CONTROL OF MEMBRANE LIPID FLUIDITY IN *ACHOLEPLASMA LAIDLAWII*

Leaf HUANG*, Steven K. LORCH**, Gary G. SMITH and Alfred HAUG
MSU/AEC Plant Research Laboratory, Michigan State University
East Lansing, Michigan 48824, USA

Received 29 April 1974

1. Introduction

Membrane fluidity seems to play an important role in cellular processes such as regulation of enzyme activity [1,2], permeability [3], transport of nutrients [4], lateral motion of membrane constituents [5], and osmotic stability of cells [6]. In this communication we report that the microorganism *Acholeplasma laidlawaii* is able to maintain a virtually constant membrane lipid fluidity at the growth temperature when the growth temperature and the exogenous fatty acid source are varied.

2. Materials and methods

Acholeplasma laidlawii (oral strain) was originally from Dr. S. Rottem's laboratory. Cells were grown in a lipid extracted tryptose broth supplemented with an appropriate fatty acid (5 mg/l) [7]. Cultures were harvested by centrifugation at their late log phase (about 20 and 48 hr for 37°C and 28°C grown cultures, respectively). Part of the collected cells were lysed by osmotic shock in deionised water, and their plasma membranes were isolated according to the method described by Razin et al. [8]. The rest of the cell pellet was used for lipid extraction with chloroform–methanol (2:1, v/v) [9]. The preparation of methyl esters of fatty acids and the subsequent analysis by gas chromatography has been described [7]. The isolated plasma membranes were mixed at room temperature with an aqueous dispersion of the spin label 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl (abbreviated as 5 NS, obtained from Synvar, Palo Alto, Calif.) as reported [10]. The final concentration was about 10 mg membrane protein/ml and 0.1–0.2 mmoles 5 NS/ml. Electron paramagnetic resonance (EPR) spectra were recorded at various temperatures. The amount of carotenoid pigments in the lipid extract was spectrophotometrically determined (optical density at 450 nm, in chloroform–methanol 2:1, v/v) [10]. Protein concentration was measured according to Lowry et al. [11].

3. Results

The organism was originally grown in a medium supplemented with oleic acid, at 37°C. After 5–10 daily transfers, the cells could be adapted to media supplemented with either arachidic or lauric acid [7]. These 37°C-grown cultures were then adapted to grow at 28°C after approximately five transfers. The evidence for adaptation rather than mutation came from the fact that these cells grew up immediately after being transferred back to the original temperature.

A typical EPR spectrum of the 5 NS spin label has been shown elsewhere [12]. A greater value of the hyperfine splitting 2 $T_{\parallel}$ reflects restricted rotational motion and therefore indicates a more viscous environment around the spin probe [13]. The value

* Present address: Department of Embryology, Carnegie Institution of Washington, 115 West University Parkway, Baltimore, Maryland 21 210, USA.
** Present address: Michigan Department of Public Health, Lansing, Michigan, USA.

