gas phase or on the catalyst surface, which is not molecular oxygen.[2] This mechanism requires (i) an equilibrium of $[O^-]$ with simple alkanes to form the surface species $[R-H--O^-]$, (ii) the value of the appropriate equilibrium constants to be in the order $C_2D_6 > C_2H_6 > CH_4$, (iii) that the CH_3 or C_2H_5 radicals formed by the $[O^-]$ site are the precursors of CO_x, and (iv) that the direct oxidation of CH_4, C_2H_6, C_2H_5, or CH_3 by molecular gas-phase O_2 is not kinetically important under the chosen reaction conditions. In this mechanism, the reduction in the CH_4 conversion on addition of the C_2H_6, and the switch in the origin of the CO_x from the CH_4 to the additive, are initiated by the competition in ii above.

A full analysis of these and other similar results will be presented elsewhere. It appears, however, that the above results could complicate the analysis of isotope replacement experiments which have been interpreted[5] as showing that the rate-determining step of the oxidative coupling reaction was the rupture of the C-H bond of the CH_4 molecule. This conclusion was in contrast to other evidence,[2] which suggested that the rate of lattice oxygen exchange (and hence the formation of the active species $[O^-]$) was rate determining.

(5) (a) Buevskaya, O. V.; Suleimanov, A. I.; Aliev, S. M.; Sokolovskii, V. D. *React. Kinet. Catal. Lett.* **1987**, *33*, 223. (b) Mimms, C. A.; Hall, R. B.; Rose, K. D.; Myers, G. R. *Catal. Lett.* In press. (c) Cant, N. W.; Lukey, C. A.; Nelson, P. F.; Tyler, R. *J. Chem. Soc., Chem. Commun.* **1988**, 766.

Water-Free Self-Assembly of Phospholipid Tubules

Alan S. Rudolph,*,† Jeffrey M. Calvert,‡ Mary E. Ayers,‡ and Joel M. Schnur†

Bio/Molecular Engineering Branch, Code 6190
Naval Research Laboratory
Washington, DC 20375-5000
Geo-Centers, Inc.
10903 Indian Head Highway, Suite 502
Fort Washington, Maryland 20744

Received June 5, 1989

The rationale for synthetic modification of biological lipids, such as lecithins, to include polymerizable groups such as diacetylenes,[1-5] methacrylates, vinyl groups, and thiols[5] is to design and create inherently stable, polymerizable, self-assembling microstructures. The polymerization of these moieties has been shown to alter functional properties of these microstructures including changes in permeability and to enhance stability to extremes in temperature or hydration.[6] The synthetic modification of lecithins has also been found to result in the formation of microstructures with unique morphologies and functional properties.[2,8] One such assembly is the formation of hollow cylinders (or tubules) from the diacetylenic phosphatidylcholines.[7] Tubules are typically 5-150 μm in length, with a 0.5-μm core surrounded by two to seven bilayers.

The sequestering of nonpolar moieties of biological amphiphiles from water is thought to be an important driving force for self-assembly into supramolecular structures.[9] We have discovered

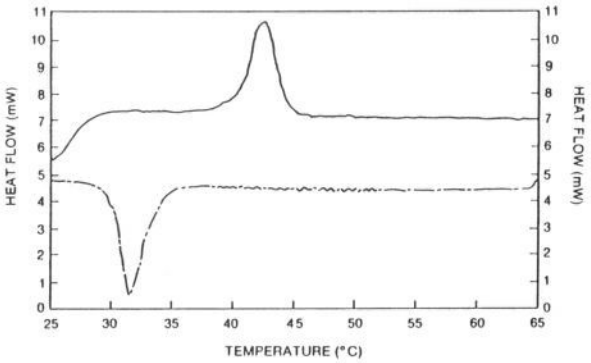


Figure 1. Calorimetric trace of heating and cooling of the diacetylenic phosphatidylcholine, 1,2-di-10,12-tricosadiynoyl-*sn*-glycero-3-phosphocholine in acetonitrile. The transition temperature on heating was observed at 42.5 °C upon heating with an enthalpy of 17.8 kcal/mol. Cooling revealed on exothermic event at 31.5 °C with an enthalpy of 18.9 kcal/mol. All experiments were performed at a scan rate of 1 °C/min and sample weights determined by gravimetric analysis before and after loading of a pan with the solution of lipid and acetonitrile in a glove box purged with dry nitrogen.

