases was found to be relatively rapid, and this permitted compression isotherms to be obtained within a few minutes using compression rates of between 0.01 and 0.06 (nm<sup>2</sup>/mol) · min<sup>-1</sup>. Reproducibility of the surface pressure measurements was within 0.5 mN/m, while with the molecular area determinations it was within 0.02 nm<sup>2</sup>/mol for water and subphases with electrolyte concentrations less than 1 M; with NaCl concentrations greater than 1 M surface pressure determinations were reproducible within 1 mN/m, whereas with area measurements the reproducibility was within 0.5 nm<sup>2</sup>/mol.

## Results

Compression isotherms, i.e. surface pressure versus mean molecular area curves of phosphatidylglycerol phosphate (PGP) and glycolipid sulphate (GLS) recorded on a subphase of distilled water (pH 5.6) are presented in Figs. 2 and 3, respectively. The surface characteristics, including the limiting molecular area ( $A_o$ ) obtained by extrapolating the high surface pressure linear portion of the isotherm to  $\pi = 0$ , the collapse pressure ( $\pi_c$ ) and the collapse molecular area ( $A_c$ ) obtained as the co-ordinates of the point on the isotherm where a sudden slope change of the curve occurs at high  $\pi$  values, were derived from these curves. The values are collated in Table I for the two lipids under designated pH conditions. The surface compressional modulus, defined as:

$$C_{so}^{-1} = -A_o(\partial\pi/\partial A)_T = A_o(\pi_c/(A_o - A_c))$$

has been calculated from the monolayer parameters and is also included in Table I.

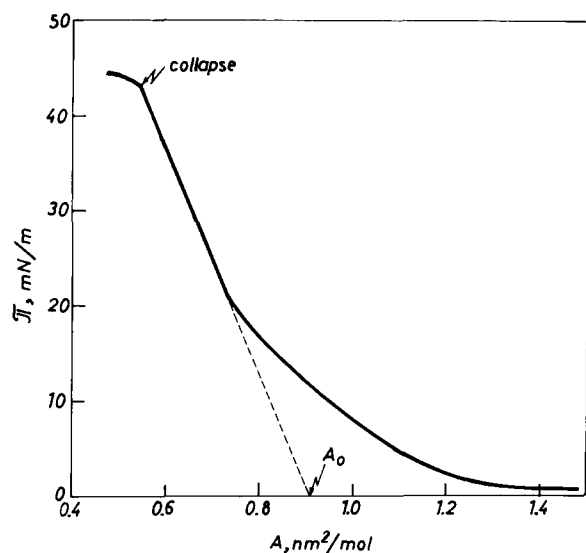


Fig. 2. Compression isotherm of phosphatidylglycerol phosphate on a subphase of water (pH 5.6).

To examine the effect of surface charge on monolayer characteristics of the two lipids, compression isotherms were constructed on subphases adjusted to pH values above and below the  $pK_a$  3.25 [19] of the ionisable groups of the lipids. The compression isotherms of phosphatidylglycerol phosphate and glycolipid sulphate on subphases of varying pH are presented in Figs. 4 and 5, respectively. Protonation of the two primary ionisable groups on each of the phosphate residues of phosphatidylglycerol phosphate does not greatly affect the surface properties of the monolayer with the exception of a slight expansion of the film at

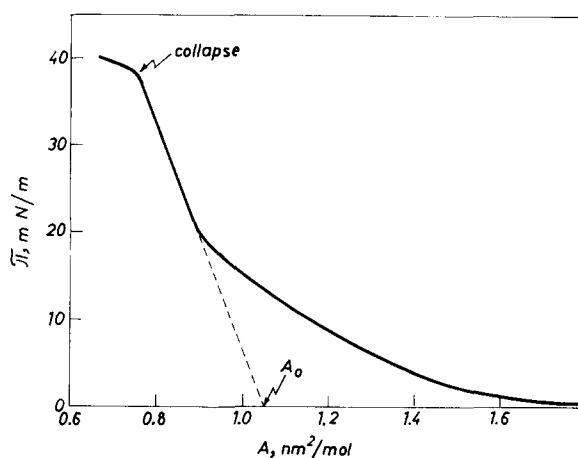


Fig. 3. Compression isotherm of glycolipid sulphate on a subphase of water (pH 5.6).

