Macromolecules 1992, 25, 7256–7260

Structure of the Condensed Phase of Methacrylic Copolymers with Lipidic Side Chains

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Received April 15, 1992; Revised Manuscript Received September 18, 1992

ABSTRACT: Structures of alternating syndiotactic copolymers of methacrylic esters with both hydrophilic and hydrophobic side chains were investigated by an X-ray diffraction method. One of the alternating side chains of the copolymers was a 2-[[2-(trimethylammonio)ethyl]phosphoryl]ethyl group with a hydrophilic phosphoryl part. The hydrophobic side chain was modified from sample to sample, i.e., a cholesteryl, lauryl, or stearyl group. The structure proposed for these copolymers is basically constructed from alternately stacked bilayers of hydrophilic and hydrophobic side chains, which is similar to that found for lipid bilayers.

Introduction

A class of block copolymers with amphiphilic nature is known to show the smectic liquid-crystalline order in a condensed phase.[1] We report here another type of smecticlike layered structure for alternating copolymers with hydrophilic and hydrophobic side chains. The hydrophilic side chain is similar to the polar head group of a phospholipid, i.e., diacylphosphatidylcholine (lecithin). Since lipid is the main constituent molecule of biomembranes, the structures and physical properties of condensed lipid phases are important for the understanding of the biological functions of membranes. The copolymers studied are polymerized analogues of the lipid, and it is interesting to compare their structures and physical properties with those of lipid–water systems.

The copolymers investigated are of polymethacrylate backbones, with hydrophilic and hydrophobic ester groups as side appendages. These polymers are amphiphilic in nature and exhibit thermotropic polymorphism, with the side chains showing a syndiotactic arrangement. On the basis of analyses of X-ray diffraction (XRD) patterns, combined with differential scanning calorimetry (DSC), a structure is proposed for the condensed phase: it is a layered structure with alternating hydrophilic and hydrophobic bilayers.

Materials

Three copolymers used are obtained by copolymerization of 2-(methacryloyloxy)ethyl-2-(trimethylammonio)ethyl phosphate with cholesteryl, lauryl, or stearyl methacrylate. Therefore, they have a backbone of a methacrylic polymer with two types of alternating lipids as side chains in a syndiotactic arrangement. The polymerization procedure has been reported elsewhere.[2]

One side chain of these polymers is always a [[2-[[2-(trimethylammonio)ethyl]phosphoryl]ethyl]oxy]carbonyl group (side chain 1). In reference to the second side chain (side chain 2) combined with the above one, we will use the abbreviation PC-CHO for the copolymer with a (cholesteryloxy)carbonyl side chain, PC-LM for that with a (lauryloxy)carbonyl side chain, and PC-SM for that with a (stearyloxy)carbonyl side chain as shown in Figure 1.

The molecular weights of the copolymers were estimated from viscosity measurements. The intrinsic viscosity $[\eta]$ of copolymers in chlorobenzene at 25 °C was 0.36 dL/g for PC-CHO, 0.34 dL/g for PC-LM, or 0.39 dL/g for PC-SM.

