Fatty-Acid Composition of Mycoplasma Lipids: Biomembrane with Only One Fatty Acid

Abstract. Mycoplasma *strain Y grew well when elaidate was the only fatty acid added to the medium, and this acid then comprised over 97 percent of the lipid fatty acids. It also grew well when elaidate was supplied together with any one of a series of straight-chain saturated fatty acids, and the lipids then contained both in approximately equimolar proportions. The ratio of cholesterol to phospholipid remained constant.*

Mycoplasmas are suitable organisms for studying the effects of variations in the fatty-acid composition of membrane lipids on membrane structure and function. The *Mycoplasma* cell is bounded by a lipoprotein membrane containing virtually all of the cellular lipids, and has no other membranous organelles (*1*). Many mycoplasmas require complex lipids for growth and incorporate exogenous fatty acids into the membrane lipids. The fatty-acid composition of the lipids of *M. laidlawii* was varied by changing the fatty-acid composition of the growth medium (*1*). However, *M. laidlawii* can synthesize long-chain saturated fatty acids from acetate (*2*). I now report that *Mycoplasma* strain Y cannot synthesize saturated or unsaturated fatty acids, that it cannot desaturate saturated fatty acids, and that it cannot alter the chain length of either. It is thus possible to control the fatty-acid composition of membrane lipids.

Mycoplasma strain Y (*3*) grew poorly in a partly defined medium (medium C) containing defatted bovine serum albumin (BSA) in the absence of added fatty acids. It grew well in this medium with the addition of oleate and palmitate. When pairs of fatty acids (one saturated, the other a monoenoic acid) were added, the range of straight-chain saturated fatty acids compatible with growth at 37°C depended on the chain length and the position and configuration of the double bond in the monoenoic acids (*3*). The results with elaidate were of particular interest because with this acid there was good growth when it was paired with saturated fatty acids varying in chain length from 10 to 20 carbon atoms. In further tests, most single fatty acids gave no growth; some, including oleate, gave poor growth followed by cellular lysis; but elaidate gave good growth.

Analyses of fatty acids by gas-liquid chromatography (*4*) in the lipids of cells grown on medium in which elaidate was the only added fatty acid, or to which it was added together with a straight-chain acid varying in length from 12 to 20 carbon atoms are shown (Table 1). Analysis of cells grown on medium containing oleate and palmitate is included in Table 1. The fatty acids added to the growth medium accounted for 90 to 96 percent of those found in the lipids, and, in the cultures to which two fatty acids were added, both were incorporated in approximately equimolar amounts. Palmitic and stearic acids accounted for most of the remainder, and these acids together with some oleic and a trace of linoleic acid were present in the defatted bovine serum albumin, which had a total fatty-acid content of 0.10 mole per mole of albumin. Only traces of palmitic and stearic acids (totaling approximately 0 02 mole per mole of albumin) were detected in the BSA after treatment with charcoal (*5*). Octadecenoic acid comprised approximately 97 percent of the fatty acids in the lipids of cells grown on medium containing charcoal-treated BSA and elaidate (Table 1, last line, and Fig. 1).

Methyl elaidate is not separated from methyl oleate by gas-liquid chromatography, but is well separated by silver-ion, thin-layer chromatography. The fatty-acid methyl esters obtained from the lipids of cells grown in medium C (to which elaidate alone, elaidate plus palmitate, or oleate plus palmitate had been added) were examined by the latter method (*6*). In each case, the only fatty-acid methyl esters detected migrated like those of the fatty acids which had been added to the growth medium, showing that elaidate is incorporated without isomerization.

The fatty-acid composition of bacterial membrane lipids is often much simpler than that of mammalian membranes, but it can vary widely. Cyclopropane and saturated methyl branched-chain acids can largely replace unsaturated acids in the 2-position of the phosphoglycerides of many species, and may account for as much as 80 to 90 percent of the total (*7*). In yeast grown anaerobically, the lack of unsaturation can be largely compensated for by the incorporation of saturated shorter-

