Phase Transitions in a Lipid Bilayer Embedded in a Crystalline Matrix

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The hydrocarbon part of $(C_{10}H_{21}NH_3)_2CdCl_4$ represents a smectic lipid bilayer exhibiting two structural phase transitions in analogy to biomembranes. Single-crystal ^{13}C NMR and ^{14}N nuclear quadrupole resonance and x-ray data show that the first transition is connected with a disordering of the polar heads whereas the second represents a partial melting of the lipid chains. A Landau theory describing the melting of bilayer membranes in terms of order parameters used in the theory of liquid crystals has been constructed to explain the obtained results.

The occurrence of phase transitions in cell membranes,[1,2] which are one of the principal organizational structures of living matter, has recently attracted a great deal of attention. It has been convincingly demonstrated[3,4] that the transitions in living-cell membranes are due exclusively to the lipid component of the bilayer membrane. Most phase-transition studies have been therefore performed on "model lipid membranes" which are similar in their structural properties to biomembranes but can be much better characterized physically and exhibit sharper phase transitions. Lipid bilayers of dipalmitoyl phosphatidyl choline (DPPC) in particular clearly exhibit two first-order phase transitions,[5] the so-called main transition at $T_{c2} = 42$ °C connected with a melting of the chains and the pretransition at $T_{c1} = 33$ °C. Below T_{c1} the lipid chains are well ordered and tilted with respect to the bilayer normal. X-ray–scattering,[6] calorimetric,[7] NMR,[8-10] and EPR[11] studies have provided a great amount of information on the microscopic nature of these transitions, but several important questions are still open. One of them is the nature of the pretransition at T_{c1}. Another is the detailed state of motion of the hydrocarbon chains in the high- ($T > T_{c2}$) and the intermediate-temperature phase ($T_{c1} < T < T_{c2}$) and the origin of the difference[12] in the values of the nematic order parameter $S_n = \frac{1}{2}\langle 3\cos^2\Theta_n - 1\rangle$, where Θ_n is the angular deviation of the nth C-C bond from its orientation in a hydrocarbon chain oriented parallel to

a preferred direction, as measured by NMR[9,10] and EPR.[11]

In this Letter we report on a ^{13}C NMR and ^{14}N nuclear-quadrupole-resonance (NQR) and x-ray–scattering study of the phase transitions in the pseudo–two-dimensional layer structure perovskite[13] $(C_{10}H_{21}NH_3)_2CdCl_4$—henceforth designated C10Cd—which we believe represents the first known example of a smectic lipid bilayer embedded in a crystalline matrix. The two first-order phase transitions in this system at $T_{c1} = 35$ °C and $T_{c2} = 39$ °C are closely analogous to the ones[5] in DPPC. The obtained results yield a detailed microscopic picture of the two phase transitions in bilayer membranes and allow for the construction of a Landau theory of the "melting" of membranes in terms of order parameters used in the theory of liquid crystals.

The projection of the structure of C10Cd on the b-c plane at room temperature ($T < T_{c1}$) is shown in Fig. 1. The structure consists of $CdCl_4^{2-}$ layers sandwiched between well-ordered alkyl-ammonium chains which are tilted by 40° with respect to the normal to the layer. The ammonium end of each chain is linked to the layer by N-H$\cdots$Cl hydrogen bonds and each chain is coordinated by six others. In C10Cd the chains in subsequent bilayers form a zig-zag arrangement parallel to the crystal c axis (Fig. 1), whereas all the chains are parallel to a single direction in C10Cu and C10Mn. The entropy change at the lower phase transition T_{c1}, $\Delta S = (0.9 \pm 0.3)R$ per

mole chains, can be explained by an order-dis-
order transition of rigid chains between two
equivalent sites. The entropy change at the high-
er phase transition T_{c2}, on the other hand, cor-
responds to $\Delta S = 0.8R$ per R-C-C-R bond and
can be only explained by a "melting" of the chains,
or what is equivalent, by rapid chain isomeriza-
tion via kink diffusion.