pH 0. Thus the presence of charged groups on the lipid does not greatly affect stability of the monolayer as judged from the collapse pressure and collapse molecular area nor, rather surprisingly, the compressional modulus and limiting molecular area. In general, the effect of discharging the ionised sulphate of the glycolipid sulphate also does not markedly affect the characteristics of these monolayers but there is a considerable expansion of films spread on a subphase of pH 0. This is evident from increased values of the limiting molecular area and decreased compressibility modulus. It is noteworthy, however, that the area per molecule observed at collapse pressure of the film is not greatly changed at very low pH.

TABLE I

SURFACE CHARACTERISTICS DERIVED FROM COMPRESSION ISOTHERMS

$A_o$ ,  $A_c$  in  $\text{nm}^2/\text{mol}$ ;  $\pi_c$ ,  $C_{so}^{-1}$  in  $\text{mN}/\text{m}$ . PGP, phosphatidylglycerol phosphate; GLS, glycolipid sulphate.

Compound	Surface characteristics	pH					[NaCl] (mol/litre) (pH 5.6)					
		0	1	2	5.6	8	0.01	0.1	1	2	3	4
PGP	$A_o$	1.05	0.90	0.83	0.91	1.02	1.00	1.06	1.15	—	—	—
	$A_c$	0.60	0.56	0.54	0.54	0.66	0.60	0.64	0.66	—	—	—
	$\pi_c$	42	44	45	44	45	42	43	43	—	—	—
	$C_{so}^{-1}$	98	116	129	108	128	105	108	101	—	—	—
GLS	$A_o$	1.70	1.21	1.07	1.05	1.12	1.30	—	2.30	4.10	5.20	5.80
	$A_c$	0.77	0.77	0.76	0.76	0.78	0.80	—	1.35	1.75	2.30	3.20
	$\pi_c$	35	37	43	38	41	37	—	33	32	30	21
	$C_{so}^{-1}$	65	102	148	138	135	96	—	80	56	54	47

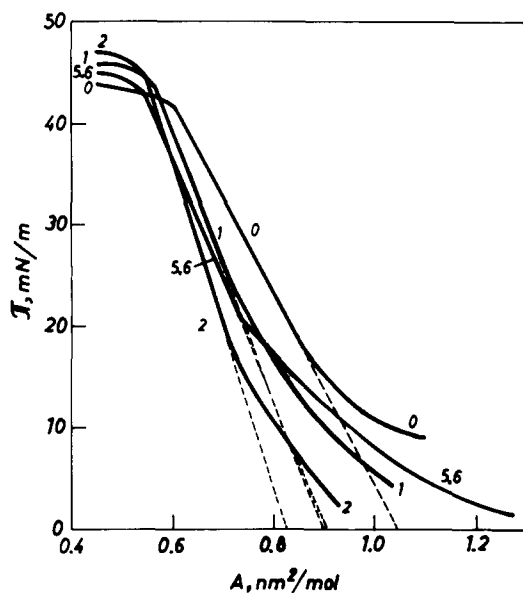


Fig. 4. The effect of subphase pH (indicated on each curve) on compression isotherms of phosphatidylglycerol phosphate.

The effect of electrolytes on monolayer characteristics was examined by constructing compression isotherms of the two lipids on subphases of varying concentration of NaCl. These data are shown in Figs. 6 and 7 for phosphatidylglycerol phosphate and glycolipid sulphate, respectively. It can be seen that the effect of subphase NaCl concentrations on the monolayer characteristics of phosphatidylglycerol phosphate is to cause a slight

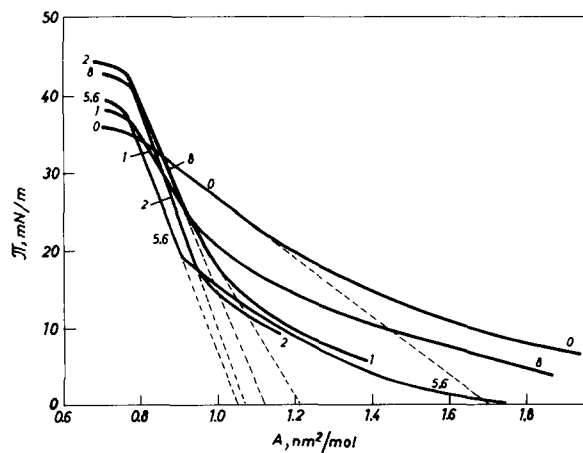


Fig. 5. The effect of subphase pH (indicated on each curve) on the compression isotherms of glycolipid sulphate.