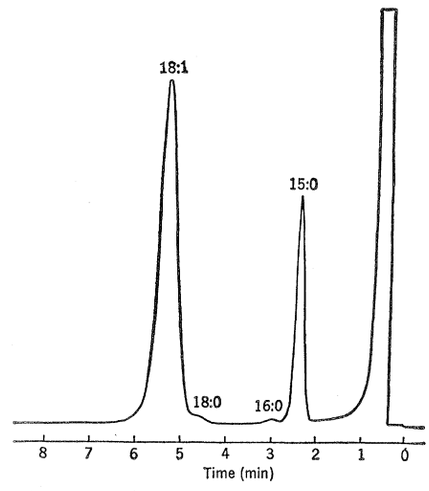

Fig. 1. Gas-liquid chromatogram (15 percent EGS column) of the fatty-acid methyl esters obtained from the lipids of *Mycoplasma* strain Y. The growth medium contained charcoal-treated (*6*) bovine serum albumin; elaidate was the only fatty acid added. A solution of the methyl esters in hexane, containing methyl pentadecanoate as an internal standard, was injected at 0 minutes.

Table 1. Fatty-acid composition and cholesterol–lipid phosphorus ratio in the lipids of *Mycoplasma* strain Y grown with different fatty acids. Cells were grown on medium C containing either fatty-acid poor (F) or charcoal-treated (CH) bovine serum albumin (BSA), 900 mg/liter; cholesterol, 60 μmole/liter, and added fatty acids as indicated. Fatty acids were added in a total concentration of 100 μmole/liter, and where two were added, they were in equimolar concentrations. About 50 percent of the added fatty acids were incorporated into the cellular lipids during growth. The composition of medium C has been described (*1*). Fatty acids are designated by the number of carbon atoms and the number of double bonds; the configuration is indicated by *c (cis)* or *t (trans)*.

Additions to medium		Fatty acids in lipids (mole per 100 mole)						Cholesterol (mole/g-atom P)
Fatty acids	BSA	12 : 0	14 : 0	16 : 0	18 : 0	20 : 0	18 : 1	
10 : 0 + 18 : 1*t*	F							0.78
12 : 0 + 18 : 1*t*	F	48.8	0.0	2.9	4.0	0.0	44.0	.71
14 : 0 + 18 : 1*t*	F	0.0	49.2	2.5	4.9	.0	43.4	.73
18 : 0 + 18 : 1*t*	F	.0	0.0	2.9	48.0	.0	49.4	.69
20 : 0 + 18 : 1*t*	F	.0	.0	2.0	4.5	47.2	46.3	.65
18 : 1*t*	F	.0	.0	1.9	4.6	0.0	93.5	.74
16 : 0 + 18 : 1*c*	CH	.0	.0	47.0	1.9	.0	51.0	
18 : 1*t*	CH	.0	.0	0.6	2.2	.0	97.2	

chain acids in the 2-position of the phosphoglycerides (*8*). It appears from these experiments that a functional biomembrane can be formed in which a single fatty acid—elaidic acid—forms at least 97 percent of the total, and that extremely wide variations in fatty-acid composition are possible.

Unlike bacteria, most mycoplasmas (including strain Y) require sterol for growth and incorporate large amounts of sterol into their membranes. It has been postulated that cholesterol stabilizes biological membranes by causing a closer packing of the fatty-acid chains (*9*). The nature of the component fatty acids has a marked influence on the condensing effect of cholesterol (*9*). A phospholipid with a *trans* monoenoic acid in the 2-position may form a condensed layer, and cholesterol may not be needed to perform this condensing function in membranes in which a *trans* acid occupied the 2-position in the phosphoglycerides (*10*). Although nothing is known about the positional distribution of fatty acids in the phospholipids of *Mycoplasma* strain Y when grown with different pairs of fatty acids, the ratio of cholesterol to lipid phosphorus did not vary significantly with these alterations in fatty-acid composition (Table 1, last column).

