

of MAO. In addition, the dynamic redistribution of phosphatidylcholine might further affect the functional level of the MAO-A type activity.

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³¹P NMR STUDIES OF ORIENTED MULTILAYERS FORMED FROM ISOLATED SARCOPLASMIC RETICULUM AND RECONSTITUTED SARCOPLASMIC RETICULUM EVIDENCE THAT "BOUNDARY-LAYER" PHOSPHOLIPID IS NOT IMMOBILIZED

A. C. MCLAUGHLIN

Department of Biology, Brookhaven National Laboratory, Upton, New York 11973, U.S.A.

L. HERBETTE AND J. K. BLASIE

Department of Chemistry and Biochemistry/Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania 19104, U.S.A.

C. T. WANG, L. HYMEL, AND S. FLEISCHER

Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235, U.S.A.

Sarcoplasmic reticulum is one of the most intensively studied membrane systems. As isolated in highly purified form, it is capable of energized calcium uptake and has a relatively simple composition. The major protein constituent (>90%) is the calcium pump protein (1, 2), which has been dissociated from the sarcoplasmic reticulum membrane and reconstituted to form functional membrane vesicles (3, 4). Such membranes of defined lipid content make possible detailed studies aimed at correlating membrane composition with structure and structure with function (4-7).

³¹P NMR has been used previously to study the motion of the polar head group region of model phospholipid membranes (8-10). Oriented multilamellar systems have proved particularly useful for this purpose (8, 9, 11). The angular dependence of the position of the ³¹P NMR signal from oriented membranes can be used to calculate the phosphorus chemical shift anisotropy and the direction of the symmetry axis for the motion of the phosphate group. The angular dependence of the width of the ³¹P NMR signal can be used to calculate the dipolar interaction between the phosphorus nucleus and the protons on the

two adjacent methylene groups, and the direction of the symmetry axis for the motion of these two groups.

RESULTS AND DISCUSSION

Fig. 1 shows the angular dependence of the ³¹P NMR signal from oriented sarcoplasmic reticulum membranes. Similar spectra were obtained from oriented reconstituted sarcoplasmic reticulum membranes with lipid-to-protein ratios ranging from 42:1 to 110:1 and from oriented bilayer membranes formed from sarcoplasmic reticulum phospholipids (12). We used the dependence of the ³¹P NMR spectra on the alignment of the membranes with respect to the magnetic field to draw two conclusions about the motion of the phospholipid molecules that contribute to the observed spectra. First, the phosphate group and the two adjacent methylene groups are able to rotate rapidly (i.e., faster than 10⁻⁵ s) around the normal to the plane of the membrane. Second, the restricted internal motion of the phosphate group and the glycerol CH₂OP group is very similar to that found in liposomes formed from sarcoplasmic reticulum phospholipids. Calibration experiments showed that all (100 ± 7%) of the

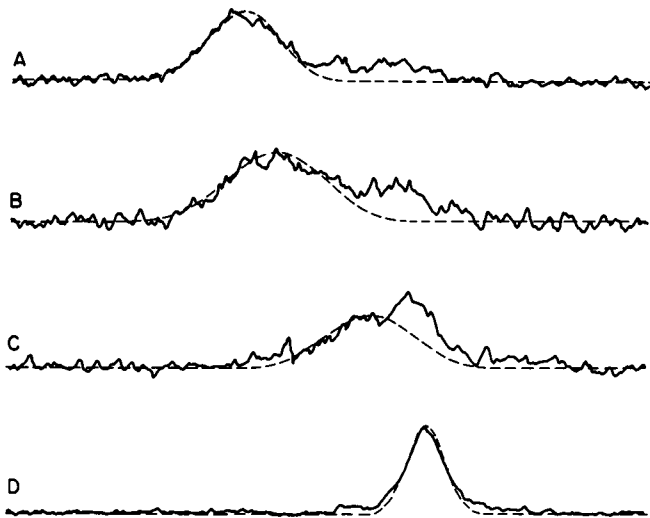


FIGURE 1 The angular dependence of the ^{31}P NMR signal from oriented sarcoplasmic reticulum membranes. The spectra shown in traces A–D were taken with membranes aligned so that the angle between the magnetic field and the normal to the plane of the membrane was 0° , 30° , 55° , and 90° , respectively. The dashed lines are theoretical Gaussian curves which were used to define the position and the width of the component of the signal arising from planar regions of the flattened spherical vesicles (12). The spectra were taken at 145.7 MHz. The total sweep width is 20 kHz. Each spectrum was accumulated for approximately two hours at $8 \pm 1^\circ$.

phospholipid molecules in the membrane can be accounted for in the observed spectra. Thus, essentially all the phospholipid molecules in the sarcoplasmic reticulum and the reconstituted sarcoplasmic reticulum membranes have the same motion in the polar headgroup region as found in model bilayer membranes. Since a large fraction of the phospholipid molecules (between one-quarter and one-half depending on the lipid-to-protein ratio) are immediately surrounding the calcium pump protein, we conclude that the calcium pump protein does not perturb the motion of these “boundary-layer” lipids.

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