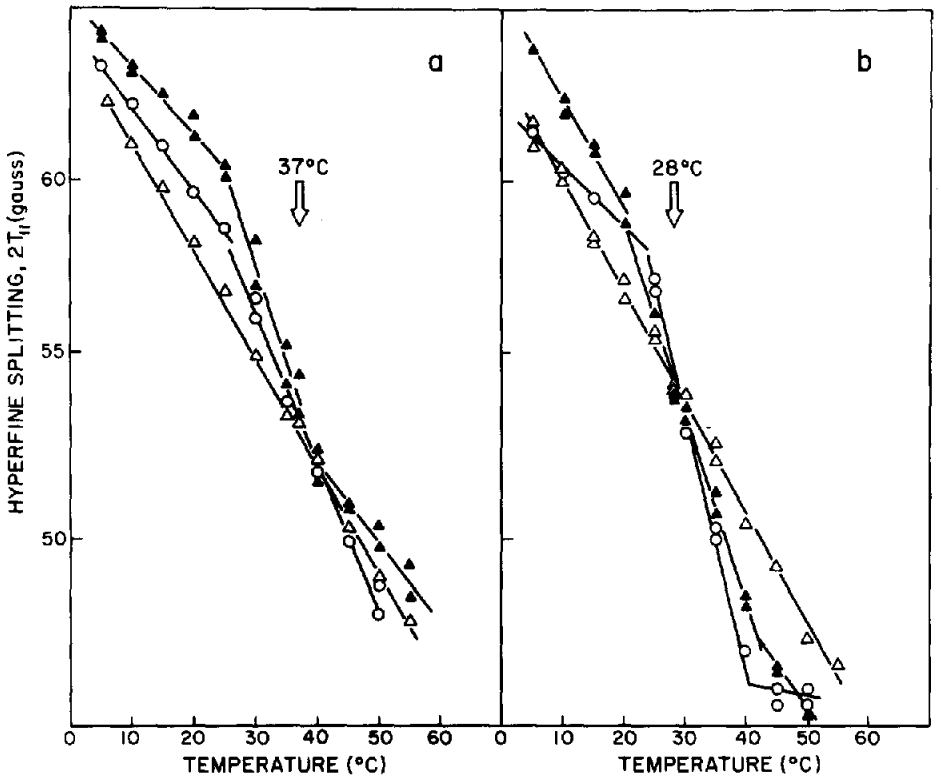

Fig. 1. Temperature dependence of the hyperfine splitting of *Acholeplasma laidlawii* membranes labelled with 5 NS. Cells were grown at (a) 37°C, (b) 28°C, in a nutrient medium supplemented with either arachidic acid (○–○–○), or lauric acid (▲–▲–▲), or oleic acid (△–△–△).

of the anisotropy parameter 2 $T_{\parallel}$ was temperature dependent (fig. 1). Cells grown at 37°C in a medium containing lauric acid showed the lowest lipid fluidity in a temperature range from 5–25°C. At temperatures above 25°C, the difference in fluidity became smaller for the three types of membranes studied (fig. 1). At the growth temperature of 37°C, the value of 2 $T_{\parallel}$ varied only within one gauss (between 53 and 54 gauss) for the three types of membranes investigated. Above the growth temperature, the variations increased.

For cells grown at 28°C, the membranes were more fluid at a given temperature compared to the corresponding cells grown at 37°C (fig. 1). Although the membrane lipid fluidity of the cells differed a great deal both above and below the growth temperature (28°C), the values of 2 $T_{\parallel}$ again varied within one gauss (about 53 to 54 gauss). Note that the values of 2 $T_{\parallel}$ at the two growth temperatures are very close to each other. These results indicate that *Acholeplasma laidlawii* cells are able to maintain their membrane lipid fluidity within a narrow range.

Acholeplasma laidlawii cells are known to incorporate a large amount of fatty acid supplied exogenously into their polar lipids [14]. When cells were cultured at 37°C, the lipids were enriched (50–60 mole %) with the fatty acid supplemented to the medium, either arachidic or oleic acid (table 1). If lauric acid was supplemented to the medium, the lipids were enriched with myristoyl ($C_{14:0}$) and palmitoyl groups ($C_{16:0}$). After adaptation to 28°C, there was a pronounced decrease in arachidoyl ($C_{20:0}$) groups in cells grown in a medium supplemented with arachidic acid. When cells were grown at 28°C in a lauric acid supplemented medium, the percentage of myristoyl groups increased and that of the palmitoyl

Table 1
Total lipid fatty acyl composition and carotenoid content of *A. laidlawii* membranes

Fatty acid supplemented (5 mg/l	Growth temperature (°C)	Fatty acyl composition (mole %)												Saturated/ unsaturated fatty acyl groups (mole/mole)	Carotenoid content (OD_{450}/mg membrane protein)
		$C_{10:1}$	$C_{12:0}$	$C_{12:1}$	$C_{14:0}$	$C_{14:1}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$		
Arachidic ($C_{20:0}$)	37	–	9.6	0.8	12.1	–	7.6	4.8	1.7	4.2	2.2	–	57.0	7.3	0.46
	28	–	9.0	1.1	19.4	2.4	16.8	8.0	1.9	5.1	3.2	–	33.2	4.1	0.43
Lauric ($C_{12:0}$)	37	0.3	0.5	–	44.9	0.8	42.4	1.2	2.7	5.4	1.8	–	–	9.5	0.17
	28	0.2	2.4	–	52.7	1.5	33.0	2.7	1.6	5.5	1.1	–	–	8.2	0.19
Oleic ($C_{18:1_{cis}}$)	37	–	2.6	0.3	7.0	0.9	28.6	0.5	2.7	50.3	1.4	5.9	–	0.69	0.25
	28	–	1.4	–	7.6	1.0	29.4	0.5	2.1	52.6	1.3	4.2	–	0.68	0.14

groups decreased. Thus, upon lowering the growth temperature, the average chain length of acyl groups was shortened for cells grown in a medium supplemented with saturated fatty acids. For oleic acid supplementation, however, no change in fatty acyl composition was observed.

