of Connecticut, toward the purchase of the computerized fluorescence spectrometer and the UV-vis diode array spectrometer used in this work. Competent technical assistance by Claudia Beretta is also acknowledged. Gratitude is extended to Dr. H. Sun and Prof. J. Bobbitt of the Chemistry Department for making model compounds. We also thank Dr. G. Hagnaur and D. Dunn of AMMRC for the gift of purified DGEBA and their generous help in the fractionation of the model compounds by HPLC. We are happy to acknowledge the kind help by Prof. H. Morawetz and Prof. N. H. Sung on the manuscript.

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(12) Present address: IBM, Est Fishkill, Hopewell Junction, NY 12533.

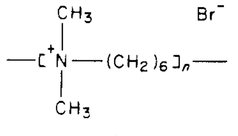
Chong Sook Paik Sung,* In-Joo Chin,[12] and Wei-Ching Yu

Department of Chemistry
Institute of Materials Science
University of Connecticut
Storrs, Connecticut 06268

Received March 29, 1985

Observation of an Interdigitated Gel Phase in Dipalmitoylphosphatidylglycerol Bilayers Treated with Ionene-6,6

We have been interested for some time in modification of phospholipid bilayers by synthetic polymers.[1-3] With molecular dimensions comparable to or greater than the thickness of the lipid bilayer and many times the intermolecular spacing in the bilayer plane, synthetic polymers might be expected to serve as potent modifiers of bilayer structure and function. We have reported recently that synthetic poly(carboxylic acids) behave as "molecular switches" in phosphatidylcholine bilayers, triggering the release of bilayer-entrapped substances in response to small changes in ambient pH.[1,2] We describe herein the observation of an interdigitated gel phase in dipalmitoylphosphatidylglycerol (DPPG) bilayers treated with ionene-6,6 (1).



$$\left[\overset{+}{N}(CH_3)_2-(CH_2)_6\right]_n \quad Br^-$$

1

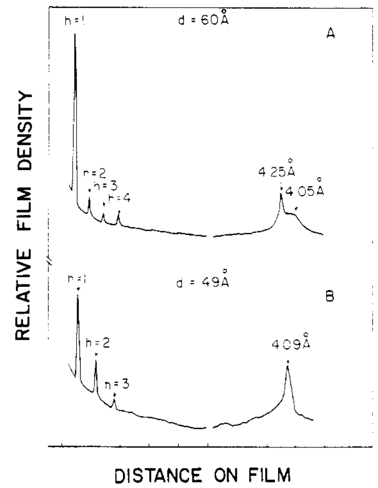


Figure 1. Densitometer traces of X-ray diffraction patterns of DPPG hydrated at a concentration of 1 mg/mL in 50 mM sodium phosphate buffer, pH 7.4 (A), or in similar buffer containing 1 mg/mL of ionene-6,6 (B). In trace A there are four orders of a lamellar repeat period of 60 Å and a double wide-angle reflection at 4.25 and 4.05 Å. In trace B there are three orders of a lamellar repeat period of 49 Å and a single wide-angle reflection at 4.09 Å.

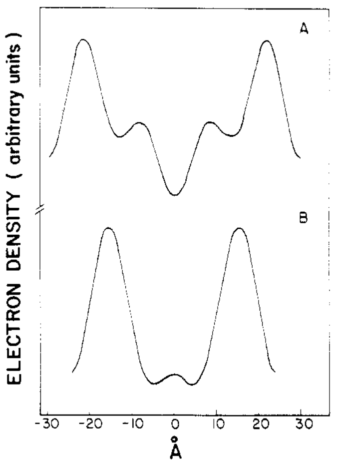


Figure 2. Electron density profiles generated from the diffraction patterns of Figure 1 and the same phase angles as in ref 12 and 13: (A) DPPG; (B) DPPG/ionene-6,6.

Dispersion of DPPG in 50 mM sodium phosphate buffer, pH 7.4, provides a turbid suspension which melts sharply at 39.8 °C, with a smaller pretransition at 32 °C (Microcal MC-1 scanning calorimeter, heating rate 10 °C/h, [DPPG] = 1 mg/mL). These results are in good agreement with those of previous workers.[4-6] In contrast, hydration of the lipid in phosphate buffer which contains 1 mg/mL of ionene-6,6 produces an aggregated suspension which melts at 43.6 °C, with no pretransition. Identical results are obtained if the polymer is added subsequent to the lipid hydration step.

