Formation of giant vesicles from diacylmannosylerythritols, and their binding to concanavalin A†

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The structures of diacylmannosylerythritols, MEL-A, -B and -C, are determined; these microbial glycolipid biosurfactants efficiently self-assemble in water to form giant vesicles, which show excellent binding affinity towards the mannose-binding protein, concanavalin A.

The 'diacyl-mannosylerythritol' lipids (MELs) are very promising 'biosurfactants',[1] owing to their attractive properties (*e.g.* easy production conditions, low toxicity, biodegradability, biological activity) compared to most synthetic surfactants. MELs are abundantly produced by the yeast strain *Candida antarctica* T-34,[2a] and mainly consist of three components: MEL-A, -B and -C.[2b] These yeast glycolipids show not only excellent surface-activity[2c] but also remarkable cell differentiation and growth inhibition activities against human leukemia[2d] and mouse melanoma cells.[2e] On the other hand, glycolipids have received much attention as leading materials for drug-carrying microcapsules and artificial cells,[3] owing to their stabilizing effect on liposomes.[4] Finally, glycolipids also carry out vital functions in biomembranes, *e.g.* cell recognition, histocompatibility, antigenicity, and are of particular interest in studying surface recognition processes.[4c,5]

Here we report the complete structural characterization of the MEL glycolipids (MEL-A, -B and -C). We also describe, for the first time the spontaneous formation of giant vesicles from each of these glycolipids; finally, we show that these giant vesicles show an excellent binding affinity towards a mannose-binding protein, concanavalin A (Con A).

The three MELs consist of 4-*O*-[β-D-mannopyranosyl] *meso*-erythritol, esterified by two medium-chain fatty acids (C_8 to C_{12}) and one or two acetic acids; the individual esterified positions, however, remained to be determined precisely.[2b] Further structural characterization of the MELs was thus carried out. The mixture of MELs was obtained as reported previously,[2a] and purified by silica-gel column chromatography with modifications of the elution conditions.

The esterified positions of the individual acetyl and acyl groups on the mannosyl back-bone were determined by ^{1}H and ^{13}C 2D NMR ($CDCl_3$, 500 MHz) (Fig. 1). HMBC (heteronuclear multiple bond connectivity) long-range correlations ($^3J_{C-H}$) between the carbonyl carbons of the acyl groups and H-2′ and H-3′ of mannose were clearly observed on all the MELs,‡ indicating that the fatty acids are linked to the hydroxy groups at C-2′ and C-3′ of mannose. $^3J_{C-H}$ correlations between the acetyl carbons (δ_{CO} 169.6 and 170.9) and the mannose protons ($\delta_{H\text{-}4'}$ 5.24 and $\delta_{H\text{-}6'}$ 4.23), respectively, were observed exclusively on MEL-A, which was thus shown to be 4-*O*-[(4′,6′-di-*O*-acetyl-2′,3′-di-*O*-alkanoyl)-β-D-mannopyranosyl] *meso*-erythritol. On the other hand, a $^3J_{C-H}$ correlation between the acetyl carbon (δ_{CO} 171.8) and H-6′ (δ 4.44) was observed with MEL-B, while a correlation between the acetyl carbon (δ_{CO} 170.1) and H-4′ (δ 5.16) was observed only with MEL-C. From these results, MEL-B and -C were shown to be 4-*O*-[(6′-*O*-acetyl-2′,3′-di-*O*-alkanoyl)-β-D-mannopyranosyl] *meso*-erythritol and 4-*O*-[(4′-*O*-acetyl-2′,3′-di-*O*-alkanoyl)-β-D-mannopyranosyl] *meso*-erythritol, respectively. It was also confirmed that none of the three hydroxy groups in the erythritol moiety is esterified in any of the MELs.

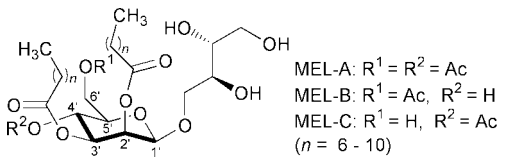

Fig. 1 Structure of diacylmannosylerythritols.

Membrane-forming properties of these MELs were then examined by phase contrast microscopy. All the MELs, when dispersed in water (pH 7.0) at 25 °C, spontaneously formed giant vesicles.§ Unilamellar vesicles of diameter larger than 10 μm were observed, beside multilamellar structures and tubules. Vesicle formation was confirmed by fluorescence microscopy, after addition of the lipophilic fluorescent probe Nile Red to the pre-formed vesicles (5 mol% to MEL) (Fig. 2). Some synthetic and natural glycolipids bearing a disaccharide or larger hydrophilic head group have been reported to form by themselves vesicular systems;[3] however, with the only exception of rhamnolipids,[3e] microbial glycolipids do not appear to have been reported to do the same.