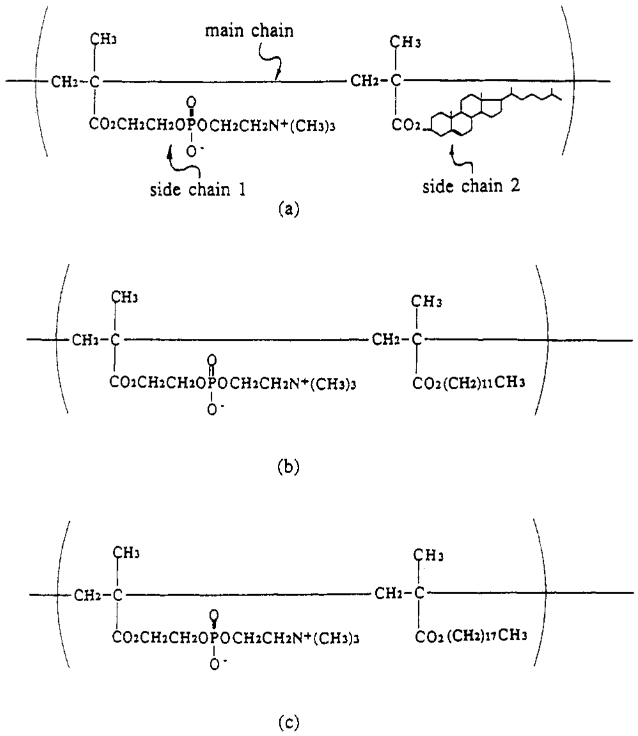

Figure 1. Chemical structures of copolymers: (a) PC-CHO, (b) PC-LM, (c) PC-SM.

All the samples were used as polymerized, i.e., powderlike samples. For PC-SM, an oriented sample was prepared by rolling above 40 °C.

Experiments

An X-ray powder diagram for each sample was obtained using nickel-filtered Cu $K\alpha$ radiation (37.5 kV, 20 mA) using a flat-plate camera with a 4–8-cm specimen-to-film distance and recorded at room temperature. For PC-SM, the diffraction pattern was also taken at 40 °C.

DSC measurements were carried out to monitor phase transition behaviors of samples between −39 and +200 °C.

Viscosity measurements were carried out at 25 °C using a Ubbelohde type viscometer with chlorobenzene as the solvent.

Results and Discussion

All the XRD patterns obtained for the three samples consist of one or two rings with a strong intensity at the

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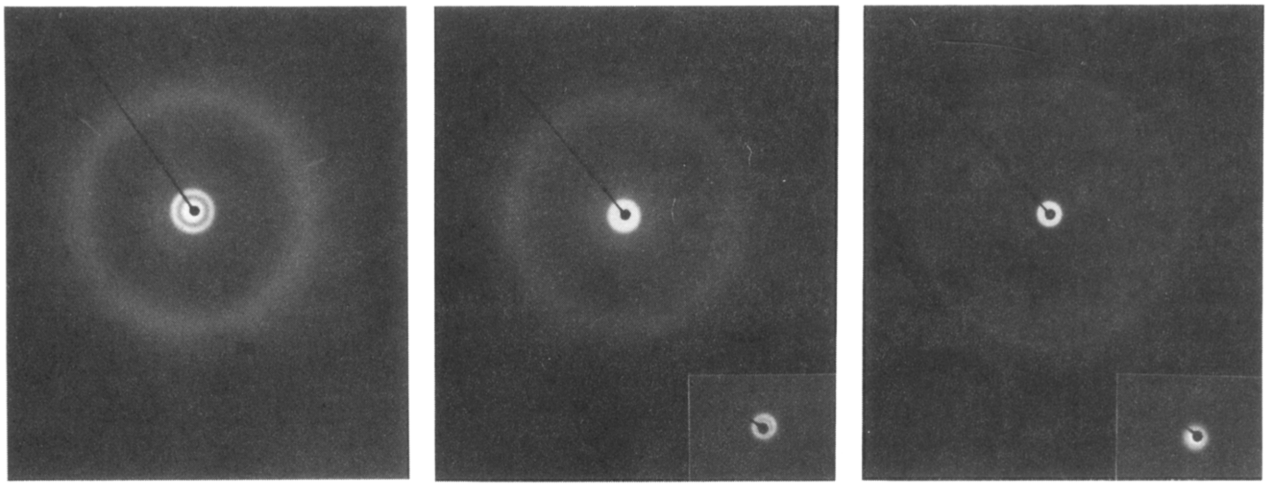

(a) PC-CHO (b) PC-LM (c) PC-SM

Figure 2. X-ray diffraction patterns obtained with a flat camera by Ni-filtered X-rays of Cu Kα: (a) PC-CHO (camera length = 78.0 mm), (b) PC-LM, (c) PC-SM (camera length = 63.1 mm). The insets at the bottom right of b and c show small-angle parts obtained with a camera length of 78.0 mm.

