

bomethoxy-4-(unsubstituted, 3-methyl-, 4-methyl-, 3-nitro-, 4-nitro-, and 2,4-dinitrophenyl)-1,4-dihydropyridine. *J. Med. Chem.* 25:126-131.

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## X-RAY DIFFRACTION STUDIES OF THE CHOLERA TOXIN RECEPTOR, $G_{M1}$ .

ROBERT V. MCDANIEL\* AND THOMAS J. MCINTOSH†

\*Department of Physiology and Biophysics, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York 11794; and †Department of Anatomy, Duke University Medical Center, Durham, North Carolina 27710

Gangliosides are anionic glycolipids that are found in the outer monolayer of many plasma membranes. In particular, the polar head group of ganglioside  $G_{M1}$  plays an important role in human physiology as it is the receptor for cholera toxin (1) and it regulates growth factor receptors (2). Gangliosides will not form bilayers by themselves, presumably because their head groups are large (3-5). However, multilamellar liposomes formed from mixtures of phosphatidylcholine (PC) and  $G_{M1}$  (4) have been used as models of the electrokinetic properties of human erythrocyte membranes (6). In this report, the structure of PC: $G_{M1}$  liposomes is analyzed by x-ray diffraction techniques to determine the distance by which the polar head group of  $G_{M1}$  extends from the bilayer surface. We label the sialic acid moiety of  $G_{M1}$  with europium (Eu) (5) and use difference profiles to determine the distance between the fixed charge on  $G_{M1}$  and the phosphate group of PC.

### MATERIALS AND METHODS

We formed bilayers from a 7:3 mol:mol mixture of 1-palmitoyl-2-oleyl phosphatidylcholine (PC) with  $G_{M1}$ . PC was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL).  $G_{M1}$  (>90% by TLC) was prepared from upper Folch extract of bovine brain (7). Eu-labeled  $G_{M1}$  was prepared by adding 1 mol europium chloride/mol  $G_{M1}$  in methanol:water 33:1, followed by evaporation of solvent. Europium chloride (99.9%) was obtained from Alfa Inorganics, Inc. (Beverly, MA). Swelling experiments were performed by gravimetrically adding dry lipid to increasing amounts of aqueous 0.1 M NaCl, pH 7.5. The x-ray patterns were recorded and analyzed by standard techniques, as described previously (8).

### RESULTS

For a range of water contents of ~15-50 wt %, each diffraction pattern contained five or six orders of a single lamellar repeat period and a broad wide-angle band at 4.6 Å, indicating a single multilamellar phase of liquid crystalline bilayers. We obtained lamellar repeat periods of 62-70 Å for unlabelled bilayers and 62-78 Å for Eu-labelled bilayers. At repeat periods larger than this range, only two or three lamellar diffraction orders were recorded. At repeat periods smaller than this range, phase separation

(4) resulted, as indicated by additional low-angle reflections that did not index on the lamellar repeat period, as well as an additional sharp wide-angle reflection at 4.2 Å (3). The points in Fig. 1 indicate the observed structure amplitudes. The curves in Fig. 1 are derived from a sampling theorem analysis and are proportional to the absolute value of the Fourier transform of the electron density distribution across the PC: $G_{M1}$  bilayer. The two transform curves are similar in shape, indicating that the Eu label did not drastically alter the bilayer structure. The correct phase angle, either  $\pi$  or 0, was assigned to each region of the transforms by using the sampling theorem (8). The resulting electron density profiles are shown in Fig. 2. The top profile is of a bilayer labelled with Eu and the middle profile is of an unlabelled bilayer. The Eu difference profile (bottom of Fig. 2) was obtained by subtracting the scaled unlabelled profile from the labelled profile, with a scaling factor that assumes no penetration of Eu ions into the hydrocarbon region of the bilayer (5). The

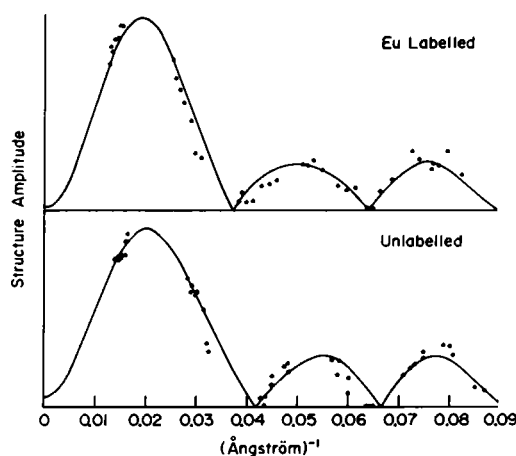


FIGURE 1 Structure amplitudes for a series of swelling experiments for unlabelled and europium-labelled PC: $G_{M1}$  multilayers. The smooth curves were calculated using the sampling theorem.