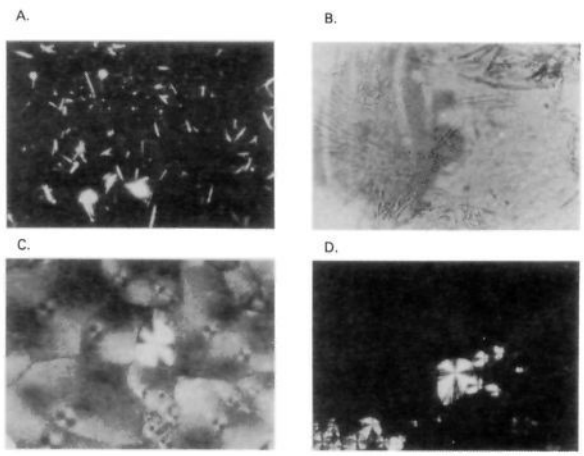


Figure 2. (a) Dark-field optical micrograph of tubules formed in acetonitrile taken from the DSC pan. Note the pieces of incompletely formed tubules and material that appears nontubular. Magnification = 125×. (B) Light-field optical micrograph of longer tubules (30–50 μm) formed on the thermal stage in acetonitrile. Magnification = 125×. (C) Light-field optical micrograph under cross polarization of spherulites and focal domains of $DC_{8,9}PC$ in dry acetonitrile. (d) Dark-field micrograph of spherulites formed in the DSC pan. Magnification = 125×. In these experiments, dry lipid was dissolved in dry acetonitrile at 50 °C and vortexed. An aliquot of this solution was then pipetted onto a glass slide and sealed with a glass coverslip.

that the self-assembly of tubules does not depend on the presence of bulk water. The inherent self-assembly of this lipid into lamellar structures in the absence of bulk water is also manifest by the observation of spherulitic structures (with uniaxial symmetry). The observation that self-assembly of this lecithin will occur in the absence of water may have important technological implications in the development of these microstructures for use in organic solvents and under controlled environmental conditions.

The polymerizable phosphatidylcholine we have studied, 1,2-di-10,12-tricosadiynoyl-*sn*-glycero-3-phosphocholine, has two diacetylenic groups at the C10 and C12 positions along the fatty acyl chains.[3] The diacetylenes will polymerize when exposed to UV light, resulting in a polymer with variable conjugation length.[3] Previous spectroscopic and calorimetric studies led us to believe that bulk water may not be required for the formation of tubules.[7,11-13] We therefore chose acetonitrile for these studies, an

* Address all correspondence to Dr. Alan S. Rudolph.
† Naval Research Laboratory.
‡ Geo-Centers, Inc.

(1) Smith, W. N.; Beumel, O. F., Jr. *Synthesis* **1974**, *24*, 441.
(2) Singh, A.; Schnur, J. M. S. *Synth. Commun.* **1986**, *16*, 847-852.
(3) Benz, R.; Prab, W.; Ringsdorf, H. *Eur. Biophys. J.* **1986**, *14*, 83-84.
(4) Regen, S. L.; Czech, B.; Singh, A. *J. Am. Chem. Soc.* **1980**, *102*, 6638.
(5) Lopez, E.; O'Brien, D. F.; Whitesides, T. H. *J. Am. Chem. Soc.* **1982**, *104*, 305-307.
(6) Johnston, D. S.; Mclean, L. R.; Whitam, M. A.; Clark, A. D.; Chapman, D. Biochemistry **1983** *22*, 3194-3202.
(7) Yager, P.; Schoen, P. *Mol. Cryst. Liq. Cryst.* **1984**, *106*, 371-381.
(8) Nakashima, N.; Asakuma, S.; Kunitake, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 509-510.
(9) Tanford, C. *The Hydrophobic Effect*; John Wiley and Sons, Inc.: New York, 1981; pp 371-381.
(10) Gray, G. W.; Goodby, J. W. *Smectic Liquid Crystals*; Leonard Hill: Philadelphia 1984; pp 9-17.

organic solvent with a moderately high dielectric. Some care was taken to remove trace water from acetonitrile by refluxing with phosphorus pentoxide for 1-1.5 h at 82 °C. The thermodynamics of tubule formation in dry acetonitrile was investigated by using differential scanning calorimetry (DSC). Following thermal cycling of the acetonitrile-lipid suspension, the DSC pan was opened at room temperature and the sample examined with light microscopy. Parallel experiments were performed in a temperature bath and on a temperature controlled microscope stage.

