

THE MOLECULAR STRUCTURE OF THE MEMBRANE OF *ACHOLEPLASMA LAIDLAWII*

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The following discussion is a survey of experiments with the membrane of *Acholeplasma laidlawii* (formerly *Mycoplasma laidlawii*)³⁻⁵). This organism is particularly suitable for membrane structural studies, since it has only a single membrane which is easily isolated by osmotic lysis and since it lacks the specialized wall structures of the bacteria. Furthermore, the fatty acid composition of the membranes can be varied by supplementation of the medium with the desired fatty acid, and under the usual growth conditions there is essentially no cholesterol present. The application of X-ray diffraction methods to the study of these membranes has led, over the past three years, to a number of limiting ideas concerning the average structure of the membrane.

I. The lipid phase transition

A thermotropic phase transition of the membrane was first reported by Steim et al.¹), who observed it in differential scanning calorimetric traces of native membranes and isolated lipids. As the scanning calorimetric measurement could not distinguish which of the several known lipid transitions²) was involved, X-ray measurements were undertaken³). A striking change in the wide angle pattern was observed: Above the phase transition a diffuse band was seen at 4.6 Å; below the transition this band disappeared and a sharp line appeared at 4.15 Å. These changes were observed in intact organisms, isolated membranes, and isolated lipids, in each case the transition temperature range (~15°) and the high temperature end of the transition were the same. The basic observations regarding the transition are:

(a) The transition temperature varies with the fatty acid composition of the membrane lipids; longer chains and increased saturation acting to raise the temperature.

(b) In a rich growth medium the organism selects fatty acids so that the transition is near the growth temperature; however, the high-temperature end of the transition is often found to be above the growth temperature.

(c) The transition is quite broad, occurring over a 10–20° range in many cases.

(d) Below the transition, grain size calculations based on the half-width of the 4.15 Å line show that the ordered regions are approximately 200–400 Å in extent.

(e) In partially dehydrated preparations an additional diffraction line is seen at 2.4 Å, which is the next spacing expected for a hexagonal lattice with a primary spacing of 4.15 Å.

(f) Above the transition the broad diffraction band at 4.6 Å resembles the diffraction from liquid *n*-paraffins.

(g) Of the lipid chains producing the 4.6 Å band, 70–100% participate in the transition. The uncertainty in this measurement arises from the difficulty of establishing a baseline against which to measure the band; however, one may say that the lower bound of 70% is a firm one. If other lipid is present which does not participate in the transition, it is not contributing to the 4.6 Å diffraction.

These observations show that the phase transition is the gel-liquid crystal transition known to occur in lipid lamellar phases. As described below, the low-angle pattern does not indicate the formation of separate multilayered lipid phases or non-lamellar configurations. The transition, then, is a change of the hydrocarbon chains from a close-packed hexagonal array with long range order to a more liquid state.

II. The lipid bilayer

An interpretation of the low angle diffraction from dispersions of membranes has recently been reported⁴). The basic idea is that scattering from lipid bilayers will be dominated by the contribution of the two electron-dense layers of lipid characteristic groups. The interference of X-rays scattered by these layers will produce a $\cos 2\pi\theta D/\lambda$ modulation of the low angle intensities, and one therefore expects to see a series of maxima at $\theta = h\lambda/2D$, where $h = 1, 2, 3, \dots$ and D is the spacing between the headgroup layers. Furthermore, the contribution of the two conformations of the fatty acid chains can be assessed; the rigid chains below the transition would have their electron-deficient terminal methyl groups more localized near the center of the bilayer than would the liquid-like chains above it. This localization will shift the intensities of the peaks, accentuating those with h odd.

These features of lipid bilayer diffraction are seen in the low angle patterns from dispersions of acholeplasma membranes. The relevant points of evidence are⁵):

(a) A series of low-angle bands are seen both above and below the tran-

sition, and their spacings are at submultiples of the first ($h=1$) peak spacing.

(b) The changes in pattern as the membrane goes through the phase transition are as predicted for a bilayer; below the transition the first and third bands are strong relative to the second, and above the transition the third is very weak relative to the second.

(c) The absolute bilayer dimensions implied by the basic spacing, D , of the bands are appropriate for the lipids known to be present. For example, model measurements of a bilayer of 16:0 supplemented acholeplasma lipids in the low temperature form predict $D \sim 50 \text{ \AA}$, and the experimental value is $D = 47 \text{ \AA}$.

