PRELIMINARY NOTES

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The effect of alterations in fatty acid composition and cholesterol content on the permeability of *Mycoplasma laidlawii* B cells and derived liposomes

The fatty acid composition and the cholesterol content of the membrane lipids of *Mycoplasma laidlawii* B can be systematically and dramatically altered by the addition of exogenous fatty acids or sterols to a lipid-poor growth medium[1]. The ratio of the membrane lipid classes in this organism is not significantly affected by these variations in fatty acid composition or sterol content (unpublished observation). It is thus possible to investigate the effect of variations in the structure of the paraffin chains and the cholesterol content on the permeability of a biological membrane and to compare the permeabilities of intact cells with liposomes prepared from total membrane lipids of the same composition. Some preliminary results from such a study are presented here.

M. laidlawii B cells were grown in lipid-poor growth medium and harvested in late log phase by centrifugation as previously described[2]. Suitable amounts of palmitic acid *plus* either elaidic, oleic, or linoleic acid were added to the growth medium so that the composition of the total membrane lipids of the harvested cells was approx. 50 mole % each of palmitic and unsaturated fatty acid. In the cholesterol experiment cells were grown in equimolar amounts of palmitic and oleic acid with and without the addition of 25 mg/l of cholesterol. Collected cells were washed and equilibrated in 200 mM sucrose and the permeability of these cells was then determined by optical measurements of the initial swelling rate in 200 mM glycerol solution as already described[3,4]. The total membrane lipid was quantitatively extracted from the remainder of the harvested cells by the method of BLIGH AND DYER[5] and freed from traces of non-lipid contaminants by silicic acid column chromatography. Liposomes were prepared from this purified lipid as previously described[4], except that the lipid was dispersed in 50 mM KCl–50 mM $MgSO_4$ solution. Mg^{2+} was found to reduce the Zeta potential and increase the osmotic response of the liposomes. The glycerol permeabilities of these liposomes were measured by the same technique utilized for the intact cells.

The initial swelling rates in isotonic glycerol solutions of intact cells in which the fatty acid composition was altered, and of the derived liposomes of corresponding lipid composition, are presented in Fig. 1. A similar strong temperature dependence and marked influence of the structure of the paraffin chains on glycerol permeation is noted in both systems. For both intact cells and liposomes the permeability was dependent on the geometrical configuration and the number of double bonds present in the paraffin chains; the rate of glycerol permeation increased in the order elaidic $<$ oleic $<$ linoleic, in agreement with trends noted for synthetic phospholipids containing similar paraffin chains[4,6]. An Arrhenius plot of the data in Fig. 1 revealed

linear relationships between the log of the initial swelling rates and the reciprocal of the absolute temperature, and permitted the calculation of an activation energy for the overall permeation process. The value obtained for both the intact cells and liposomes was approx. 18–20 kcal/mole, and did not appear to be significantly dependent on the nature of the paraffin chains in either the biological or artificial membrane system.

In Fig. 2 the initial swelling rates in isotonic glycerol solutions of intact cells and derived liposomes grown in the presence and absence of cholesterol are presented. It can be seen that the presence of cholesterol lowers the rate of glycerol penetration in both liposomes and intact cells. The activation energy of glycerol permeation, calculated as just described, did not appear to be appreciably altered when cholesterol was present in the membrane system.

The experiments described here reveal a number of close similarities between the permeability behaviour of the biomembrane of *M. laidlawii* B and an artificial

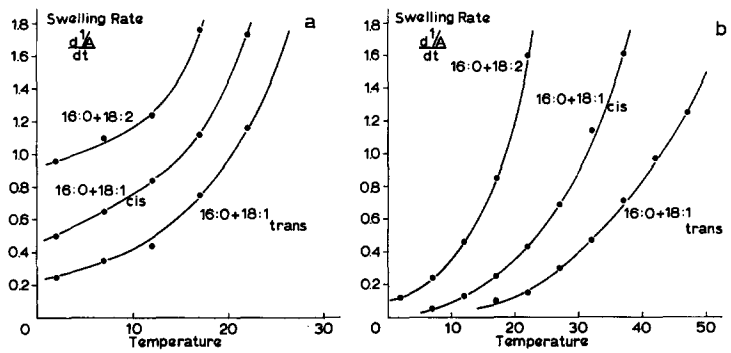

Fig. 1. (a) Initial swelling rates in isotonic glycerol of intact cells of *Mycoplasma laidlawii* B, grown in the presence of different combinations of fatty acids, as a function of temperature. (b) Initial swelling rates in isotonic glycerol of liposomes, prepared from total membrane lipids of *M. laidlawii* B, as a function of temperature. For the details of liposome preparation and initial swelling rate determination, see text.

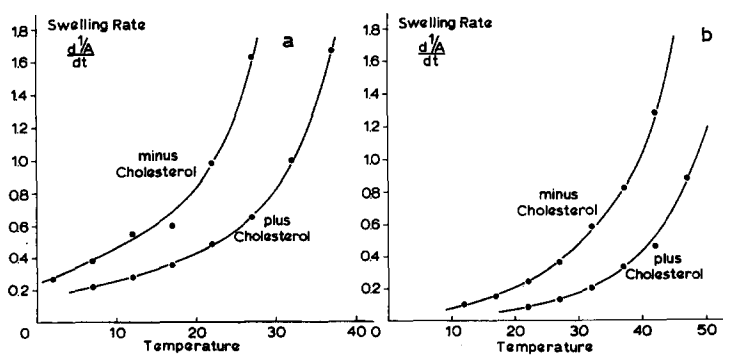

Fig. 2. (a) Initial swelling rates in isotonic glycerol of intact cells of *M. laidlawii* B, grown in equimolar amounts of palmitic and oleic acids with and without 25 mg/l of cholesterol, as a function of temperature. Cholesterol accounted for 12% by weight of the total membrane lipid of cells grown in the presence of cholesterol but was not detectable in the lipid of cells grown in the absence of cholesterol. (b) Initial swelling rates in isotonic glycerol of liposomes prepared from total membrane lipids of *M. laidlawii* B, as a function of temperature.

bilayer membrane system having the same lipid composition. Because of differences in size, ionic environment, and osmotic response between the whole cells and liposomes quantitative comparisons between the two systems can not be safely made, although the biomembrane in all cases appears to be somewhat more permeable than the corresponding artificial membrane system.

The results reported here are consistent with several recent investigations which indicate that the major portion of the lipids in the membrane of this organism are arranged in a bilayer-like structure[7,8]. The effect of cholesterol in reducing glycerol permeability is in line with a recent study in which cholesterol-depleted erythrocytes showed an increase in glycerol permeability[9] and with several studies demonstrating that cholesterol lowers hydrocarbon chain mobility[10] and reduces the permeability of artificial lipid bilayer membranes[4,6].

The effect of a number of other fatty acids and sterols on the permeability of *M. laidlawii* B cells and liposomes to several other nonelectrolytes is currently being investigated.

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