Figure 1 shows the calorimetric result of heating and cooling the lipid-acetonitrile suspension. A transition temperature of 42.5 °C is observed upon heating with an enthalpy of 17.8 kcal/mol. Cooling scans reveal a transition at 31.5 °C with an enthalpy of 18.9 kcal/mol. The enthalpy values for these same transitions previously examined in water depend somewhat on the thermal history of the sample[7,11,12] but generally are 3-5 kcal/mol higher than those observed in this study. Parts A and B of Figure 2 show light micrographs of tubules formed in the DSC pan and on the thermal stage of the microscope, respectively. These tubules have similar macroscopic dimensions to those tubules formed in water (10-50 μm in length, 0.5μm in diameter). The conversion to tubules is not complete, with some precipitated lipid present in a nontubular form, which may account for the difference in enthalpy values observed in dry acetonitrile and water. Spherulites and focal conic domains also appear under cross polarization in the samples cooled on the thermal stage and from the DSC pan (Figure 2C,D). The spherulites show a characteristic pattern (maltese cross) under cross polarization, which may be similar to the patterns commonly observed in the smectic phase of other liquid crystals.[10] The observed pattern in the spherulites may indicate that the crystalline form may have uniaxial symmetry. The observation of spherulites and focal conic domains reveals the tendency of this lipid to self-assemble into lamellar structures in the absence of water. The spherulites may be similar to a low-temperature phase observed in the diacetylenic lecithins in water which appears in freeze fracture replicas as stacked lamellar sheets.[11]

Although we have taken great care to minimize the amount of water present in these experiments, it is possible that some residual water remains (e.g., the water of hydration of the lipid) and that the dry lipid itself is not completely anhydrous. It is significant, however, that self-assembly of tubules occurs under these dramatically reduced water conditions. The two previously reported methods of tubule formation rely on the presence of bulk water.[8,12,13] One of these methods involves the isothermal precipitation of tubules from ethanol-water solution.[14] Spectroscopic characterization and X-ray diffraction analysis of tubules reveal the presence of highly crystalline acyl chains.[14,15] The crystalline nature of the tubules and the observations presented in this work suggest that bulk water may not play a major role in the self-assembly of tubules. This is in contrast to the major role water does have in the formation of other self-assemblies formed from biologically derived amphiphiles. It may be that the dielectric of acetonitrile is sufficient to drive the assembly of this chemically modified phospholipid. We are continuing our examination of tubule formation and what conditions govern the formation of the spherulites to further understand forces that drive the self-assembly of this lipid.

Acknowledgment. We thank the Defense Advanced Projects Research Agency for partial support of this work. Many discussions with Professor R. Shashidhar (Georgetown University, Department of Chemistry) are gratefully acknowledged.

(11) Rudolph, A. S.; Burke, T. G. *Biochim. Biophys. Acta* **1987**, *902*, 347-359.
(12) Burke, T. G.; Rudolph, A. S.; Price, R. P.; Singh, A. S.; Singh, B. P.; Schoen, P. E. *Chem. Phys. Lipids*, in press.
(13) Georger, J. H.; Singh, A.; Price, R. P.; Schnur, J. M.; Yager, P.; Schoen, P. E. *J. Am. Chem. Soc.* **1987**, *109*, 6169.
(14) Rhodes, D. G.; Bechner, S. L.; Yager, P.; Schoen, P. S. *Chem. Phys. Lipids* **1988**, *49*, 39-47.
(15) Sheridan, J. S. Naval Research Laboratory Memorandum Report, No. 5975; 1988.

Efficient, Specific Cross-Linking and Cleavage of DNA by Stable, Synthetic Complementary Oligodeoxynucleotides[1]

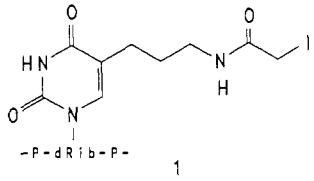
Rich B. Meyer, Jr.,* John C. Tabone, Gerald D. Hurst, Todd M. Smith, and Howard Gamper

MicroProbe Corporation, 1725 220th Street SE Bothell, Washington 98021

Received June 2, 1989

"Antisense" oligodeoxynucleotides (ODNs) have generated significant recent interest as potential chemotherapeutic agents.[2] Despite theoretical appeal, the potency necessary for animal or clinical trials has not yet been shown. We have been investigating the attachment of a side chain, bearing an alkylating electrophile, to ODNs as a method of achieving irreversible inactivation of the complementary target nucleic acid.[3] These ODNs can, as shown here, function as exceptionally selective cleavage reagents for DNA.