(d) Changing the average fatty acid chain length by varying the medium supplement produces changes in the basic spacing which are as expected for a bilayer:

Supplement	# carbons in average fatty acid	D (in \AA) above transition	D (in \AA) below transition
16:0	16	39	47
22:1 <i>cis</i>	20	41	52
18:1 <i>cis</i>	17.5	40	46

The evidence, then, leads strongly to the conclusion that the main features of the diffraction pattern are those of a lipid bilayer, and that the bilayer is the main lipid structure of the membrane.

III. The membrane protein

Unfortunately, the protein of the membrane (about 50% of the membrane mass) does not contribute sharp features to the diffraction pattern and interpretation of the data is more difficult; nonetheless, certain aspects of the protein distribution and lipid-protein interactions can be inferred from the behavior of the lipid. Clearly these arguments are indirect and less satisfying than those regarding the bilayer structure, but they are important to consider in the absence of much information from any source regarding membrane proteins.

(a) The area per lipid molecule below the phase transition can be got directly from the packing of the fatty acid chains. The chains must be nearly perpendicular to the bilayer plane (since D is appropriate for full extension) and the area is then $40\text{--}43 \text{ \AA}^2$ per lipid. This packing is very close and penetration of protein side chains past the lipid headgroups to make hydrophobic contact seems unlikely. On the other hand, the presence of a protein

embedded in the lipid is not excluded, since it could substitute for a group of lipids in the lattice.

(b) Above the transition an area per lipid can be calculated by taking $D = 10 \text{ \AA}$ as the thickness of the hydrocarbon layer, assigning volumes to the groups in the lipid chains⁶), and calculating the area by dividing the volume of two chains by the hydrocarbon layer thickness. This procedure leads to values of $60\text{--}70 \text{ \AA}^2/\text{lipid}$ for the various supplements. This range of values is similar to areas found for isolated phospholipids in smectic mesophases, and it does not seem that the presence of the membrane protein is constraining the lipid headgroups. That this is the case is further established by the change in area of the lipids – a 60% increase on melting of the fatty acid chains. Clearly models for lipid-protein interactions cannot be such as to require a specific area per lipid molecule.

(c) If the membrane protein were present as layers on the surfaces of the bilayer as in the Danielli model, it would be expected to contribute an additional interference term to the low angle pattern. This term, reflecting the correlation of the protein masses with each other and with the bilayer, would be most prominent in the $h=1$ band and die out rapidly toward smaller spacings. It is not observed. It follows that most (but one cannot say all) of the protein is not in layers at the surfaces of the bilayer.

A possible model for the membrane protein structure which is consistent with the evidence is that the proteins are inserted almost entirely into the lipid bilayer, some perhaps penetrating it completely, some extending half way through. The hydrophylic groups on the proteins would be in contact with the aqueous phase and the hydrophobic groups would be localized in the lipid hydrocarbon region. The central feature of membrane (as compared to non-membrane) proteins would then be that they can be substituted for groups of lipid molecules in the bilayer without greatly perturbing it.

References

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Discussion

The phase transitions suggested by Engelman's results attracted considerable comment.

Thus, LUZZATI argued convincingly that the data implies segregation of more from less saturated lipids.

This point was reinforced by VAN DEENEN, who also pointed to the fact that *Mycoplasma laidlawii* can be enriched selectively with fatty acids containing 2, 4 or even more double bonds, although the proportion of incorporation declines with the degree of unsaturation; in such cases there would assuredly be a marked heterogeneity of fatty acid distribution within the membrane plane, and one possibly facilitating protein penetration.

In the same vein, CHAPMAN cited work in his group, using differential thermal analysis and NMR of incorporated, deuterated fatty acids, indicating that in *Mycoplasma* a large proportion of lipid chains are stiff at growth temperature, although fluidizing at higher temperatures. He suggested that perhaps only small parts of the membrane need to be fluid to permit growth.

WALLACH enquired as to the proportion of lipid in the bilayer and also suggested lipid segregation into two categories, previously noted for erythrocytes by Parpart and Ballentine, as well as Van Deenen's group, i.e., one loosely bound, perhaps bilayer, and another closely associated with proteins.

The latter point was reinforced by CHANGEUX, who suggested that the membrane proteins might act like a sponge into which the lipid chains fit by apolar associations.

ENGELMAN argued that the sponge model is not compatible with the observed phase-transitions, but that his data suggests - 50% of the membrane surface to be lipid bilayer and the rest penetrating protein, with very little protein on the surfaces.

This proportion was disputed by FINEAN, whose red cell data is not consistent with more than 30% penetration and who favours the Wallach-Zahler concept previously discussed by Da Silva, where an appreciable proportion of the protein is viewed as lying at the surface.

The concept of protein penetration of the membrane was also supported by BANGHAM, citing Haydon's work on black films, whose properties should, in principle, approach those of biomembranes more closely if penetrated at intervals by proteins.