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# Notes

## Primary Termination of Radical Chain Polymerizations in Bilayer Membranes

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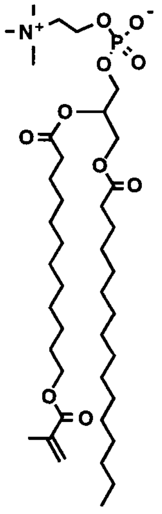
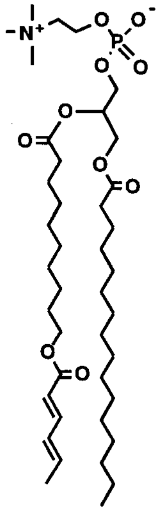
### Introduction

The radical chain polymerization of hydrated lipid assemblies can yield quite large polymer chains depending on the mole ratio of the monomeric lipid to initiator.[1,2] Systematic investigation of these reactions found that at high conversion the number-average degree of polymerization, $X_n$, was proportional to $[M]^2$ and $[I]^{-1}$, where [M] and [I] were the initial monomer and initiator concentrations, respectively.[3−5] The data indicate that the termination of radical chain polymerizations in organized media is likely to involve reaction of initiator fragments with the growing end of the polymer chains, i.e., primary termination or so-called head addition. Here we report a further test of this hypothesis by the use of a $^{13}$C-enriched initiator to label the polymers formed during the bilayer polymerization of monomethacryloylphosphatidylcholine (MethPC) as well as monosorbylPC (SorbPC). Tail addition of the initiator to a monosubstituted vinyl monomer was expected to produce a magnetic environment that is distinct from that created by head addition of the initiator fragment to the growing polymer chain.



**MethPC**



**SorbPC**

Various $^{13}$C-enriched azo initiators have been used to examine the structure of end groups in polymers and copolymers,[6] the reactivity of monomers in copolymerizations,[7] and the tacticity of the repeat units adjacent to the end of polymer chains. The use of AIBN as well as several related azo initiators containing enriched carbons was reviewed by Bevington.[6] These labeled initiators include $^{13}CH_3$-*β*,*β*-AIBN,[6,8,9] $^{13}$CN-AIBN,[6,10] and $^{13}$C-α-AIBN.[11] The mode of termination was inferred from the $^{13}$C NMR of polymers with labeled end groups resulting from the polymerization of methyl methacrylate (MMA), vinyl acetate (VAc), and vinyl formate (VF). Both poly-VAc and poly-VF showed $^{13}$C signals that indicated both head and tail addition of the initiator fragment to the growing polymer chain, whereas the MMA polymerization proceeded without head addition.

### Experimental Section

**Lipid Polymerization**. Both dimyristoylphosphatidylcholine (DMPC) (45.0 mg, 0.064 mmol) and MethPC (49.1 mg, 0.065 mmol) were dried from individual stock solutions and combined together in $CHCl_3$, followed by the addition of $^{13}$CN-AIBN initiator (4.3 mg, 0.0262; M/I = 4.9) from a benzene stock solution. Solvent was evaporated with a stream of argon followed by 6 h under high vacuum in the dark to ensure complete removal of organic solvent. The dried lipid film was hydrated with 20 mL of degassed Milli-Q water, warmed above the phase transition, and vortexed to uniformity (40 s), followed by cooling in an ice bath for 30 s. The above freeze−thaw cycle was repeated a total of three times while purging the sample with argon before the first and after the last cycle. Polymerization was carried out for 72 h at 60 °C under argon.

SorbPC was also polymerized by initiation with $^{13}$CN-AIBN using similar reaction conditions and isolation methods as those used for MethPC.

**Molecular Weight Determination.** After polymerization the sample was lyophilized to remove water. The unreactive DMPC could be extracted from the sample with $CHCl_3$, because the polylipid was insoluble in $CHCl_3$. The polymer solubility was increased by transesterification of the lipid headgroup with MeOH−HCl (5%) (1 mL of acetyl chloride/10 mL of anhydrous methanol) as previously described.[5] The polymer molecular weight (relative to PMMA) was determined in triplicate by size exclusion permeation chromatography. The number-average degree of polymerization was 697 ± 45 and 134 ± 10 for poly-MethPC and poly-SorbPC, respectively.

