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THE DYNAMIC STRUCTURE OF THE POLAR-GROUP OF CARDIOLIPIN IN E.COLI PHOSPHOLIPID BILAYER AS MONITORED BY ^{2}H-NMR

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The glycerol part of E.coli phospholipid was specifically deuterated by supplying $^{2}H_5$-glycerol in growth media of glycerol-requiring auxotroph.[1] The ^{2}H-spectra of cardiolipin were obtained for dispersion of the purified deuterated cardiolipin mixed with non-deuterated other components. The structure of the whole polar region of cardiolipin in the lipid bilayer appeared to be dynamically symmetric, and quite rigid.

Cardiolipin, which occurs widely in bacteria, plants and animal tissues to a various extent, is one of the major phospholipid classes. As can be seen in Fig. 1, cardiolipin has an unique structure with a glycerol moiety to which two phosphatidyl groups are attached.

The polymorphic properties of pure cardiolipin-water system are well known. In X-ray and ^{31}P-NMR studies, it has been shown that cardiolipin takes on mainly the bilayer phase in the absence of divalent cations, whereas in the presence of the cations the hexagonal phase(H_{II}) is observed.[2,3] However, the structural study on the unique polar region has not been reported at all. In this paper, we present a ^{2}H-NMR analysis of the polar group region of cardiolipin for the first time.

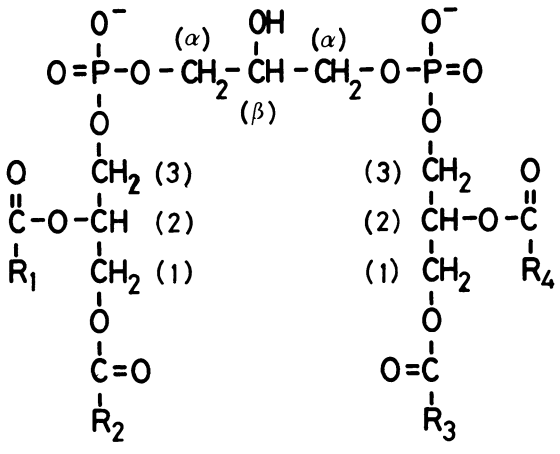

Fig. 1. The head-group structure of cardiolipin. Numberings of the glycerol carbons are shown in ( ).

An auxotroph requiring unsaturated fatty acids[4] and glycerol[5]

(designated as E.coli K-12 UFA^{ts}, glpA) was used for the specific deuteration of the head group region of the phospholipids. The auxotroph was grown at 42 °C in a basal medium containing 1.0% casamino acids, 2.0% KCl and 0.002% $^{2}H_{5}$-glycerol (98% deuterated, from the CEA). The cells were cultured in Jar-fermenter (100 l), and harvested at the late log-phase. To obtain the non-deuterated phospholipids, $^{2}H_{5}$-glycerol was replaced with non-deuterated glycerol. Phospholipids were extracted from the cells according to the method of Bligh and Dyer,[6] and further purified by silicic acid column chromatography. The phospholipid composition is 76 mol% phosphatidylethanolamine(PE), 20% cardiolipin(CL), and 4% phosphatidylglycerol(PG).

Figure 2 shows the ^{2}H-NMR spectra of the total lipid extract in chloroform/methanol (3:2, vol), recorded on a Bruker WM 360wb NMR spectrometer at 55.3 MHz. TMS was used as the internal standard. Deuterium chemical shift is usually the same to the corresponding proton chemical shift. The large peaks, around 4.0 and 5.3 ppm, can be assigned to the deuterons of C(1) and C(3), and that of C(2) of the glycerol segment, respectively.[7] The relative intensity was approximately 4:1. Small signals due to the naturally abundant deuterons of methyl and methylene groups are also observed around 1.0 ppm. Comparing the intensities of these signals, it is certain that deuterons were specifically introduced into glycerol segments of phospholipids.

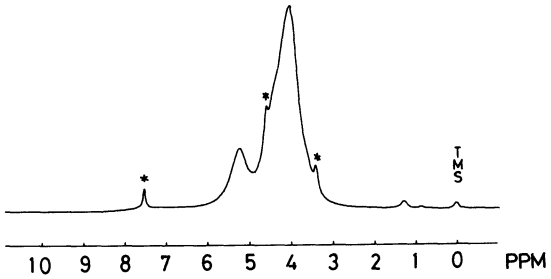

Fig. 2. The high resolution ^{2}H-NMR spectrum of the total lipid extract in chloroform:methanol(3:2).

