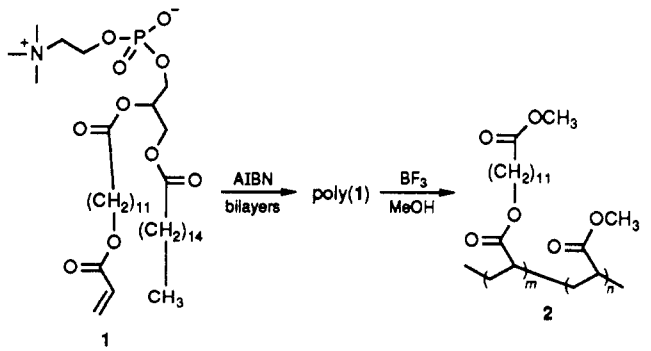
Degree of Polymerization in Two-Dimensional Assemblies

The last decade has seen the introduction of several methods to polymerize supramolecular assemblies (see ref 1-5 for reviews). These assemblies, e.g., monolayers, vesicles (liposomes), extended bilayers, cast multilayers, BLMs, and tubules, are each two-dimensional arrays of amphiphiles. A host of reactive moieties can be utilized to modify lipids to make them polymerizable, e.g., diacetylene, methacryloyl, dienoyl, sorbyl, styryl, vinyl, thiol, and lipoyl. Polymerizable groups may be incorporated into one or both of the hydrophobic tails near the middle or at the end of the chain(s). Reactive groups may also be attached to the hydrophilic head group or electrostatically associated with a charged lipid. These new and evolving chemistries open opportunities to prepare new molecules and assemblies with particular properties of interest to both biological and material sciences.

Success in these endeavors requires a clear understanding of the processes used to modify the assemblies; however, little effort has been devoted to characterization of the polymerization process in two dimensions. Preliminary molecular weight data are available for some vesicle and extended bilayer systems, e.g., the degree of polymerization of linear polymers from methacryloyl lipids was about 500,[6,7] from styrene-substituted lipids was 10-20,[8] and from thiol-substituted lipids was 17-25.[9] These scattered studies do not reveal which factors control the polymer size in two-dimensional polymerizations. The lack of systematic studies has made it difficult to clearly define the fundamental principles that underlie these important new polymerizations. Accordingly we have examined the variables that control the size of polymers formed in supramolecular assemblies. This first report of these studies describes the relationship between initiator concentration and the polymer size for acryloyl-substituted lipids.

A monosubstituted acryloylphosphatidylcholine was prepared for these experiments. Phosphatidylcholines (PC) are well-known for their ability to form hydrated bilayer assemblies. The polymerization of mono- and bis-substituted PCs yields linear and cross-linked polymers, respectively. The monoacryloylphosphatidylcholine (1) was synthesized by acylation of L-α-lysopalmitoylphosphatidylcholine with the anhydride of 1-acryloyloxy-12-dodecanoic acid. This fatty acid was formed by oxidation of 1-acryloyloxy-12-dodecanol, which is the product of the stoichiometric reaction of acryloyl chloride with 1,12-dodecanediol.



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A hydrated sample of 1 is milky and shows a phase-transition temperature (T_m) at 31.7 °C (ΔH = 8.9 kcal/mol). This cooperative lipid behavior is similar to that normally observed for other hydrated PC bilayers. Ultrasonication or extrusion of the hydrated bilayers of 1 at temperatures > T_m converts the sample into a translucent suspension of vesicles.

Purified lipid stock solution was combined with the desired amount of AIBN stock solution and then taken to dryness. The lipid film was hydrated with MilliQ water to form extended bilayers, which were flushed with argon and then sealed with a rubber septum. The samples were heated at 70 °C for 18 h (~4 AIBN half-lives) and then cooled and freeze-dried. The dried polymer products were poorly soluble in the standard solvents for GPC analysis; therefore, the lipid polymer was cleaved by BF_3 in MeOH/benzene. This methodology removes the lipid head group, glycerol backbone, and most of the connecting acyl chains from the poly(acryloylphosphatidylcholine) to yield a copolymer (2) of methyl acrylate and methylcarboxyundecyl acrylate. The compositions of the copolymers were determined by ^1H NMR spectroscopy (data not shown). This cleavage procedure not only is applicable to linear polylipids but also will be useful for the cleavage of cross-linked polymers formed from bisacryloylphosphatidylcholine. The removal of the glycerol backbone breaks the covalent links between polymer chains. A report on the cross-linked lipids will be forthcoming.

The molecular weights were estimated by GPC calibrated from the number-average molecular weight of poly(methyl methacrylate) standards of narrow polydispersity. Each polymerization was repeated at least twice, the BF_3 cleavage on each polymer sample was performed at least twice, and the GPC analysis of each copolymer was done in triplicate. The dependence of the apparent molecular weights on the copolymer distribution (value of n) introduces an error that was no greater than the observed variation between polymer samples. The relative molecular weights were used to calculate a relative number-average degree of polymerization, X_n. When the monomer to initiator molar ratio, [M]/[I], was varied from 5 to 40, holding [M] constant, the X_n increased from about 200 to nearly 2000. The experimental data for the X_n of poly(1) is shown vs $[I]^{-1}$ (Figure 1). The correlation coefficient for the linear relationship is 0.983, whereas it is less than 0.92 for X_n vs $[I]^{-0.5}$. Although there is some scatter in the experimental data, they indicate that the X_n is inversely proportional to the first power of the initiator concentration.