In the intermediate phase the polar NH_3 groups
are flipping between two possible orientations,[13]
$p = \pm 1$. The alkyl chains also flip by 90° around
their chain axes so that neighboring chains move
in opposite directions like a two-dimensional ar-
ray of connected gears.

The onset of the disordering of the polar heads
in the low-temperature phase can be quantitatively
followed[14] by measuring the temperature depen-
dence of the ^{14}N quadrupole coupling constant,
e^2qQ/h and asymmetry parameter η. Since the
motion between the two sites with electric-field-
gradient (EFG) tensors $T(1)$ and $T(2)$ is fast on
the NQR time scale, the ^{14}N NQR spectrum is
determined by the time-averaged value of the EFG

tensor:

$$\langle T(t) \rangle = \tfrac{1}{2}(1+p)T(1) + \tfrac{1}{2}(1-p)T(2). \qquad (1)$$

Knowing the EFG tensors $T(1)$ and $T(2)$ from the
completely ordered state, the temperature de-
pendence of $p = \langle p(t) \rangle$ can be determined from
$\langle T(t) \rangle$. The results are shown in Fig. 2.

The Fourier map in the high-temperature phase
shows in addition to the $CdCl_4$ octahedra well-
defined N and C(1) positions whereas the remain-
ing part of the chain is parallel to the c axis and
splits symmetrically on both sides of the mirror
plane with a statistical weight 0.5 for each chain
because of the 90° flipping. The average posi-
tions of C(2) and C(3) are weakly defined whereas
the terminal part of the chain, C(4) to C(10),
forms a continuous distribution parallel to the
c axis. There is no evidence for a conelike mo-
tion around the layer normal.

The ^{13}C spectra of the low-temperature and
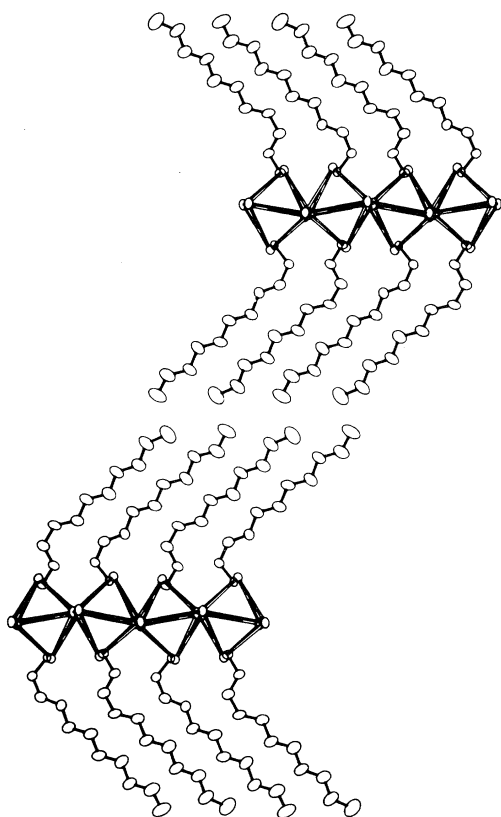the intermediate-temperature phases are near-

FIG. 1. Projection of the low-temperature structure
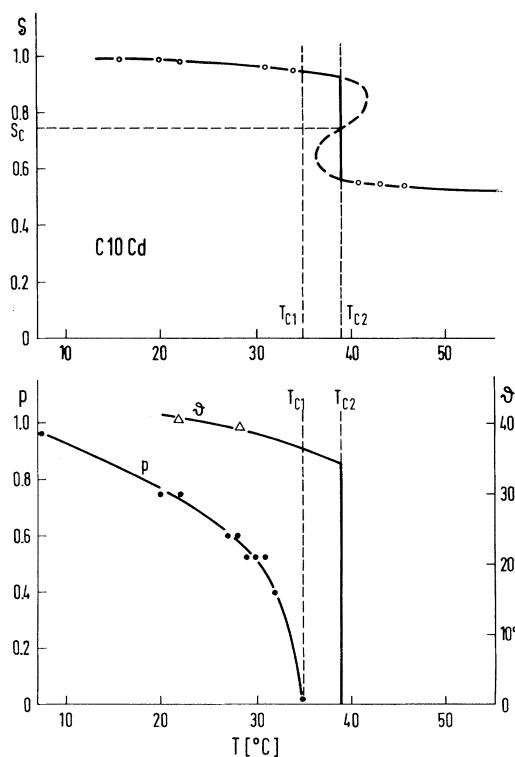of $(C_{10}H_{21}NH_3)_2CdCl_4$ on the b-c plane.