Acholeplasma laidlawii cells protein carotenoid pigments in their plasma membrane. Higher levels of these pigments lead to a more rigid lipid packing (10). For cells grown in arachidic or lauric acid, there was no change in membrane carotenoid content upon lowering the growth temperature (table 1). In case of oleic acid supplementation, however, the pigment content decreased by about 40%. Such a change is probably too small to account for the entire membrane fluidization, since previous results [10] showed a decrease of 2 $T_{\parallel}$ by 2.5 gauss (22°C) when the carotenoid content was lowered by one order of magnitude. In case of cells grown in an oleic acid supplemented medium, the microorganisms therefore seem to regulate the membrane lipid fluidity by a mechanism yet unknown.

4. Discussion

Acholeplasma laidlawii cells were shown to be capable of maintaining their membrane lipid fluidity when the growth medium is supplemented with either a long chain or a short chain saturated fatty acid (C_{20}, C_{12}), even if the growth temperature was reduced from 37°C to 28°C. These cells are also able to regulate the fluidity when the carotenoid pigment content of the membrane was drastically altered [10]. This capability allows the organism to survive in a broad spectrum of living conditions. The regulation of the lipid fluidity is achieved by modifying the membrane fatty acyl composition, when the cells are grown in a medium containing saturated fatty acids. If the medium contains oleic acid, the pigment content is somewhat varied whereas the fatty acyl composition seems to remain constant.

In various microorganisms larger quantities of unsaturated fatty acyl groups in the membrane lipids are found upon lowering the growth temperature [15–18]. A regulatory mechanism has been proposed for *E. coli* which controls the composition of saturated vs. unsaturated acyl groups in lipids [16]. Furthermore, *E. coli* incorporates higher proportions of saturated and long chain fatty acids into phospholipids upon increase of the growth temperature. Phospholipids are synthesized which have identical viscosities at the growth temperature of the cells [19]. For the temperature control of the phospholipid biosynthesis in *E. coli*, the acyl-Co A; glycerol-3-phosphate transacylase seems to be important [20]. A similar mechanism may be involved in the regulation of fluidity of *Acholeplasma* membranes.

Acknowledgement

This research was supported by U.S. Atomic Energy Commission Contract No. At-(11-1)-338.

References

[1] Lyons, J. M. (1973) Cryobiology 9, 341–350.
[2] De Kruyff, B., Van Dijck, P. W. M., Goldbach, R. W., Demel, R. A. and Van Deenen, L. L. M. (1973) Biochim. Biophys. Acta 330, 269–282.
[3] De Kruyff, B., De Greef, W. J., Van Eyk, R. V. W., Demel, R. A. and Van Deenen (1973) Biochim. Biophys. Acta 298, 479–499.
[4] Wilson, G., Rose, S. P. and Fox, C. F. (1970) Biochem. Biophys. Res. Commun. 38, 617–623.
[5] Edidin, M. (1974) Ann. Rev. Biology, in press.
[6] Rottem, S., Hubbell, W. L., Hayflick, L. and McConnell, H. M. (1970) Biochim. Biophys. Acta 219, 104–113.
[7] Huang, L., Jaquet, D. D. and Haug, A. (1974) Canad. J. Biochem. 52.
[8] Razin, S., Tourtellotte, M. E., McElhaney, R. N. and Pollack, J. D. (1969) J. Bact. 91, 609–616.
[9] Folch, J., Lees, M. and Sloane-Staney, G. H. (1957) J. Biol. Chem. 226, 497–505.
[10] Huang, L. and Haug, A. (1974) Biochim. Biophys. Acta in press.
[11] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 93, 265–275.
[12] Hsung, J. C., Huang, L., Hoy, D. J. and Haug, A., to be published.
[13] McConnell, H. M. and McFarland, B. G. (1970) Quart. Rev. Biophys. 3, 91–136.
[14] McElhaney, R. N. and Tourtellotte, M. E. (1969) Science 164, 433–434.
[15] Marr, A. G. and Ingraham, J. L. (1962) J. Bact. 84, 1260–1267.
[16] Esfahani, M., Barnes, E. M. and Wakil, S. J. (1969) Proc. Natl. Acad. Sci. U.S. 64, 1057–1064.

[17] Cullen, J., Phillips, M. C. and Shipley, G. G. (1971) Biochem. J. 125, 733–742.
[18] Chang, S. B. and Matson, R. S. (1972) Biochem. Biophys. Res. Commun. 46, 1529–1535.
[19] Sinensky, M. (1974) Proc. Natl. Acad. Sci. U.S. 71, 522–525.
[20] Sinensky, M. (1971) J. Bact. 106, 449–455.