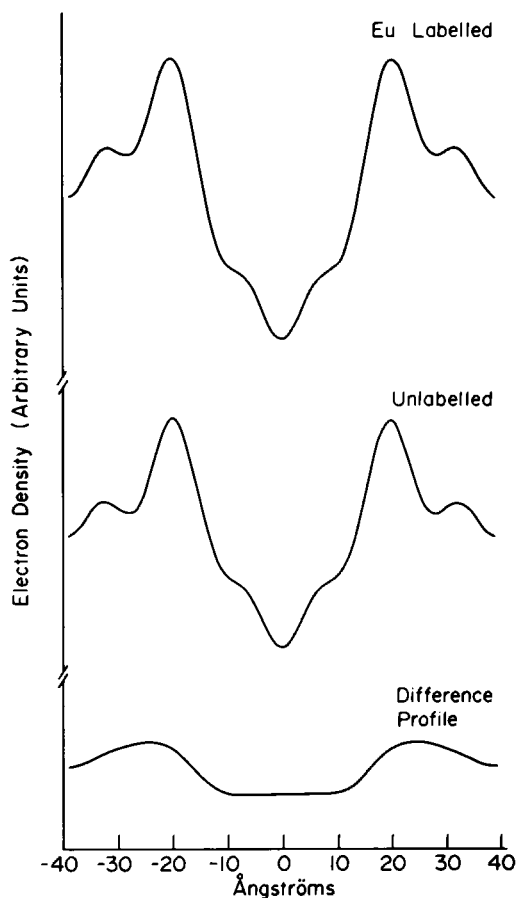


FIGURE 2 Electron density profiles for europium-labelled and unlabelled PC:G<sub>M1</sub> bilayers, showing the terminal methyl dip (at 0 Å), surrounded by the methylene chain regions (at ±10 Å), the PC head group regions (at ±20 Å), and the hydrated G<sub>M1</sub> head groups at the outer edges. The difference profile was obtained by subtracting the unlabelled profile from the labelled profile.

electron density profiles indicate that the introduction of 30 mol % G<sub>M1</sub> has little effect on the organization of the hydrocarbon core of the PC bilayer. G<sub>M1</sub> adds density to the hydrophilic region between the PC head groups and the Eu difference profile shows significant amounts of Eu in this region. Because it is known that Eu binds to the carboxylate group of the G<sub>M1</sub> sialic acid moiety (5), these profiles show that the fixed charge on G<sub>M1</sub> is spatially separated from the PC head group. The width of the peak in the difference profile indicates that Eu binds to the PC head group as well as to the sialic acid moiety.

To obtain a more quantitative interpretation of these data, we used electron density strip models (9) to analyze the unlabelled diffraction data, and a reciprocal space refinement procedure of Buldt et al. (10) to calculate the position of Eu in the labelled bilayers. For the unlabelled specimens, the data of Fig. 1 are adequately described by a simple electron density strip model (solid line in Fig. 3). The central five strips of the model correspond to the terminal methyl region, two methylene chain regions, and

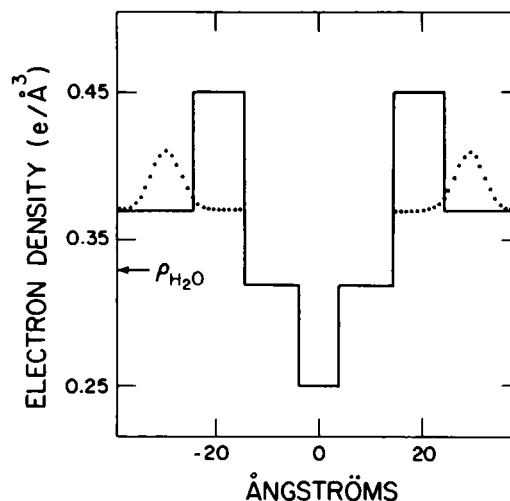


FIGURE 3 Electron density strip model for the PC:G<sub>M1</sub> bilayers shown in Fig. 2. The solid line represents the electron density distribution for the unlabelled bilayer. The outer strips have a higher electron density than water (arrow), indicating the presence of the G<sub>M1</sub> head groups in these regions. The dotted line is the best-fit gaussian distribution (9) for the Eu-labelled carboxylate groups of G<sub>M1</sub>.