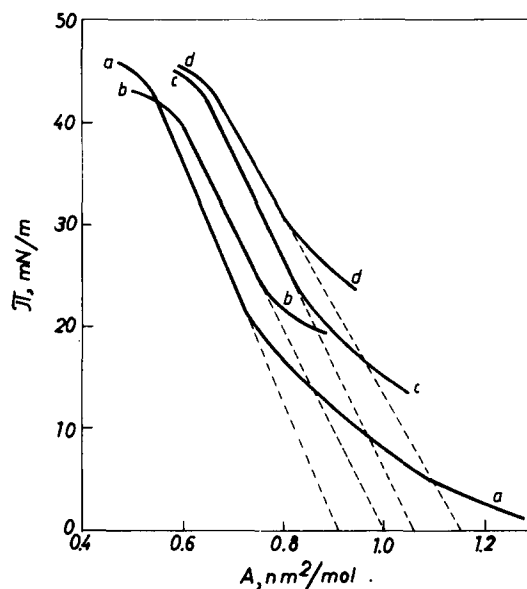


Fig. 6. The effect of subphase NaCl concentration on compression isotherms of phosphatidylglycerol phosphate. NaCl concentrations; a, 0 M; b, 0.01 M; c, 0.1 M; d, 1 M.

expansion of the monolayer in proportion to the salt concentration. This was manifest as an increase in the area per molecule at the collapse pressure and limiting molecular area; there was, however, no significant change in film compressibility or stability. By contrast, increasing concentrations of NaCl in subphases under glycolipid sulphate monolayers cause a considerable increase in both area per molecule at collapse pressure and limiting molecular area. Further-

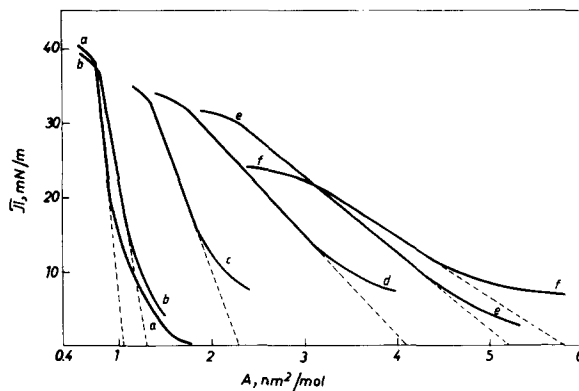


Fig. 7. The effect of subphase NaCl concentration on compression isotherms of glycolipid sulphate. NaCl concentrations; a, 0 M; b, 0.01 M; c, 1 M; d, 2 M; e, 3 M; f, 4 M.

more, there was a significant decrease in compressibility modulus and loss of film stability with increasing salt concentrations. Such effects of salt are unlikely to be due simply to the incorporation of electrolytes into the monolayer but suggest that high salt concentrations induce a conformational change in the film constituents.

So as to relate the surface characteristics of monolayers of phosphatidylglycerol phosphate and glycolipid sulphate with molecular conformation of the respective surfactant molecules the surface area occupied by molecules in two configurations have been calculated from molecular models.