Table I
Summary of Results

	PC-CHO	PC-LM	PC-SM
$[\eta]$,[a] dL/g	0.36	0.34	0.39
M_w[b]	750	549	633
X-ray data,[c] Å			
at 25 °C	62.7 31.4 *5.6	58.6 *4.7	51.0 17.0 *4.7 4.12
at 40 °C			about 60 *4.7
lattice param	a = 4.8 Å b = 7.5 Å c = 62.7 Å	a = 4.8 Å $b \sin \alpha$ = 5.3 Å $c \sin \alpha$ = 58.6 Å α = 116°	a = 4.76 Å b = 8.24 Å c = 51.0 Å
	orthorhombic	monoclinic	orthorhombic
ρ_{calc},[d] g/cm^3	1.1	(1.1)	1.1
DSC data			15 J/g (at 38.7 °C)
	160 J/g (at 70 °C)	230 J/g (at 100 °C)	230 J/g (at 110 °C)

[a] $[\eta]$ is the intrinsic viscosity obtained by use of chlorobenzene as solvent. [b] M_w is the molecular weight of one monomer unit. [c] An asterisk indicates a diffuse scattering. [d] ρ_{calc} is the density calculated from lattice parameters of the proposed model. Measured values of ρ are 1.0 g/cm^3 < $\rho_{obs} \leq$ 1.1 g/cm^3 for all three samples. The value for PC-LM was obtained by assuming α = 116°.

small-angle region and a weak diffuse scattering at the wide-angle region. Figure 2 shows typical XRD patterns for the three powder samples.

In the XRD pattern of PC-CHO, two strong rings with spacings of 62.7 and 31.4 Å and a weaker diffuse ring at 5.6 Å were observed (Figure 2a). For PC-LM, a strong ring at spacing 58.6 Å and a weaker diffuse ring at 4.7 Å were observed (Figure 2b). For PC-SM at room temperature, a strong ring at 51.0 Å, a weaker ring at 17.0 Å, and a weak diffuse ring at 4.7 Å together with a strong, sharp ring at 4.12 Å were observed (Figure 2c). In the wide-angle region of the XRD pattern of PC-SM at 40 °C, the sharp ring (4.12 Å) disappeared and the diffuse ring at 4.7 Å remained with increased intensity. At the same time the strong ring at 51.0 Å in room temperature shifted to about 60 Å at 40 °C. Table I shows a summary of the results obtained from XRD measurements together with those from DSC measurements.

In addition to the powder diagrams referred to above, we obtained X-ray fiber diagrams for PC-SM treated by roll stretching. A little block of PC-SM was drawn in one direction by rolling above 40 °C (i.e., above the phase transition temperature). The X-ray fiber diagram was recorded at room temperature after keeping the sample at 5 °C for 24 h. XRD patterns from three directions of the roll-stretched PC-SM sample are shown in Figure 3. For the incident X-ray normal to the rolling direction and parallel to the rolling plane (Figure 3a), the obtained diffraction pattern was the same as that for the incident X-ray parallel to both the rolling direction and the plane (Figure 3b), i.e., a pair of arcs with a spacing of 51.0 Å together with the third-order one of it, 17.0 Å, on the equator, a pair of sharp arcs at 4.12 Å on the meridian, and

