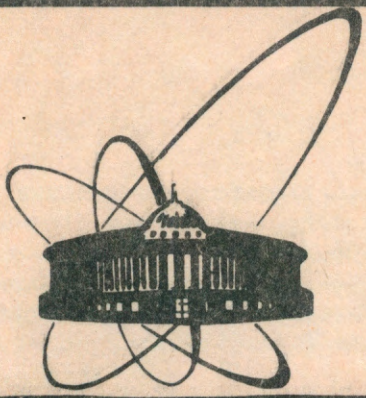

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POSITION OF P-HYDROXYBENZONITRILE IN EGG PHOSPHATIDYLCHOLINE MODEL MEMBRANES AS SEEN BY NEUTRON DIFFRACTION

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INTRODUCTION

Parahydroxybenzonitrile (PHBN) belongs to the group of phenolic herbizides which were described as inhibitory uncouplers [1-3]. The herbizidale action of these compounds is caused by a direct interaction with a chloroplast thylakoid membrane protein associated with photosystem II [4,5] as well as by a perturbation of the lipid membrane induced by the herbicides [6,7].

In order to contribute to the clearing of these problems we started systematic studies using lipid model membranes. It was evidenced by DSC [8] that PHBN has a high affinity to DPPC membranes. The interaction of PHBN with model membranes was studied also by other methods as NMR and FT-IR [9].

The ability of phenolic herbicides to form hydrogen bonds [10] points to an interaction of PHBN with the polar head group of the lipids. On the other hand, a location of PHBN in the hydrophobic core of the membrane should be favored due to the hydrophobicity of the nitrile group and the aromatic moiety. In Ref. [8] information about these interactions and their dependence on the substituents of PHBN in 3,5-position were obtained and from these indirect conclusions about the mean localisation of the herbizides in the membrane were drawn.

In the present paper we directly determined the mean position of PHBN in egg phosphatidylcholine (EPC) membranes applying the powerful and well established method of neutron diffraction. The certain knowledge of its mean position in the membrane reversely allows conclusions on the interactions between PHBN and the lipid molecules.

EPC is an appropriate lipid for our purpose because it forms a fluid lamellar phase within a wide range of water concentrations [11,12] which is not drastically disturbed by PHBN [9].

MATERIALS AND METHODS

EPC was separated from hen eggs according to the method of Singleton [13]. PHBN was synthesized by SYS Schwarzheide,

GDR. The deuterated compound, PHBN-d_4, was obtained from Isocommerz, Berlin, GDR.

PHBN or PHBN-d_4 and EPC in molar ratios $R_{A/L} = 0.4$ were dissolved in ethanol (5 wt% EPC). About 0.2 ml of this solution were spread on a polished quartz slide of (24*24) mm^2. The solvent was evaporated under vacuum (4.66 Pa) at a temperature of 30 °C as described in [13]. A multilamellar film was obtained which was composed of about 5000 bilayers. The mosaic spread of the bilayer structure was about 1°.

The experimental conditions and the procedure for obtaining neutron diffractograms as well as their theoretical analysis are described elsewere [15]. The measurements were performed at 21 °C at the relative humidity of RH = 85% using a saturated water solution of potassium chloride. Diffraction peaks up to the fifth order were observed and used for the analysis.

The distribution of the deuterium labels was approximated according to the procedure of Büldt et al.[16] by one Gaussian. The mean label position x_0 and the distribution width ν were determined by a least square fit of:

$$\sum_h w_h \left[F^M_{DL}(h) - F^E_{DL}(h) \right]^2 , \qquad (1)$$

where

$$F^M_{DL}(h) = \int_{-\infty}^{+\infty} \rho_{DL}(x) \cos\left(\frac{2\pi h x}{d}\right) dx = 2t_N \exp\left\{-\left(\frac{\nu\pi h}{d}\right)^2\right\} \cos\left(\frac{2\pi h x_0}{d}\right) , \qquad (2)$$