These calculations assume that all the molecules are oriented at the interface with their polar groups in contact with the aqueous subphase and the phytanyl chains directed up into the air. Because the molecules are very flexible, there is a very large number of possible conformations when the molecules are present on the surface at relatively low density. As the area occupied is reduced by compressing the film, however, lateral constraints and interactions with neighbouring molecules limit the range of conformations that can be adopted. Near the collapse pressure, it may be expected that the molecules of the monolayer will form a fairly regular lattice in which either the phytanyl chains or the polar group will determine the minimum area,  $A_c$ , that the molecule can occupy. If this condition is determined by the phytanyl chains it is most likely that these will be in a fully extended state and directed vertically from the interface. The orientation of the polar groups in an extended configuration into the subphase, however, may not be the preferred conformation particularly if the limiting molecular area is determined by the hydrophilic component since electrostatic and hydration factors may complicate the situation. Such factors, moreover, may dominate the equation of state of the monolayer and, in turn, determine the value of  $A_c$ . In our previous studies [12–16] we found that, as a general rule, the experimental value of  $A_c$  was very close to the minimum area required for vertically oriented, close-packed, non-rotating molecules in their most extended conformation designated  $A_p$ . We also showed that the experimental value,  $A_o$ , is related to a molecular area referred to  $A_4$ , which is the theoretical molecular area of an array of

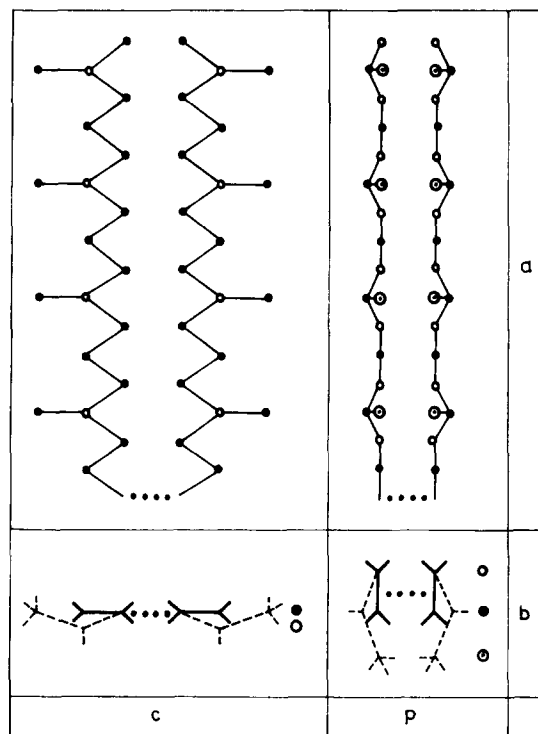


Fig. 8. Extreme conformations of the phytanyl chains. c, co-planar conformation; ●, common-plane C atoms; ○, out-of-plane C atoms; p, parallel conformation; ○, first-plane C atoms; ●, second-plane C atoms; ○, third-plane C atoms. (a) 'Lateral' view; (b) 'top' view, i.e. projection onto the interface. Solid lines show the bond in the overlapping  $-\text{CH}_2-\text{CH}_2-$  residues, dashed lines show the bonds of the out-of-plane C atoms carrying an  $-\text{H}$  and a  $-\text{CH}_3$  group. Dotted line represents the glycerol backbone.

tetragonally close-packed molecules rotating about an axis perpendicular to the plane of the monolayer. In the present study, theoretical molecular areas have been calculated for the phytanyl chains (this is the same for phosphatidylglycerol phosphate and glycolipid sulphate) and for each of the polar head groups. Two extreme conformations of the phytanyl chains have been considered. The first case is when all the carbon atoms of the phytanyl chains are located in the same vertical plane except four of them, carrying the side chain methyl groups. This conformation, designated co-planar or c conformation is illustrated in Fig. 8, both in 'lateral' view (a) and in 'top' view (b). The second case, also shown in Fig. 8, consists of a parallel arrangement of the two phytanyl chain

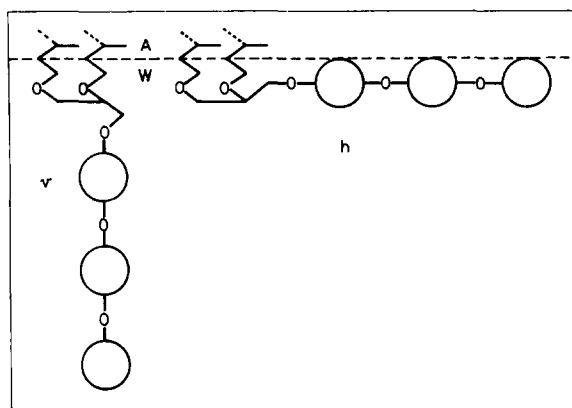


Fig. 9. The polar head group of glycolipid sulphate in extreme vertical (v) and horizontal (h) conformations. The relative position of the air (A)/water (W) interface is shown.

planes. This conformation is referred to as the parallel or p conformation. In each of these conformations the glycerol backbone of the molecule is shown as a dotted line. In the 'top' view, unbroken lines represent the bonds in and between the overlapping methylene groups of the phytanyl chain in projection onto the interface and dashed lines indicate the position of the bonds of the out-of-plane carbon atoms carrying the side chain methyl and hydrogen residues.