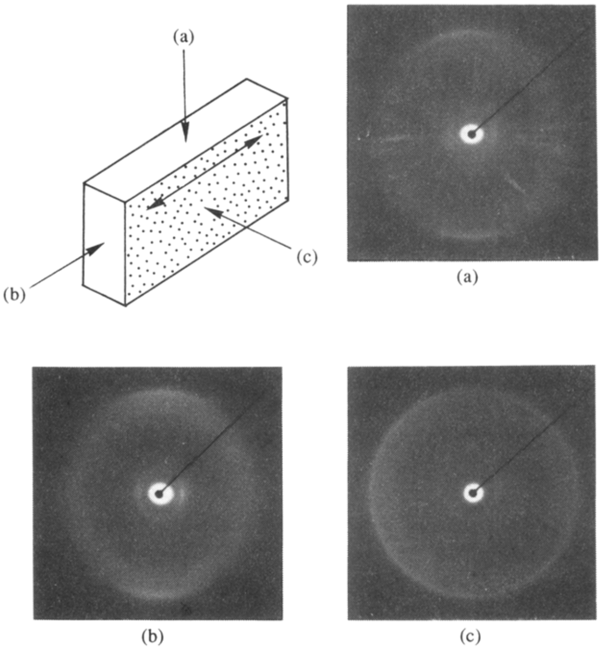

Figure 3. X-ray fiber patterns of PC-SM obtained by a flat camera with an incident beam perpendicular to the rolling direction and parallel to the rolling plane (a), parallel to both the rolling direction and the rolling plane (b), and perpendicular to both the rolling direction and the rolling plane (c). The camera length is 63.1 mm. The double-headed arrow is for the rolling direction, the single-headed arrows for the incident X-ray beam direction, and the dotted plane shows the rolling plane. Radial background reflections in a and c are due to the supporting aluminum foil.

a diffuse ring at 4.7 Å. When the incident X-ray was normal to both the rolling direction and the plane, a ring with a spacing of 51.0 Å and the third-order reflection of it in the small-angle region and a sharp ring at 4.12 Å together with a diffuse ring at 4.7 Å in the wide-angle region were observed (Figure 3c). The intensity distribution along the diffuse ring (4.7 Å) was almost homogeneous for this sample.

The X-ray results show the existence of an ordered structure with a spatial repeating period of about 63, 59, and 51 Å for PC-CHO, PC-LM, and PC-SM, respectively, and that of 4.12 Å for PC-SM at room temperature, an order along the orthogonal direction to that of the long spacing. The observed values of the long spacing are similar to that of the thickness of a phospholipid bilayer.[3,4] The meridional reflection at 4.12 Å implies an arrangement of hydrocarbon chains in the crystalline state, and the diffuse scattering with an average dimension of 4.7 Å can originate from a packing of hydrocarbon chains in the disordered liquidlike state. Thus the XRD results imply the following:

(1) A stacked bilayer structure of hydrophilic and hydrophobic side chains exists, which is similar to that found in lipids. The bilayer plane, and also the main chain, can be oriented parallel to the roll plane in PC-SM with roll stretching.

(2) Hydrocarbon chains in the crystalline state are oriented normal to the bilayer plane for PC-SM at room temperature.

(3) The diffuse scattering at 4.7 Å for PC-SM at room temperature comes from a packing of hydrocarbon chains in the region excluded from stacked bilayers, since it is not affected by roll stretching and the intensity is weaker compared to that of the 4.12-Å reflection. The diffuse scattering at 4.7 Å for PC-LM mainly comes from disordered hydrocarbon chains in the bilayered region, since the scattering pattern is similar to that of PC-SM in the high-temperature phase where a 4.12-Å reflection of the room-temperature phase disappears, giving rise to the 4.7-Å diffuse scattering.

(4) The diffuse scattering at 5.6 Å for PC-CHO comes from a disordered packing of cholesteryl groups.