two PC head group regions, with densities and widths similar to published models (10, 11) for PC bilayers. The outer strip extends from the edge of the PC head group to the edge of the unit cell and has a larger electron density than water. The electron density of this strip remains constant as water is added, suggesting that the G<sub>M1</sub> head group swells as it is hydrated. This high-density strip extends to the edge of the unit cell for every data set, even those with repeat periods of 70 Å. Thus, the G<sub>M1</sub> head group can extend at least 15 Å from the center of the PC head group or 21 Å from the hydrocarbon-water interface. Based on a molecular model (6), this is the maximum extension of the G<sub>M1</sub> head group. The x-ray analysis therefore indicates that the hydrated G<sub>M1</sub> head group is fully extended, approximately perpendicular to the plane of the bilayer. In contrast, the head groups of gangliosides in the hexagonal phase extend 10 Å from the hydrocarbon-water interface (3). For the Eu-labelled bilayers, we obtained the best fit (9) to the measured difference structure factors with the G<sub>M1</sub> carboxylate groups (5) located 10 Å from the PC head group, as shown by the dotted line of Fig. 3.

## DISCUSSION

Our x-ray data indicate that the polar head group of G<sub>M1</sub> is fully extended in hydrated PC:G<sub>M1</sub> bilayers. This extension of mass and charge away from the bilayer surface has two important consequences for the electrostatic and electrokinetic properties of these bilayers. Because the fixed charge on G<sub>M1</sub> is located away from the bilayer surface, the surface potential of PC:G<sub>M1</sub> vesicles decreases compared with charged phospholipid vesicles (6). Also, when PC:G<sub>M1</sub> vesicles move in an electric field, the extended head group

of  $G_{M1}$  exerts a hydrodynamic drag force that slows the movement of the vesicles (6).

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# NEUTRON CRYSTALLOGRAPHY OF A MEMBRANE PROTEIN

## Localization of Detergent and Protein at 20-Å Resolution

M. ZULAUF,\* P. A. TIMMINS,<sup>†</sup> AND R. M. GARAVITO<sup>‡</sup>

\*European Molecular Biology Laboratory, 38042 Grenoble Cedex, France; <sup>†</sup>Institut Laue-Langevin, 38042 Grenoble Cedex, France; and <sup>‡</sup>Biozentrum, CH-4056 Basel, Switzerland

Matrix Porin (1) is a transmembrane protein in the outer membrane of *Escherichia coli* that allows passive diffusion of nutrient molecules through pores of 10 Å diam. It can readily be solubilized as a trimer ( $3 \times 35,000$ ) in the presence of detergents including *n*-octyl- $\beta$ -D-glucopyranoside ( $\beta$ -OG), and it crystallizes from these solutions in space group  $P4_2$  ( $a = b = 154$  Å;  $c = 172$  Å) with two trimers/asymmetric unit (2, 3). Crystals contain ~40% protein, 40% aqueous solvent and 20% detergent, and diffract x-rays to 2.8 Å resolution; the structure analysis has been hampered for some time by the difficulty in obtaining suitable heavy atom derivatives. Neutron diffraction studies with  $\text{D}_2\text{O}/\text{H}_2\text{O}$  contrast variation at 16 Å resolution have been carried out to distinguish between, and localize, the protein and detergent moieties.

## MATERIALS AND METHODS

Crystals for neutron diffraction were grown from 1%  $\beta$ -OG in the presence of 18% polyethyleneglycol (PEG 4,000), 0.5 M NaCl, 0.1 M NaPO<sub>4</sub>, pH 7.0 and various  $\text{D}_2\text{O}/\text{H}_2\text{O}$  percentages: 0, 25, 60, and 100%. Sizes were up to 0.5 mm in linear dimension. The crystals were fixed with glutaraldehyde before mounting.

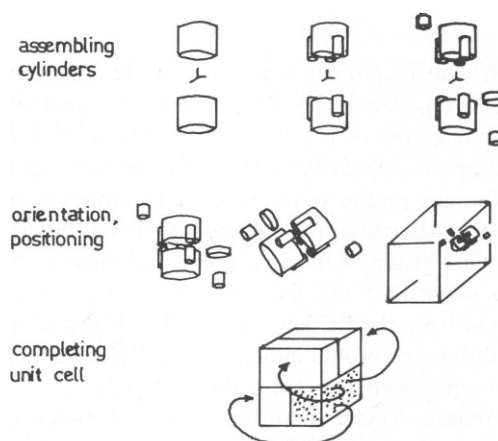


FIGURE 1 Schematic representation of the steps involved in calculating a model of arrays of cylinders. Cylinder dimensions, densities, and positions with respect to a fixed coordinate system are entered successively. Each cylinder may or may not be doubled by any kind of symmetry operation. The assembly is then oriented in space by specifying three Euler angles, and its center is positioned in a quarter of the unit cell. The complete unit cell is finally assembled and the Fourier transform is calculated at the desired resolution.