The conformations of the polar headgroups have also been considered in the context of two extreme cases, one in which the polar residue is oriented vertically down into the subphase in its most extended conformation (vertical conformation, v) and the other where the head group is

located within the interfacial plane, again in its most extended conformation (horizontal conformation, h). These two conformations are illustrated schematically for glycolipid sulphate in Fig. 9. For simplicity the sugar residues are drawn as circles. Note also that the glycerol residues are oriented parallel to the plane of the monolayer, however, a vertical orientation is also possible but the effect of this on the calculated molecular areas would be trivial. The values of theoretical molecular areas calculated for co-planar and parallel configurations of the phytanyl chains and the vertical and horizontal orientation of the polar groups of phosphatidyl glycerol phosphate and glycolipid sulphate are presented in Table II.

## Discussion

One of the most conspicuous features of the red and purple membranes of extreme halophilic organisms such as *Halobacterium cutirubrum* is that their integrity depends on the presence of NaCl or KCl in concentrations of 3 M or greater in the surrounding medium [17,18]. The effect of charge shielding on the phase behaviour of phosphatidylglycerol phosphate and glycolipid sulphate in aqueous dispersions is therefore of importance in understanding the underlying factors responsible for the stability of these membranes under conditions in which the halophiles grow. Phosphatidylglycerol phosphate is a dianion at neutral pH [19,20] with each phosphate group possessing a single charge. The  $pK_a$  of these two anionic groups is about 3.25 [19] and the  $pK_a$  of the third ionised group on the terminal phosphate residue must be higher than 8 when oriented at an aqueous interface. With the polar group in the vertical conformation the charged groups would exist in two separate planes. Studies of cation and pH dependence of charge neutralisation in total polar lipid extracts of *H. cutirubrum* have indicated that the penetration of counter ions into the Stern layer requires a spacing out of the molecules with uncharged lipid [21]. These experiments were based on co-dispersion of the polar lipid fraction with squalene which is present together with other neutral lipids in a proportion of about 8–10% by weight of the total membrane lipid. The monolayer experiments reported in the present study

TABLE II

### THEORETICAL MOLECULAR AREAS OF VERTICALLY ORIENTED LIPID MOLECULES IN THEIR EXTREME CONFORMATIONS

A in nm<sup>2</sup>/mol. PGP, phosphatidylglycerol phosphate; GLS, glycolipid sulphate.

Surfactant	Type of area	Hydrocarbon chain (C)		Polar headgroup (H)	
		c	p	h	v
PGP	$A_4$	1.44	0.68	3.69	0.52
	$A_p$	0.58	0.56	0.92	0.35
GLS	$A_4$	1.44	0.68	4.00	0.85
	$A_p$	0.58	0.56	1.68	0.77



TABLE III

## COMPARISON OF CHARACTERISTIC MOLECULAR AREAS

$A$  in nm<sup>2</sup>/mol. PGP, phosphatidylglycerol phosphate; GLS, glycolipid sulphate. Numeric subscripts refer to subphase pH or NaCl concentration (in square parenthesis); H and C refer to head group and hydrocarbon chain; v, h and p, c are the presumed conformation of the head and chains, respectively.