Since the number of reflections observed by XRD measurements was small, it was necessary to refer to the reported information on each part of the sample molecule for estimation of the molecular packing. Ordinary phospholipids with two hydrocarbon chains and one bulky polar head group, which corresponds to side chain 1 of the present sample, have a stable paired structure of two lipid molecules directing each polar head group outward and each hydrocarbon chain facing inside, which results in the so-called lipid bilayer.[3,4] However, PC-SM has only one hydrocarbon chain for one polar head group. We therefore expect the structure of PC-SM to be similar to the crystal structure of lysophosphatidylcholine,[5] which also has only one hydrocarbon chain per one polar head group. Taking the crystal structure of lysophosphatidylcholine into consideration, we propose as the molecular packing of PC-SM the model shown in Figure 4. In the proposed model, hydrocarbon chains of top and bottom main chains shown in Figure 4a are interdigitated with each other, giving a stable bilayer structure. In the low-temperature phase of PC-SM, hydrocarbon chains are in the crystalline state with the planar zigzag conformation. Thus the observed long spacing, i.e., the bilayer thickness, of 51.0 Å corresponds to the sum of about 30 Å of the interdigitated hydrocarbon chain part plus 2 × 10 Å of the bilayered polar head parts. As for the length of the polar head part of 10 Å, i.e., side chain 1, the estimation was made based on the reported values for the lengths of the glycerophosphorylcholine group[6] and the polar head group of phospholipid.[3,4,7] Assuming that the reflection at 4.12 Å comes from the side-by-side packing of hydrocarbon chains in a closed hexagonal structure and taking the orientation of them to be normal to the bilayer plane, we can calculate $a = 4.76$ Å and $b = 2 \times 4.12$ Å from the area occupied by one hydrocarbon chain in hexagonal packing (i.e., 19.6 Å^2 $\approx 4.76 \times 4.12$ Å^2) on the bilayer plane in a c-axis projection of the proposed model (Figure 4c). Therefore, the length of one repeating unit along the main chain is 4.76 Å. Consequently, lattice parameters of the present model are $a = 4.76$ Å, $b = 8.24$ Å, and $c = 51.0$ Å (from XRD result), where for the lattice including the main chain an orthorhombic symmetry is assumed for convenience. These values give the density $\rho_{\text{calc}} \simeq 1.1$ g/cm^3, which is consistent with the observed density $1 < \rho_{\text{obs}} \leq 1.1$ g/cm^3 (see Table I).

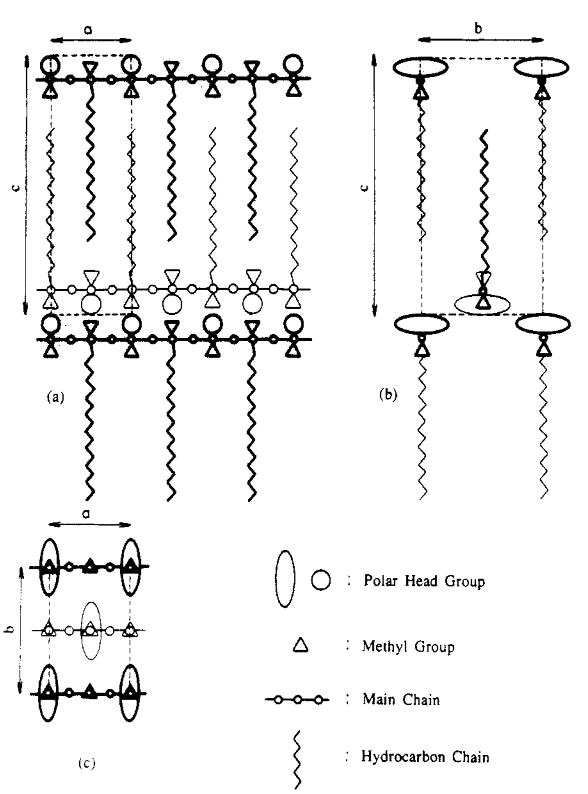

Figure 4. Schematic representation of the three-dimensional packing for a proposed structure of PC-SM at low temperature.

For the case of PC-LM, the length of a lauryl hydrocarbon chain (side chain 2) is shorter than that of a stearyl chain of PC-SM by 6 carbon atoms (12 carbon atoms for the bilayer). Moreover, the observed diffuse scattering at 4.7 Å implies a disordered hydrocarbon chain with a shorter chain length compared to the expanded planar zigzag case. Therefore, it is difficult to explain the observed long spacing of 58.6 Å, a value much longer than that of PC-SM (i.e., 51.0 Å), from the interdigitated structure proposed for PC-SM at room temperature. Above the thermotropic phase transition, PC-SM gives an XRD pattern with a long spacing of about 60 Å; i.e., the thickness of the bilayer increases by thermotropic phase transition. At the same time the sharp reflection at 4.12 Å from crystalline hydrocarbon chains changes to the diffuse scattering at 4.7 Å as described before. In addition, as will be described later, the latent heat by this phase transition obtained from DSC measurement is not as large as would be expected for a conformational change of the main chain.