Formation of giant vesicles should lead to surfaces covered by the multiantennary mannopyranoside residues, which could act as high affinity receptors for mannose-binding proteins. We therefore investigated the interaction between these giant vesicles and concavalin A. Succinyl Con A,[6] labeled with a fluorescence probe (FITC), was dissolved in a phosphate buffer

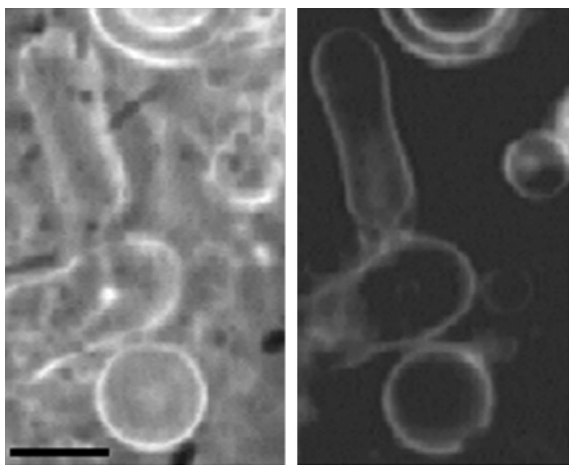

Fig. 2 Phase contrast (left) and fluorescence (right) microscope images of MEL-C giant vesicles stained with Nile Red. The bar represents 10 μm.

† Electronic supplementary information (ESI): preparation, optical microscopy details and NMR data for MELs. See http://www.rsc.org/suppdata/cc/b0/b000968g/

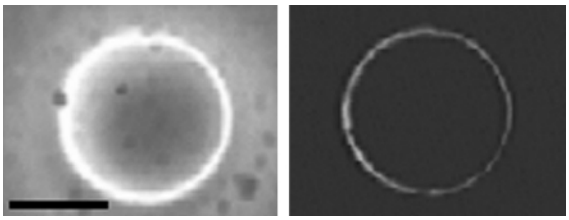

Fig. 3 Phase contrast (left) and fluorescence (right) microscope images of MEL-B giant vesicles coated with FITC-labeled concanavalin A. The bar represents 10 μm.

(pH 7.0) and added to the pre-formed vesicles, and were observed under fluorescence microscope. With MEL-B and -C vesicles, a clear fluorescent ring was observed around the outer edge of the vesicle. This clearly indicates the recognition by the fluorescence labeled Con A of the mannose residues of the glycolipid head-groups that are located on the vesicular surface (Fig. 3). The coating, as judged by the abundance of fluorescent vesicles, appeared to be efficient on the giant vesicles of MEL-B and -C; however, no clear coating was observed on those made of MEL-A.

Interactions of Con A with disk-like assemblies[4c] or small vesicles[5] of synthetic glycolipids have been previously reported. However, these small lipidic assemblies showed a tendency to aggregate and precipitate when treated with Con A, presumably due to interactions of one ConA molecule with several lipidic systems.[5,7] In the case of giant vesicles of MEL-B and -C, coating of the outer surface was not accompanied by aggregation and precipitation : the giant vesicles presumably provide a very large surface relative to the molecular size of Con A, which probably impedes the binding of the lectin to more than one vesicle at the same time, and thus aggregation does not occur.

In addition, most of the carbohydrate–receptor interactions observed are weak,[8] and in order to compensate for this low binding affinity, different strategies based on multivalent interactions have been designed, including carbohydrate clusters, glycopolymers and glycodendrimers.[5c,9] Our work describes yet another way of enhancing the interaction between a receptor (Con A) and a carbohydrate moiety (mannose), which is based on the spontaneous self-assembly of the glycolipids onto vesicles, thereby generating a multivalent surface. It should be noted that only the giant vesicles prepared from MEL-B and -C, both of which carry a free OH at C-4′ or C-6′, bind efficiently with Con A. There must therefore be a specific mode of binding, like multivalent interactions, on the vesicular surface, because Con A mainly recognizes and binds to the hydroxy groups at C-3′, C-4′ and C-6′ in D-mannose or D-glucose.[10]

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Notes and references

‡ The following correlations ($^3J_{C-H}$) were observed between the carbonyl carbon of acyl groups and mannose protons: MEL-A, (δ_{CO} 173.8 and $\delta_{H\text{-}2'}$ 5.51) and (δ_{CO} 172.9 and $\delta_{H\text{-}3'}$ 5.06); MEL-B, (δ_{CO} 173.8 and $\delta_{H\text{-}2'}$ 5.49) and (δ_{CO} 173.7 and $\delta_{H\text{-}3'}$ 4.91); MEL-C, (δ_{CO} 173.6 and $\delta_{H\text{-}2'}$ 5.50) and (δ_{CO} 172.8 and $\delta_{H\text{-}3'}$ 5.09).