Figure 1 shows densitometer traces of X-ray diffraction patterns recorded at room temperature from DPPG suspensions prepared in phosphate buffer (A) and in the same buffer to which ionene-6,6 was added at a concentration of 1 mg/mL (B). The pattern in Figure 1A is in good agreement with that reported by Watts et al.[7] and reveals a lamellar repeat period of 60 Å. The double wide-angle reflection at 4.25 and 4.05 Å is characteristic of the $L_{\beta'}$ phase, in which the hydrocarbon tails of the lipid are tilted by approximately 30° from the bilayer normal.[7] Addition of ionene-6,6 produces the pattern shown in Figure 1B, with a lamellar repeat period of 49 Å and a single wide-

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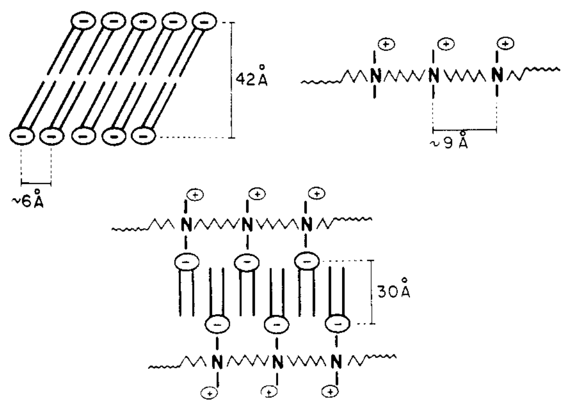
Scheme I



angle reflection at 4.09 Å. Comparison of these features with the diffraction data of previous workers[7-14] identifies this as a lipid phase in which the hydrocarbon chains of apposed monolayers are normal to the bilayer and fully interdigitated.

Acyl chain interdigitation is demonstrated more clearly by a comparison of the electron density profiles calculated from the diffraction patterns in Figure 1. Figure 2A is the profile of the unmodified DPPG bilayer and indicates a phosphate-to-phosphate separation across the bilayer of 42 Å, which is the expected thickness for an $L_{\beta'}$ bilayer of this chain length.[15] Treatment with ionene-6,6 reduces the bilayer thickness to 30 Å, a result which can be reconciled only with the formation of a gel phase in which the hydrocarbon tails are deeply interpenetrated.

Interdigitation has been observed previously in samples of DPPG neutralized with organic counterions.[8-11] Specifically, preparation of the choline, acetylcholine, or polymyxin salts of DPPG affords interdigitated gel phases. McIntosh and co-workers[13,14] have discussed the requirements which must be met by small molecules which induce the interdigitated phase: such molecules must displace water from the interfacial region without penetrating deeply into the bilayer interior.

Thus the observation of an interdigitated phase in ionene-treated DPPG bilayers—the first indication that *polymeric* additives may induce interdigitation—is consistent with previous results concerning the formation of this unusual gel phase. In fact, the picture of the ionene-6,6-treated bilayer as an interdigitated structure is a pleasing one. One imagines that the approach of ionene-6,6 to the bilayer surface is driven by strong electrostatic forces between the negatively charged phosphate groups of the surface and the ammonium cations bound to the polymer chain. But effective ion pairing at the surface would place the hydrophobic polymethylene spacer of ionene-6,6 in juxtaposition with the polar, hydrated diglycerylphosphate portion of the lipid head group. A position in the hydrocarbon core of the bilayer would be energetically preferred for the spacer, but access to the core is precluded by the high energetic cost of burying an ammonium cation. The solution is a lateral expansion of the bilayer, accompanied by deep interpenetration of the hydrocarbon chains of the lipid (Scheme I). This allows terminal methyl groups of the hydrocarbon chains to take interfacial positions, in contact with the hydrophobic polymethylene spacer, while remaining dehydrated as a result of protective "capping" by the polymer chain.

We are currently exploring the relation of polymer chain structure and the induction of acyl chain interdigitation in DPPG and related bilayers. The consequences of interdigitation in terms of bilayer stability and permeability are also under investigation.

Acknowledgment. This work was supported by 3M Co., St. Paul, MN (D.A.T.), and by a Presidential Young Investigator Award of the National Science Foundation (D.A.T.).

Registry No. 1, 29322-35-8; 1, SRU, 31622-88-5; DPPG, 4537-77-3.

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David A. Tirrell*
Department of Polymer Science and Engineering
University of Massachusetts
Amherst, Massachusetts 01003

Anne B. Turek
Department of Chemistry
Carnegie-Mellon University
Pittsburgh, Pennsylvania 15213

D. Allan Wilkinson
Allegheny-Singer Research Institute
Pittsburgh, Pennsylvania 15212

Thomas J. McIntosh
Department of Anatomy
Duke University Medical Center
Durham, North Carolina 27710

Received March 18, 1985

Effect of Ultrasonic Irradiation on Ion Binding by Synthetic Polyelectrolytes Using Terbium(3+) as a Fluorescence Probe[1,2]

Interest in the absorption and fluorescence of rare earth metal ions in solution has been greatly stimulated because of their possible use as lasers. In an effort to obtain stronger fluorescence, two general approaches appear possible. The first involves the addition of an agent to the solution, usually an organic ligand, which will absorb energy and transfer it to the rare earth ion. An alternative method is to enhance the absorption and fluorescence of the rare earth ion by suitable changes in its environment.

The extreme sensitivity of certain lanthanide absorption bands to their solution environment is well documented and the phenomenon is referred to as "hypersensitivity".[3] The hypersensitive absorption is greatly increased by three factors: an increasingly basic character of the coordinating

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