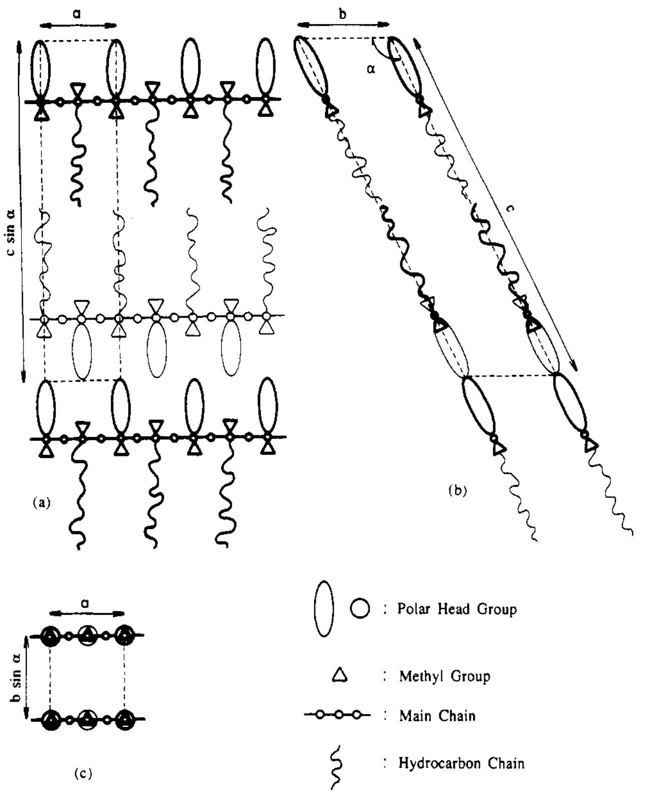

Figure 5. Schematic representation of the three-dimensional packing (a) for PC-LM at room temperature, (b) for PC-SM in a higher temperature phase above the phase transition temperature, and (c) for PC-CHO at room temperature.

Therefore, the change associated with this phase transition may be a local change in side-chain packing.

Figure 5 shows a proposed model of molecular packing for PC-LM where hydrocarbon chains are not interdigitated. Assuming that the 4.7-Å reflection corresponds to the average lattice distance of the hexagonally packed hydrocarbon chains in a disordered state, we obtain the value 25.5 Å^2 as the area per hydrocarbon chain (i.e., area per monomer unit). Therefore, referring to the *c*-axis projection of the present model (Figure 5c) and putting the lattice constant *a* along the main chain as 4.8 Å ($\approx$4.76 Å) as before, we can obtain the value of $b \sin \alpha$ as 5.3 Å (i.e., 25.5 Å^2 $\approx$ 4.8 × 5.3 Å^2), where α is the tilt angle of the lattice (see Figure 5b). The density ρ calculated for an orthorhombic lattice (i.e., $\alpha = 90°$) is $\rho_{\text{calc}} \cong 1.2$ g/cm^3. This value is larger than the observed value of $\rho \leq 1.1$ g/cm^3. We, therefore, have proposed a tilted packing with a monoclinic lattice as shown in Figure 5 with a tilt angle α. If we take $\alpha = 116°$, we have $b = 5.9$ Å and $c = 65.2$ Å with $\rho_{\text{calc}} \cong 1.1$ g/cm^3. As for the high-temperature phase of PC-SM, essentially the same packing with that of PC-LM can be proposed.

For the case of PC-CHO, if we take the diffuse scattering at 5.6 Å to be from the side-by-side hexagonal packing of side chains 2 (i.e., cholesteryl group), we obtain the area per monomer unit as 36.2 Å^2. Putting $a = 4.8$ Å and $b = 7.5$ Å (i.e., 4.8 × 7.5 Å^2 $\approx$ 36 Å^2) as before and assuming $\alpha = 90°$, we obtain $\rho_{\text{calc}} \cong 1.1$ g/cm^3 by use of the value 62.7 Å from the long spacing of XRD as the lattice parameter for the *c*-axis, which is consistent with the observed density of $\leq$1.1 g/cm^3. The long spacing, 62.7 Å, corresponds to the sum of about 20 Å × 2 (i.e., the length of two cholesteryl groups[8]) and 10 Å × 2 (i.e., the bilayered polar head parts[3,4]). Therefore, we propose for the molecular packing of PC-CHO the same one as shown in Figure 5 with $\alpha = 90°$ (i.e., an orthorhombic lattice).