§ A typical procedure was as follows: MEL (10 mg) was dissolved in 1 mL of a 2:1 mixture of chloroform and methanol. An aliquot (5–10 μL) of the solution was dropped on a glass microscope slide with a well of 15 mm diameter. After 10 min drying (argon flow), 0.5 mL of water or a buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 6.98) was added to the film, and the slide was incubated for 30 min at 25 °C to allow lipid hydration. Optical (phase contrast) and fluorescence microscopies were carried out according to the procedures described in ref. 11.

This showed unilamellar and multilamellar vesicles (diameter > 10 μm) as well as tubules. This was confirmed by the fluorescence observed after addition of Nile Red (5 mol%/MEL) to the pre-formed vesicle suspension.

The vesicles observed retained their structures at room temperature for at least 2 days.

1 J. D. Desai and I. M. Banat, *Microbiol. Mol. Biol. Rev.*, 1997, **61**, 47; S.-C. Lin, *J. Chem. Tech. Biotechnol.*, 1996, **66**, 109; I. M. Banat, *Acta Biotechnol.*, 1995, **15**, 251.

2 (*a*) D. Kitamoto, H. Yangishita, K. Haraya and H. K. Kitamoto, *Biotechnol. Lett.*, 1998, **20**, 813; (*b*) D. Kitamoto, S. Akiba, T. Hioki and T. Tabuchi, *Agric. Biol. Chem.*, 1990, **54**, 31; (*c*) D. Kitamoto, H. Yanagishita, T. Shinbo, T. Nakane, T. Nakahara and C. Kamisawa, *J. Biotechnol.*, 1993, **29**, 91; (*d*) H. Isoda, H. Shinmoto, D. Kitamoto, M. Matsumura and T. Nakahara, *Lipids*, 1997, **32**, 263; (*e*) X. Zhao, Y. Wakamatsu, M. Shibahara, N. Nomura, C. Geltinger, T. Nakahara, T. Murata and K. K. Yokoyama, *Cancer Res.*, 1999, **59**, 482.

3 (*a*) T. Endo, K. Inoue and S. Nojima, *J. Biochem.*, 1983, **93**, 1; (*b*) B. Maggio, J. Albert and R. K. Yu, *Biochim. Biophys. Acta*, 1988, **945**, 145; (*c*) Y. Ishigami and H. Machida, *J. Am. Oil Chem. Soc.*, 1989, **66**, 599; (*d*) C. Guedj, B. Pucci, L. Zarif, C. Coulomb, J. G. Riess and A. A. Pavia, *Chem. Phys. Lipids*, 1994, **72**, 153; (*e*) Y. Ishigami, Y. Gama, H. Nagahora, M. Yamaguchi, H. Nakahara and T. Kamata, *Chem. Lett.*, 1987, 763.

4 (*a*) T. M. Allen, C. Hansen and J. Rutledge, *Biochim. Biophys. Acta*, 1989, **981**, 27; (*b*) S. K. Huang, K.-D. Lee, K. Hong, D. S. Friend and D. Papahadjopoulos, *Cancer Res.*, 1992, **52**, 5135; (*c*) S. Takeoka, K. Sou, T. Ohgushi and E. Tsuchida, *Supramol. Sci.*, 1998, **5**, 159.

5 (*a*) W. Curatolo, *Biochim. Biophys. Acta*, 1987, **906**, 111; (b) A. Varki, *Glycobiology*, 1993, **3**, 97; (c) R. Roy, *Trends Glycosci. Glycotechnol.*, 1996, **8**, 79.

6 G. R. Gunther, *Proc. Natl, Acad. Sci. USA*, 1973, **70**, 1012.

7 T. Suzuki, K. Inoue, S. Nojima and H. Wiegant, *J. Biochem.*, 1983, **94**, 373; R. Sundler, *FEBS Lett.*, 1982, **141**, 11; H. Bader, H. Ringsdorf and J. Skura, *Angew. Chem., Int. Ed. Engl.*, 1981, **20**, 91.

8 E. J. Toone, *Curr. Opin. Struc. Biol.*, 1994, **4**, 719; D. A. Mann, M. Kanai, D. J. Maly and L. L. Kiessling, *J. Am. Chem. Soc.*, 1998, **120**, 10 575.

9 K. Ozaki, R. T. Lee, Y. C. Lee and T. Kawasaki, *Glycoconjugate J.*, 1995, **12**, 268; A. Kichler and F. Schuber, *Glycoconjugate J.*, 1995, **12**, 275; R. Roy and J.-M. Kim, *Angew. Chem., Int. Ed.*, 1999, **38**, 369.

10 J. H. Naismith and R. A. Field, *J. Biol. Chem.*, 1996, **271**, 972.

11 F. M. Menger and S. J. Lee, *Langmuir*, 1995, **11**, 3685; F. M. Menger, S. J. Lee and J. S. Keiper, *Langmuir*, 1996, **12**, 4479; T. Ueda, S. J. Lee, Y. Nakatani, G. Ourisson and J. Sunamoto, *Chem. Lett.*, 1998, 417.