PGP		GLS	
$(A_4)_{Hv} = 0.52$	$(A_p)_{Hv} = 0.35$	$(A_4)_{Cp} = 0.68$	$(A_p)_{Cp} = 0.56$
$(A_4)_{Cp} = 0.68$	$(A_c)_2 = 0.54$	$(A_4)_{Hv} = 0.85$	$(A_p)_{Cc} = 0.58$
$(A_o)_2 = 0.83$	$(A_c)_{5,6} = 0.54$	$(A_o)_{5,6} = 1.05$	$(A_c)_2 = 0.76$
$(A_o)_1 = 0.90$	$(A_c)_1 = 0.56$	$(A_o)_2 = 1.07$	$(A_c)_{5,6} = 0.76$
$(A_o)_{5,6} = 0.91$	$(A_p)_{Cp} = 0.56$	$(A_o)_8 = 1.12$	$(A_c)_1 = 0.77$
$(A_o)_{[0.01]} = 1.00$	$(A_p)_{Cc} = 0.58$	$(A_o)_1 = 1.21$	$(A_c)_0 = 0.77$
$(A_o)_8 = 1.02$	$(A_c)_0 = 0.60$	$(A_o)_{[0.01]} = 1.30$	$(A_p)_{Hv} = 0.77$
$(A_o)_0 = 1.05$	$(A_c)_{[0.01]} = 0.60$	$(A_4)_{Cc} = 1.44$	$(A_c)_8 = 0.78$
$(A_o)_{[0.1]} = 1.06$	$(A_c)_{[0.1]} = 0.64$	$(A_o)_0 = 1.70$	$(A_c)_{[0.01]} = 0.80$
$(A_o)_{[1]} = 1.15$	$(A_c)_{[1]} = 0.66$	$(A_o)_{[1]} = 2.30$	$(A_c)_{[1]} = 1.35$
$(A_4)_{Cc} = 1.44$	$(A_c)_8 = 0.66$	$(A_4)_{Hh} = 4.00$	$(A_p)_{Hh} = 1.68$
$(A_4)_{Hh} = 3.69$	$(A_p)_{Hh} = 0.92$	$(A_o)_{[2]} = 4.10$	$(A_c)_{[2]} = 1.75$
		$(A_o)_{[3]} = 5.20$	$(A_c)_{[3]} = 2.30$
		$(A_o)_{[4]} = 5.80$	$(A_c)_{[4]} = 3.20$

provide some evidence for the penetration of counter ions into the interfacial region. In the case of phosphatidylglycerol phosphate monolayers the values of  $A_o$  and  $A_c$  are considerably greater when spread on subphases of NaCl compared with HCl of equivalent concentration. This trend is even more pronounced in the case of the glycolipid sulphate but interpretation is complicated by the effects of the solvent on the conformation of the polar sugar residues of this lipid.

The conformational transitions of the lipid molecules during compression of the monolayers can be judged by comparing experimental values of  $A_o$  and  $A_c$  with the respective theoretical values of  $A_4$  and  $A_p$ . All characteristic molecular areas presented in Tables I and II are summarized in Table III in order of increasing area occupied by the respective conformers. It can be seen from Table III that for monolayers of phosphatidylglycerol phosphate, values of  $A_o$  are considerably less than  $(A_4)_{Hh}$  and somewhat greater than the  $(A_4)_{Hv}$  value. This suggests that the limiting molecular area is determined by the phytanyl chains rather than by the polar group which presumably assumes a conformation that can be accommodated within the surface area prescribed by the chains. The  $A_o$  value in fact is close to the arithmetical mean of  $(A_4)_{Cc}$  and  $(A_4)_{Cp}$  areas of

the phytanyl chains supporting the idea of either a combination of each of the extreme chain conformers or a chain conformation that is intermediate between c and p. Furthermore, the values of  $A_c$  are similar to areas  $(A_p)_{Cp}$  and  $(A_p)_{Cc}$  suggesting that near the collapse pressures the phytanyl chains are in a close-packed configuration.