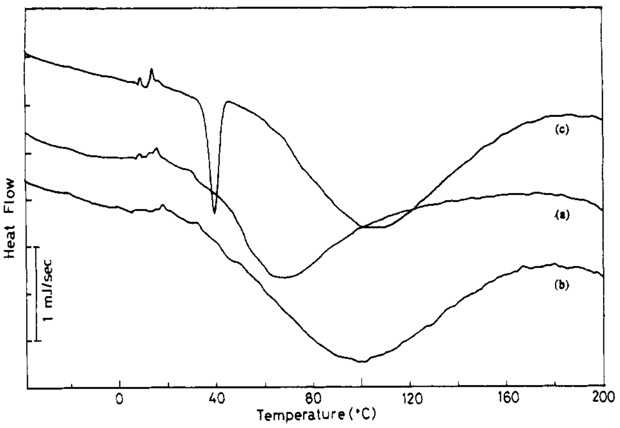

Figure 6. DSC curves for (a) PC-CHO, (b) PC-LM, and (c) PC-SM.

Figure 6 shows the results of DSC measurements for all the three samples, which are summarized in Table I. As shown in Figure 6, a DSC plot of PC-SM shows a sharp endothermic peak at 38.7 °C with a latent heat of about 15 J/g. The value of the latent heat is an order of magnitude smaller than that for the melting phase transition and about half of that for the rotational phase transition of a paraffin with a similar chain length.[9] The observed change in the XRD pattern in the wide-angle region (i.e., the disappearance of the sharp reflection at 4.12 Å at about 40 °C), therefore, indicates that the arrangement of hydrocarbon chains (side chain 2) is transformed from a crystalline state to a more or less disordered liquid-crystalline state by the phase transition, giving rise to the endothermic peak. A similar phase transition is observed for monohydrate DSPC (L-distearoylphosphatidylcholine) at around 75 °C.[10] The shift of the phase transition temperature to the lower temperature may come partly from the characteristic structure of PC-SM with hydrocarbon chains in an interdigitated state pinned by main chains at one end.

A broad endothermic peak was observed in the high-temperature region of a DSC plot for all the samples examined. The location of the endothermic peak and the latent heat were about 70 °C and 160 J/g for PC-CHO, 100 °C and 230 J/g for HC-LM, and about 110 °C and 230 J/g for PC-SM. These broad endothermic peaks may correspond to the disordering of the stacked layer structure constructed by main chains and side chains. The broadening of the peak can partly come from the distribution of the main-chain length. For PC-CHO, the shift of the endothermic peak to a lower temperature than that of PC-LM or PC-SM comes probably from the bulkier side chains which may impose more or less disordered packing compared to the other copolymers. The smaller magnitude of latent heat can come from a smaller conformational change of the cholesteryl side chain by increasing temperature compared to hydrocarbon side chains of the other two samples. The phospholipid–water system can easily be transformed from a layered structure to a liposome structure and/or other structures through thermotropic and/or lyotropic phase transitions.[11] However, the present polymers, which have a hydrophilic polar head group of phospholipid and hydrophobic hydrocarbon chains as side chains, cannot easily change the structure because of the restriction imposed by the main chain. The present system with a stable bilayer structure can be considered as a stable model system of a biological membrane.

Acknowledgment. The authors are grateful to Dr. T. Sakurai of the Institute of Physical and Chemical Research

(RIKEN) and Dr. M. Minobe of Tsukuba Research Laboratory, Sumitomo Chemical Co., Ltd., for their valuable discussion.

References and Notes

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Registry No. (PC)(CHO) (copolymer), 144514-06-7; (PC)(LM) (copolymer), 144514-07-8; (PC)(SM) (copolymer), 144514-08-9.