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References and Notes

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4. Lipids were extracted from the cells with 19 volumes of a mixture of chloroform and methanol (2 : 1), and the extracts were washed with 0.1*M* KCl. Methyl esters of fatty acids were prepared by direct alkaline methanolysis [J. L. Foote, R. J. Allen, B. W. Agranoff, *J. Lipid Res.* **6**, 518 (1965)] of the lipids and separated from cholesterol by chromatography on Florisil columns [K. C. Kopaczyk and N. S. Radin, *ibid.*, p. 140 (1965)]. An F and M 810 instrument with flame-ionization detector was used for gas-liquid chromatography. Columns were packed with 15 percent ethylene glycol succinate (EGS) or 10 percent Apiezon L on Diatoport S and were calibrated with National Heart Institute (N.H.I.) standard mixtures. Methyl pentadecanoate was the internal standard. Cholesterol was eluted from the Florisil (activated magnesium silicate preparation manufactured by Floridin Co., Tallahassee, Florida) columns with a chloroform-methanol system (3 : 1) and was determined colorimetrically [H. D. Wycoff and J. Parsons, *Science* **125**, 347 (1957)]. Lipid phosphorus was determined in the water-soluble products of alkaline methanolysis.
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6. The fatty-acid methyl esters were prepared, and were separated from cholesterol as described in (*4*). They were chromatographed on thin layers of silica gel G impregnated with silver nitrate and developed in a diethyl ether–light petroleum system (5 : 95) [L. J. Morris, in *New Biochemical Separations*, A. T. James and L. J. Morris, Eds. (Van Nostrand, London, 1964), p. 296]. Spots were located by spraying with sulfuric acid and charring.
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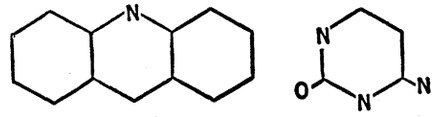
Crystal Structure of a Complex of Acridine, Cytosine, and Water (1 : 1 : 1)

Abstract. *X-ray structural analysis of a crystalline complex between acridine $C_{13}H_9N$, cytosine $C_4H_5N_3O$, and water (1 : 1 : 1) has been completed. The cytosine and water molecules form a sheet-like structure through a series of hydrogen bonds. The acridine molecules are bound to this layer through a hydrogen bridge from the water to the ring nitrogen. The acridine molecules stack in a parallel fashion normal to the cytosine-water sheets, with an average interplanar spacing of 3.5 angstroms.*

Many members of the acridine family elicit mutagenic effects through their interaction with DNA. Lerman (*1*) has proposed from a wide variety of experimental evidence that the acridine dyes bind by intercalation between adjacent hydrogen-bonded base pairs in the helix of DNA. Optical studies (*2*) with various dyes have indicated that electrostatic forces may be of primary importance. Recently, Pritchard *et al.* (*3*) presented a modified version of the Lerman model to account for the electrostatic interactions. However, the exact mode and site of the acridine interaction is uncertain. In order to provide more definitive information about these interactions, I have made a crystallographic study of a complex of acridine (I), cytosine (II), and water (1 : 1 : 1).



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Small needles of the complex were obtained from a solution of water and alcohol containing equal molar quantities of acridine and cytosine. These crystals were determined to be orthorhombic (space group *Pbca*), having cell dimensions of *a* equal to 19.95 Å, *b* equal to 9.327 Å, and *c* equal to 16.908 Å. In attempts to measure the density of the crystals in nonaqueous solvents, it was found that the crystals rapidly changed to anhydrous cytosine. The intensity data were collected on a GE XRD-6 diffractometer equipped with a single-crystal orienter. These intensities were measured by the stationary counter-stationary crystal technique with balanced filters used for the Cu$K\alpha$ radiation. A marked drop in the number of measurable reflections at two theta values greater than 100°, as a result of the thermal motion of the complex, made it feasible to only collect data to this value. Of the 1614 independent reflections measured, 1037 had values significantly greater than their respective background count. The intensities were converted to structure factors (*F*'s) and normalized structure factors (*E*'s) in the usual manner.

The phases of 230 reflections having *E* greater than 1.4 were determined by use of the Sayre relationships (*4*). A program written by Long (*5*) was used for this purpose. An *E* map calculated with the most consistent set of signs determined for the 230 planes revealed the positions of all the nonhydrogen atoms. The structure was then refined by the least-squares method using a block diagonal approximation to the normal equations. At the present stage of refinement, the *R* value (usual discrepancy index) is 0.112 for the observed data and with all the nonhydrogen atoms having anisotropic temperature factors. Because of the high temperature factor of the complex (average isotropic B approximately 8 Å^2), difficulty in obtaining accurate hydrogen positions is being experienced. It is through analysis of bond lengths and angles that the hydrogen bonding scheme has been proposed.

The bond lengths and angles obtained for the acridine and cytosine molecules agree well with those found in x-ray analyses of crystals of these