**Model Compound Synthesis**. AIBN (7.2 g, 44 mmol) and methyl-2,2′-azobis(isobutyrate) (10.2 g, 44 mmol) were dissolved in 75 mL of distilled toluene (purged with argon) and refluxed at 110 °C for 3 h until the evolution of $N_2$ ceased. The reaction mixture was concentrated under vacuum to an oily precipitate. The mixture was taken up in ethanol (60 mL), heated to dissolve the precipitate, and stored in the freezer for 20 h to form a crystalline precipitate, which was filtered and dried. The solid, tetramethylsuccinonitrile, was identified by mp 167−168 °C (lit. 167 °C). $^1$H NMR ($CDCl_3$) 1.52 ppm (singlet, $CH_3$); $^{13}$C NMR 23.10 ppm (−$CH_3$), 39.09 ppm (C), 121.26 ppm (−CN). MS: *m/e* 137 $C_8H_{12}N_2^+$ (M + 1), *m/e* 110 $C_7H_{11}N^+$ (loss of HCN (27)), *m/e* 83 $C_6H_{10}^+$ (loss of 2 HCN (54)). The filtrate was reduced under vacuum to an oil and separated by flash silica gel chromatography with a hexane/ethyl acetate gradient. The first fractions contained dimethyl tetramethylsuccinoate. Subsequent fractions contained methyl 2,2,3-trimethyl-3-cyano-butanoate: $R_f$ 0.65 (7:3 Hex/EtOAc). $^1$H

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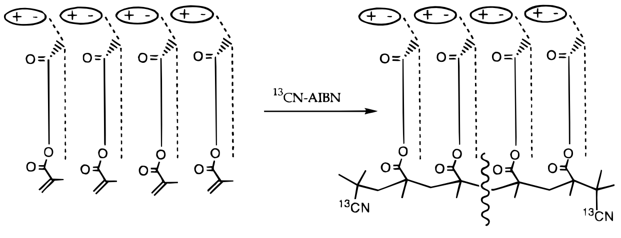
NMR 3.66 ppm of $OCH_3$ (s), 1.32 ppm of $H_3C-C-CO_2$ (s), 1.28 ppm of $H_3C-C-CN$ (s). $^{13}C$ NMR 174.59 ppm of $\mathit{C}O_2CH_3$, 123.77 ppm of $-CN$, 52.01 ppm of $-OCH_3$, 46.92 ppm of $-\mathit{C}-CN$, 38.74 ppm of $-\mathit{C}-CO_2CH_3$, 23.16 ppm of $(\mathit{C}H_3)_2-C-CN$, 21.66 ppm of $(\mathit{C}H_3)_2-C-CO_2CH_3$. MS: *m/e* 170.2 $C_9H_{15}NO_2^+$ (M + 1), *m/e* 144 $C_8H_{15}O_2^+$ (loss of $-CN$ (26)), *m/e* 110 $C_7H_{12}N^+$ (loss of $CO_2CH_3$ (59)), *m/e* 101 $C_5H_9O_2^+$ (loss of $(H_3C)_2-C-CN$ (68)), *m/e* 83 $C_6H_{11}^+$ (loss of HCN (27) and $CO_2CH_3$ (59)), *m/e* 68 $C_4H_6N^+$ (loss of $H_3C)_2-C-CO_2CH_3$ (101)).

## Results and Discussion

The radical chain polymerization of MethPC (or SorbPC) was initiated with $^{13}$CN-AIBN at 60 °C. The conditions were similar to those used in our previous studies of the degree of polymerization of monomeric lipids in hydrated bilayer membranes.[4,5] Each polymerizable lipid was hydrated with the unreactive DMPC to yield bilayers composed of nearly equal amounts of the reactive and unreactive lipid in order to shorten the polymer chains and increase the mole percent of the $^{13}$C-label in the polymers.[4] The $^{13}$C NMR spectra of transesterified polylipids was determined at 62.9 MHz (250 MHz NMR spectrophotometer). After transesterification the poly-MethPC is essentially transformed into PMMA. Two major signals were observed in the nitrile region of the $^{13}$C spectrum: 124.46 and 121.70 ppm. The $^{13}$C NMR spectrum of the transesterified poly-SorbPC was similar with two intense peaks at 124.14 and 122.90 ppm. These signals were assigned by reference to the literature and compared with model compounds.