Phospholipids were dispersed at 50 °C in 0.1 M PIPES buffer at pH 7.2 containing 2 mM EDTA, and centrifuged to form pellets. Deuterium-depleted water(less than 0.2 ppm) was used in all sample preparations to eliminate the deuterium signal of natural abundance in water. ^{2}H-NMR spectra of the phospholipid dispersion were recorded on the same WM 360wb spectrometer, employing the quadrupole echo technique.[8] The pulse width for a 90° pulse was 24 μs, the spectral width 100 KHz, and the recycle time 0.15 s. To eliminate the contributions of PE and PG to the deuterium spectra and characterize the properties of CL in the total phospholipid system, the deuterated CL was

purified with thin-layer chromatography, and remixed with non-deuterated PE and PG in the same ratio of the total lipid extract. The spectra of CL obtained by this procedure at different temperatures are shown in Fig. 3. All the signals of the spectra can be ascribed to glycerol moieties of CL. Since we are looking at powder pattern spectra, single deuteron gives rise to a couple of signals separated by so-called quadrupole splitting. Each spectrum shows four discerned quadrupole doublets and a central signal. The ^{31}P-NMR spectra are characteristic of a pure lamellar structure below 60 °C. Therefore, the central signal should result from the lamellar lipids in which C-D bond vectors are either aligned to the magic angle with respect to the bilayer normal or undergoing such a fast motion as to completely average the static quadrupole splitting. The central signal could be composed of the two components, although it is not clear at this stage.

Cardiolipin has three glycerol segments, namely, the two glycerol backbones and the "bridge" segment between the two phosphate groups as shown in Fig. 1. As to the glycerol backbone, the common features of the deuterium spectra are known for various phospholipid species.[1,9] That is, all three deuterons of C(3) and C(2) positions give similar quadrupole splittings ranging 22-28 KHz, and the two deuterons of C(1) give two different quadrupole splittings of around 0 and 15 KHz. That is also the case with CL in this system. The quadrupole splittings of 25, 22, 15, and 0 KHz were observed in both spectra of CL and PE(the spectrum is not shown).

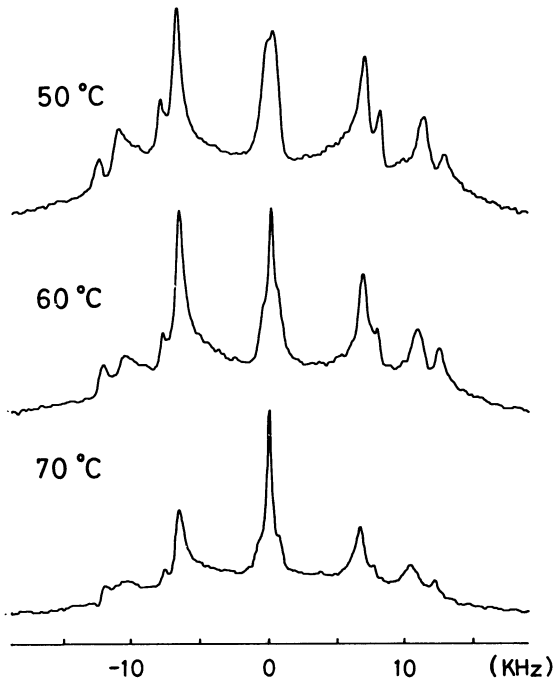

Fig. 3. The ^{2}H-NMR spectra of the glycerol segments of CL at various temperatures.

Following the assignments performed for the glycerol backbone of PE,[1] the outermost signals can be assigned to the 3-R deuteron and the broad signals with the second largest quadrupole splitting are assignable to the 2 and 3-S deuterons (nomenclatures for using the R and S system, when a proton is

replaced by a deuteron). The quadrupole splittings of around 15 and 0 KHz can be ascribed to the 1-R and 1-S deuterons, respectively. Since just one set of signals are deduced from the two glycerol backbones of CL, it can be concluded that the two backbones take the equivalent dynamic structures in the lipid bilayers.

Besides the quadrupole splittings assigned to the glycerol backbones, a strong doublet split around 13 KHz was observed. Judging from the strong intensity, it is most probable that the four deuterons of the two methylenes in the polar head give rise to the same quadrupole splitting. It means that the two methylene groups take dynamically equivalent structures. The signals of the β deuteron of the polar head is not yet clear. It should be assigned either to the strong doublet or to the central signal.

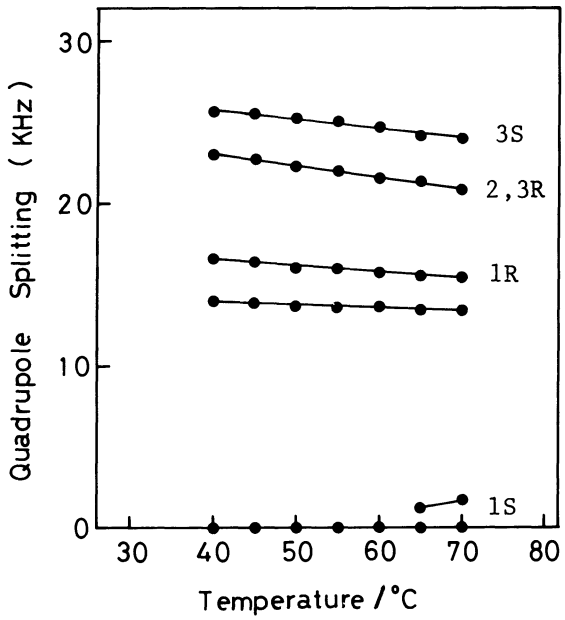

Fig. 4. Temperature changes of the quadrupole splittings.

The quadrupole splittings of CL are plotted as a function of temperature in Fig. 4. The behaviors of the backbone deuterons are close to those of PE. The large value and the small temperature dependence of the quadrupole splitting of the methylene groups in the polar head moiety suggest that the polar headgroup structure is rigid and very stable.

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