In their pioneering work, Brookes and Lawley[4] proposed that a five-atom interstrand cross-link was formed by bis(2-chloroethyl)amines between the N-7 of guanines in adjacent base pairs of GC sequences. Using molecular models of B form DNA, we found that a five- or six-atom arm should diagonally bridge the major groove from the 5-position of a pyrimidine in an ODN to the N-7 of a purine paired to the residue on the 3′-side of the pyrimidine bearing the arm.



1

To test our model, we prepared a tetradecadeoxynucleotide (ODN-I), incorporating 5-[3-(iodoacetamido)propyl]-2′-deoxyuridine (**1**) in place of a T, and a 30-mer containing the sequence complementary to ODN-I using a sequence derived from human papillomavirus (HPV) type 16, as shown below. Nucleoside **1** has the requisite six-atom chain to bridge the major groove on to the nucleophilic guanine (G-21) in the target strand.

```
                    5    10   15   20   25   30
                    |    |    |    |    |    |
Target:    5'-AGA CAG CAC AGA ATT CGC AGG AAC ATC CAG-3'
ODN-I:                          3'-CG TCC ITG TAG GTC-5'
```

5-[3-(Trifluoroacetamido)propyl]-2′-deoxyuridine[5] was converted to the 5′-*O*-dimethoxytrityl-3′-(cyanoethyl *N*,*N*-diisopropylphosphoramidite) derivative by standard methods[6] for direct use on an automated DNA synthesizer.[7] The deprotected ODN[8]

(1) This work was supported by Grants AI25959, CA40336, and CA45905 from the National Institutes of Health.
(2) (a) Zon, G. *Pharm. Res.* **1988**, *5*, 539. (b) Miller, P. S.; T'so, P. O. P. *Adv. Med. Chem.* **1988**, *23*, 295. (c) Cohen, J.; Stein, C. *Cancer Res.* **1988**, *48*, 2659-2668.
(3) For examples of cross-linking and related interstrand alkylation reactions, see: (a) Summerton, J.; Bartlett, P. A. *J. Mol. Biol.* **1978**, *122*, 145. (b) Webb, T. R.; Matteucci, M. D. *Nucleic Acids Res.* **1986**, *14*, 7661. (c) Vlassov, V. V.; Zarytova, V. F.; Kutyavin, I. V.; Mamaev, S. V. *FEBS Lett.* **1988**, *231*, 352-354. (d) Fedorova, O. S.; Knorre, D. G.; Podust, L., M.; Zarytova, V. F. *FEBS Lett.* **1988**, *228*, 273-276. (e) Iverson, B. L.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4615-4619. (f) Gamper, H. B.; Cimino, G. D.; Hearst, J. E. *J. Mol. Biol.* **1987**, *197*, 349-362. (g) Lee, B. L.; Murakami, A.; Blake, K. R.; Lin, S.-B.; Miller, P. S. *Biochemistry* **1988**, *27*, 3197-3203. (h) Pieles, U.; Englisch, U. *Nucleic Acids Res.* **1989**, *17*, 285-299.
(4) Brookes, P.; Lawley, P. D. *Biochem. J.* **1961**, *80*, 486.
(5) Ruth, J. L. European Patent Publication No. WO 84/03285, 1984. For an alternate method, see: Benkovic, S. J.; Gibson, K. *Nucleic Acids Res.* **1987**, *15*, 6455-6467. Hobbs, F. W., Jr. *J. Org. Chem.* **1989**, *54*, 3420-3422.
(6) Gait, M. J., Ed. *Oligonucleotide Synthesis a Practical Approach*; IRL Press: Oxford, 1984.
(7) An ABI 380B DNA synthesizer was used.
(8) After removal of the oligonucleotide blocking groups by ammonolysis, the ODN was purified by reverse-phase HPLC and detritylated with 80% acetic acid. Desalting was accomplished on a G-25 Sephadex column, followed by concentration.

0002-7863/89/1511-8517$01.50/0