**Half a Bilayer of MethPC** **Tail and Head Addition**

Previously, Bevington et al. reported that when the polymerization of MMA is initiated with $^{13}$CN-AIBN, under conditions that favor tail addition, the PMMA shows two $^{13}$CN signals at 125.0 and 124.8 ppm (data obtained at 25 MHz) in a ratio of 3:1. The two signals from tail addition of initiator to monomer correspond to the presence of meso dyads at some chain ends and racemic dyads at the others.[6] There was no evidence for head addition. In contrast, Bevington reported evidence for head addition of the radical derived from $^{13}$CN-AIBN during the polymerization of vinyl acetate and vinyl formate.[10] The poly-VAc spectrum showed two peaks at 124.2 and 122.8 ppm in a ratio of 4:1. The principal peak was assigned to tail addition of the initiator fragments to the monomer, whereas the smaller peak was ascribed to head addition via primary radical termination/chain transfer to initiator. Note that the relative intensities of these $^{13}$C signals are not necessarily a quantitative measure of proportion of the two labels in the polymer chains. The basis for their qualitative analysis was the similarity between the chemical shift of the polymer with that of model compounds representing tail addition (124.3 ppm) and head addition (122.4 ppm). Similar results were obtained for poly-VF: tail addition (124.5 ppm) and head addition (122.9 ppm).

Bevington and co-workers consistently observed signals for $^{13}$CN between 124.2 and 125 ppm for the tail addition of $^{13}$CN-AIBN fragment to vinyl monomers.[6,10] One of the signals, 124.46 ppm, observed for the transesterified poly-MethPC also occurs at this field strength and was assigned to tail addition that occurs during polymer chain initiation. The same basis was used to assign the signal at 124.14 ppm to the tail addition of initiator to SorbPC. The second $^{13}$CN signal in poly-MethPC as well as in poly-SorbPC signal occurs further upfield at 121.70 and 122.90 ppm, respectively. Because this transesterified poly-MethPC signal was not observed in PMMA formed via tail addition, it is reasonable that the 121.70 ppm signal is a consequence of head addition during chain termination. Moreover, the upfield signals observed in both the transesterified poly-MethPC and poly-SorbPC are similar to the values of 122.4 and 122.9 ppm reported for head addition of $^{13}$CN-AIBN fragments to vinyl acetate and vinyl formate polymer chains.[10] Finally, the two small model compounds for head addition, tetramethylsuccinonitrile and methyl 2,2,3-trimethyl-3-cyano-butanoate, show $^{13}$CN signals at 121.26 and 123.77 ppm, respectively. In summary, the data from the literature and model compounds indicate at this field strength the $^{13}$CN signal due to tail addition to this monomer would occur as a major peak between 124.2 and 125 ppm. The presence of second resonance upfield by 2−3 ppm is consistent with head addition of $^{13}$CN-AIBN fragment to the growing polymer chain, i.e., primary radical termination via coupling. The qualitative nature of these $^{13}$C NMR measurements does not allow determination of the relative amounts of primary termination by coupling and disproportionation.

We have previously noted that primary radical termination of polymerizations in bilayers could result from the constrained motion of the growing polymer chains in the bilayers.[5] Both monomer and growing polymer are confined to the two-dimensional environment of the bilayer. Polymer chain growth reduced the lateral diffusion coefficient ($D$) of a fluorescent lipid probe in the bilayer.[12] As the $X_n$ of the polylipid increased from 230 to 1900, the $D$ of the NBD-PE probe decreased from 1.4 to 0.24 $\mu m^2\ s^{-1}$. These data indicate the effective viscosity of the bilayer is increased by the polymerization process, Increased bilayer viscosity will decrease the translational and/or segmental motion of the growing polymer chains. Consequently bimolecular termination processes, i.e., coupling and disproportionation of polymer chains, will become more difficult. The apparent effect is a reduction in the probability of bimolecular termination relative to primary termination of the polymer chain by reaction with the small isobutyronitrile radical.

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