The characteristics of glycolipid sulphate monolayers and the effects of screening the electrostatic charge on the head group contrasts significantly with those observed in films of phosphatidylglycerol phosphate. Thus, the values of limiting molecular area of glycolipid sulphate molecules in all monolayers except on subphases of pH 0 and/or NaCl concentrations greater than 0.01 M are markedly less than the theoretical values  $(A_4)_{Hh}$  and less than  $(A_4)_{Cc}$  area calculated for the phytanyl chains. The experimental value, however, is greater than both  $(A_4)_{Hv}$  and  $(A_4)_{Cp}$ , suggesting that under these conditions the limiting molecular area is determined by the area occupied by the phytanyl chains. Some contribution from the polar head group of the glycolipid sulphate cannot, however, be excluded especially since the limiting molecular area is slightly greater than recorded for phosphatidylglycerol phosphate. When the charges on the sulphate are effectively

screened, such as on subphases of pH 0 or particularly on high salt concentrations, the limiting area increases very significantly and exceeds values of  $(A_4)_{Cc}$  and even  $(A_4)_{Hh}$ . The most likely explanation for this effect is that the polar head group of the lipid undergoes a decrease in affinity for the subphase and adopts a more compact arrangement in the interfacial region and possibly contains a number of hydrated sodium ions as a component of the film. This effect could be considered in terms analogous to a salting out of the sulphated sugar group which normally requires a large number of water molecules to hydrate the numerous hydroxyl groups as well as the sulphate residue.

The effect of lateral constraints of molecular packing on conformation can be determined from the relationship between the area occupied per molecule of glycolipid sulphate at collapse pressure,  $A_c$ , and the electrostatic conditions at the interface. It can be seen that on all subphases except NaCl concentrations greater than 10 mM the  $A_c$  value approximates to the theoretical  $(A_p)_{Hv}$  value of the polar group. Meanwhile, all  $A_c$  values are considerably greater than both  $(A_p)_{Cp}$  and  $(A_p)_{Cc}$  values. This is consistent with a close packing of the vertically oriented head groups which, unlike phosphatidylglycerol phosphate, determine the minimum molecular area that the molecule can occupy in the monolayer. Comparing the experimental  $A_c$  value of glycolipid sulphate on subphases of pH 0 and 10 mM NaCl with  $(A_p)_{Hp}$ , suggests that the head groups reorient from a horizontal configuration at low pressure to an extended vertical conformation during compression of the film. When the subphase consists of NaCl concentrations of 1 M or greater, the polar group apparently remains in the surface layer in a horizontal orientation. The  $A_c$  value increases with increasing concentration of NaCl and presumably it eventually exceeds  $(A_p)_{Hh}$  due to incorporation of the electrolyte into the monolayer.

The stability of monolayers of the two lipids is reflected in the surface pressure recorded at the collapse of the film. This pressure is not affected greatly by the nature of the subphase under monolayers of phosphatidylglycerol phosphate since the limiting factor in close packing of the molecule is

determined by the phytanyl chains. The values of  $\pi_c$  for glycolipid sulphate monolayers, in contrast to phosphatidylglycerol phosphate films are much less.

This emphasises the importance of dispersive-type attractive forces between the chains in stabilising the monolayer. Because the polar group of glycolipid sulphate, especially those configurations adopted in high concentrations of NaCl, determines the packing density at collapse pressure, the cohesion between the phytanyl chains is likely to be reduced and there is possibly an increase in *gauche* conformers. The difference in packing density between molecules dispersed in 4 M NaCl is also seen in wide-angle X-ray diffraction spacings which show single broad diffraction bands typical of disordered hexagonal arrangement at 0.51 and 0.55 nm for phosphatidylglycerol phosphate and glycolipid sulphate, respectively (unpublished observations). If the arrangement of the chains is in an hexagonal array, calculations of the area occupied by each molecule at the aqueous interface are 0.54 and 0.63 nm<sup>2</sup>/mol for the respective lipids.

The compressibility (reduction in surface compressional modulus) of phosphatidylglycerol phosphate monolayers increases only slightly with increasing subphase salt concentration, in contrast to glycolipid sulphate, where compressibility of the film is very sensitive to subphase electrolyte concentration. This feature can be explained by reference to the arguments used above to describe the stability of glycolipid sulphate monolayers. A looser structure dominated by the relatively flexible conformation of the sulphated sugar residue leads to a highly compressible monolayer. Such behaviour may explain, in part, why the osmotic behaviour of dispersed polar lipid extracts of *H. cutirubrum* breaks down at salt concentrations greater than 0.2 M. This is in contrast to the explanation of Chen et al. [8] who suggested that the area occupied by the polar group decreases in salt concentrations greater than 0.2 M leading to loss of bilayer stability.

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