Thermally initiated free-radical polymerizations in solution have a kinetic chain length, ν, which is usually proportional to the first power of [M] and inversely dependent on the square root of [I] according to eq 1, where

$$\nu = k_p[M]/2(fk_dk_t[I])^{0.5} \quad (1)$$

k_p is the rate constant for propagation, k_d is the rate constant for initiator homolysis, k_t is the rate constant for termination, and f is the initiator efficiency.[10] Relationship 1 is a consequence of the thermal generation of two radical fragments, R$^\bullet$, from each initiator molecule, and the termination of the growing radical chains by coupling and/or disproportionation of two chains with one another. The X_n either is equal to the kinetic chain length when two living polymer chains terminate by disproportionation or is twice the kinetic chain length when they terminate by coupling. Both coupling and disproportionation are diffusion controlled and require pairing of the electron spins. Many polymer radical chains, including those from acryloyl monomers, preferentially terminate by coupling.[11]

The experimental data for the X_n of poly(1) formed in bilayer assemblies clearly do not conform to the well-known solution behavior described above but rather they

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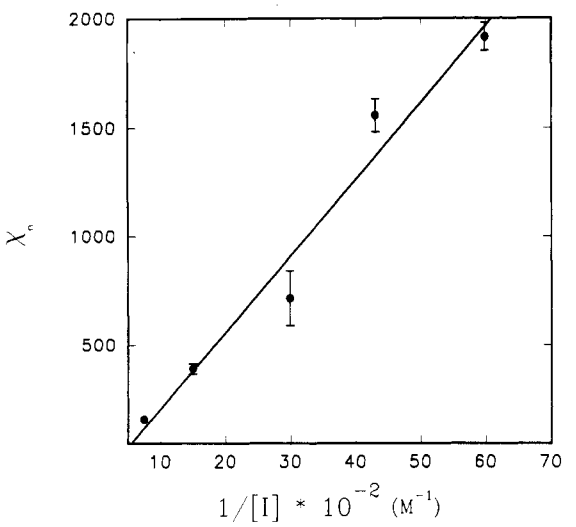

Figure 1. Relative values of number-average degree of polymerization, X_n, of poly(1) vs the reciprocal of the molar AIBN concentration: reaction temperature, 70 °C.

fit a relationship for $X_n \propto [M]^n/[I]$. These data suggest an alternate mode of termination is important. Deviations from the normal bimolecular chain-termination model for polymer chain growth are found at high initiator to monomer ratios or at high viscosity. Under these conditions the primary radicals, R•, formed from the initiator may react with the growing polymer chain and provide a third route to polymer chain termination, i.e., primary termination, which has a rate constant, k_{tp}. The rate of thermal polymerization when primary termination dominates is given by eq 2. The corresponding relationship

$$R_p = k_p k_i [M]^2/k_{tp} \quad (2)$$

for X_n is given in eq 3. Primary termination could be a

$$X_n = k_p k_i [M]^2/2fk_d k_{tp}[I] \quad (3)$$

consequence of high initiator concentration or low diffusional behavior of the polymer chains in the bilayer. Since a linear dependence on $[I]^{-1}$ was observed over the complete concentration range investigated, it is likely that the observed effects are due to reduced mobility of the polymer chains in the constrained environment of the bilayer interior. The translational mobility and/or the segmental mobility of the growing polymer chains may be decreased by the bilayer. These circumstances are expected to increase the lifetime of the polymer chains, thereby allowing the smaller primary radical fragments sufficient time to diffuse through the bilayer and terminate the growing polymer chain.

We have also probed the relationship between [M] and X_n. Since the critical micelle concentrations of the monomeric lipids are low ($<10^{-6}$ M), only the lipids in the bilayer contribute significantly to [M]. Therefore, it is necessary to add a second (or additional) lipid(s) to the monomeric lipid in order to vary [M]. When the mole fraction of monoacryloylphosphatidylcholine is progressively reduced by addition of the saturated dimyristoylphosphatidylcholine (DMPC), the X_n was substantially reduced. The initial data suggest that X_n is proportional to $[M]^2$, but further experimental confirmation (in progress) is desirable. DMPC was chosen as the colipid because it is likely to form ideal mixtures with monoacryloylphosphatidylcholine. This supposition was based on the similar chain lengths of the two lipids and is supported by our differential scanning calorimetry measurements of an equimolar mixture of the two lipids, which shows one transition at a temperature between the respective phase-transition temperatures of the pure lipids.

These results on the polymerization of monoacryloylphosphatidylcholine provide the first evidence that the growing polymer chains in the lipid bilayers are preferentially terminated by reaction with initiator fragments. This appears to occur even though there are tens of polymer chains per bilayer.[12] The data suggest that when polymer chains have a large kinetic chain length, the motion of the growing polymer chains is constrained in the bilayer and that the probability of interaction between the chain ends is minimal. What constitutes a large kinetic chain length in two-dimensional polymerizations is of course a subject of current experimental interest and will be described in the future. These findings clearly demonstrate that careful attention to the ratio of [M]/[I] is necessary to reproducibly control the size and properties of polymers formed in bilayers and other supramolecular assemblies. Furthermore, they provide a fascinating glimpse at the nature of two-dimensional polymerizations.

Acknowledgment. This research was supported by a grant from the Division of Materials Research of the National Science Foundation.

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(12) The lipid bilayers used in these studies are greater than 250 nm in diameter. Consequently the number of monomers per lipid vesicle is greater than 5×10^5. Thus if the X_n is 2×10^3, there are more than 10^2 polymer chains per vesicle.

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Received August 15, 1990
Revised Manuscript Received November 15, 1990