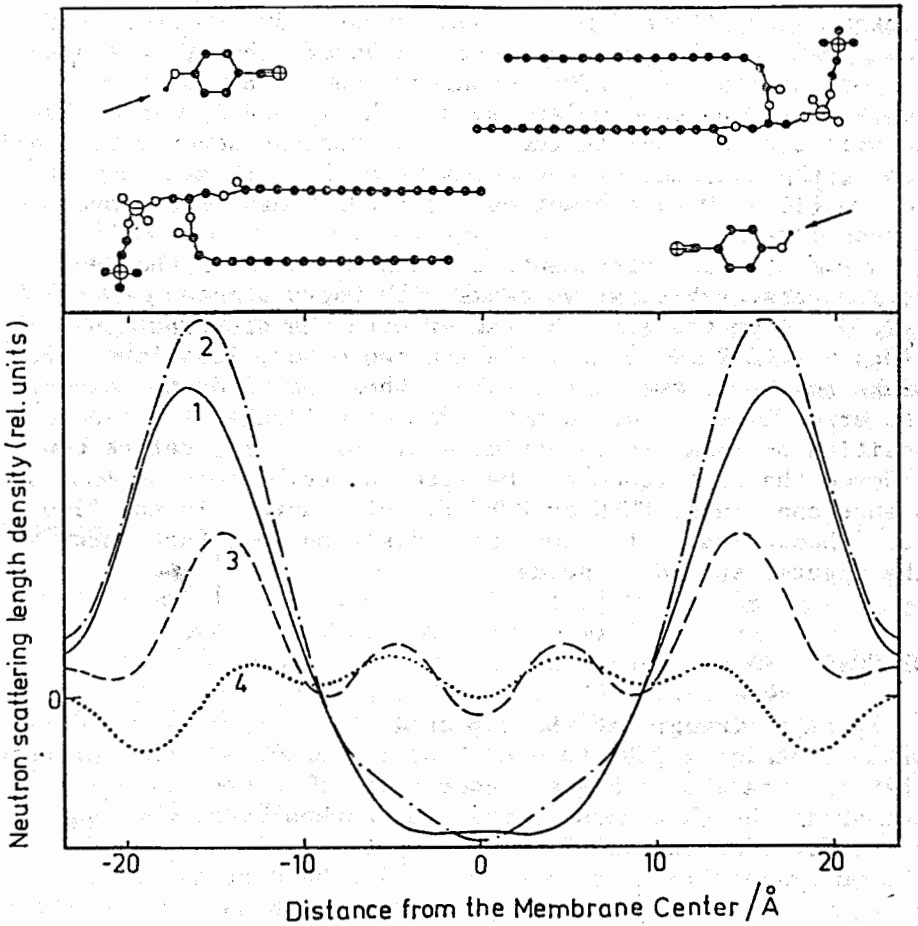
and

$$F^E_{DL}(h) = F^E_D(h) - F^E_H(h) \qquad (3)$$

are the adjustable and experimental structure factors, respectively. F^E_D and F^E_H denote the structure factors determined experimentally with PHBN-d_4 and PHBN, respectively, h is the order of the structure factor; w_h, a weighing factor and t_N plays the role of a calibration factor, $\rho_{DL}(x)$ is the scattering density of the deuterium labels in real space.

RESULTS

The structure factors obtained at different isotopic compositions of the saturated salt solutions (0, 50, and 100% ^2H_2O, respectively) depend linearly on the content of heavy



Neutron scattering density profiles across EPC bilayers containing PHBN ($R_{A/L}$=0.4) hydrated at RH=85% and measuring at T=21 °C. Curve 1 - bilayer containing PHBN, curve 2 - bilayer containing PHBN-d_4, curve 3 - deuterium label distribution (difference of curves 2 and 1), curve 4 - error level (see text). Curves 3 and 4 were vertically scaled by a factor of 2. A sketch of the mean position of PHBN is shown above. The following symbols were used: ● - carbon, O - oxygen, ⊕ - nitrogen and O - phosphorus atoms. With the exception of the hydroxyl group of PHBN(denoted by arrows) hydrogen atoms were omitted for the sake of lucidity.

water. This confirms the centrosymmetric structure of the system as expected for a lamellar phase and evidenced also by NMR (unpublished results).

In the figure the scattering density profiles across the membranes obtained by the Fourier- and Fourier difference

synthesis are shown. In the case of pure EPC membranes the main peaks of the bilayers profile locate the ester linkages of the lipid [17]. This is also true for EPC membranes containing PHBN in a molar ratio of $R_{A/L} = 0.4$, since the contribution of PHBN to the total scattering density is small. Hence, the distance of the peaks from the membranes center $\ell_{hc} = \pm(16.5 \pm 0.5)$ Å gives half the thickness of the hydrocarbon core.

Curve 3, which represents the distribution of the deuterium labels, exhibits two peaks with the distance $\ell_A = \pm(17.5 \pm 0.5)$ Å from the membrane center with the distribution width $\nu = (3.8 \pm 0.2)$ Å. There are two other, less intensive peaks nearer to the center. Since these peaks do not exceed the error level, they do not give the evidence of a second position of PHBN. As an estimate of the error level we considered the difference of the water distributions of membranes containing PHBN or PHBN-d_4 (cf. curve 4 in the figure). Theoretically the two water distributions (not shown in the figure) should coincide.

DISCUSSION

It is striking that the repeat distance of the EPC membrane containing PHBN (d = 47.2 Å) is about 4 Å smaller than that of pure EPC membranes under comparable conditions [18]. Comparing the thickness of the hydrocarbon core, the ℓ_{hc} value for EPC/PHBN (16.5 Å) is slightly smaller than that of EPC membranes (18 Å at RH = 66% [17]). The decrease of the membrane thickness seems to be a general phenomenon if additives are incorporated in the polar region of lipid membranes. It was also observed for amiodarone [19] as well as for various phosphonic acid diesters [15] incorporated in EPC. The thinning of the membranes is mainly caused by a larger disorder and/or a partial interdigitation of the lipid hydrocarbon chains. On the contrary, molecules which locate between opposing monolayers of the bilayers cause an increase of the membrane thickness [20].

The aromatic moiety of PHBN is located in the mean below the ester linkages of EPC with respect to the membrane center (cf. the figure). Since PHBN has roughly a rod-like shape, it should be partially oriented with its symmtery axis in parallel to the director of the membrane. This assumption is supported by deuterium NMR experiments using PHBN-d_4 [9]. Direct information about the direction of the PHBN molecules in the membrane cannot be gained. However,

taking into account the hydrophobicity of the aromatic moiety and the polarity of the hydroxyl group of PHBN on the basis of the neutron diffraction results it can be concluded that the nitrile group of PHBN is deeply incorporated in the hydrophobic core pointing to the membrane center. Hence, the hydroxyl group of PHBN must be located near the phosphate groups of the lipid and the formation of hydrogen bonds between PHBN and the phosphate group is very likely. The idea of such a hydrogen bond is supported by the observation that the deuterium quadrupole splitting of heavy water is reduced by the incorporation of PHBN into EPC membranes (unpublished data) which is an indication of the decreased number of water binding sites. Similar conclusions were drawn by Boden et al. [21] concerning the interactions of benzyl alcohol with model membranes.

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