FIG. 2. Comparison between the calculated and ob-
served temperature dependences of the order parame-
ters S, p, and ϑ for C10Cd. The experimental S values
were calculated from the ^{13}C data with the help of Eq.
(2) and Ref. 15 and represent the average over the rig-
id portion of the hydrocarbon chain excluding terminal
methylene and methyl groups.

ly identical but there is a sharp change in the ^{13}C spectrum on going into the high-temperature phase. The angular dependence of the (-CH$_2$)-group spectra in the low-temperature phase is characteristic of two sets of rigid (CH$_2$)$_n$ chains which are tilted by ± 40° with respect to the layer normal. The chain methylene groups display the full chemical shift anisotropy similar to the one observed in n-eicosan[15] or polyethylene.[16] In the intermediate-temperature phase the chains are still tilted by ± 40° and almost rigid (Fig. 3). The bulk of the data can be described by an axially symmetric ^{13}C chemical-shift tensor: $\sigma_\perp = \frac{1}{2}(\sigma_{11} + \sigma_{22}) = 43 \pm 3$ ppm, $\sigma_\parallel = \sigma_{33} = 15 \pm 5$ ppm with respect to tetramethyl silane. The symmetry axis $\sigma_\parallel = \sigma_{33}$ is parallel to the chain direction. The axial symmetry of the ^{13}C tensor is due to the 90° flips around the chain axis which result in a rapid exchange of σ_{11} and σ_{22} but do not affect σ_{33}. The nematic order parameter S with respect to the preferred chain direction, $\vartheta = \pm 40°$, is here about 1: $S \approx 0.95$–0.99. There is another set of ^{13}C lines belonging to the less rigid terminal methylene groups which is also axially symmetric and can be described by $S \sim 0.75$.

The angular dependence of the ^{13}C spectra in the high-temperature phase is completely different (Fig. 3) from the one in the low- and intermediate-temperature phases. It shows that all chains are equivalent and normal to the layers. Three different sets of ^{13}C lines can be resolved. The most intense line belongs to the bulk of the -CH$_2$ groups. It can be described by $\sigma_\parallel = 20 \pm 2$ ppm and $\sigma_\perp = \frac{1}{2}(\sigma_{11} + \sigma_{22}) = 34$ ppm. The anisotropy of the chemical-shift tensor is only partially removed by the molecular motion (chain isomerization via fast kink diffusion) and the nematic order is still significant:

$$S = \frac{(\sigma_\parallel - \sigma_\perp)_{obs}}{(\sigma_\parallel - \sigma_\perp)_{rigid}} = 0.55 \pm 0.10. \qquad (2)$$

Another weak line belongs to the terminal

FIG. 3. Angular dependence of the ^{13}C spectra in the high-temperature and intermediate-temperature (inset) phases of C10Cd. φ stands for the angle between the c axis and the direction of the external magnetic field.

methylene group, -CH$_2$-CH$_3$, and can be described by $\sigma_{\parallel\alpha} = 20$ ppm, $\sigma_{\perp\alpha} = 25 \pm 1$ ppm. The third line is due to the methyl group. Here $\sigma_{\parallel CH_3} = 10 \pm 1$ ppm, $\sigma_{\perp CH_3} = 14 \pm 1$ ppm. With the help of the rigid-lattice ^{13}C shift tensors of n-eicosane[15] (n-C$_{20}$H$_{42}$) we find that $S(\alpha$-CH$_2) \approx 0.25 \pm 0.05$ and $S(-CH_3) \approx 0.20 \pm 0.05$. The above results and the absence of a significant flexibility gradient along the main part of the chain is at variance with the more "liquidlike" picture of the bilayer interior suggested by EPR spin-label studies,[11] but agrees with the deuteron resonance data.[9]

The hydrocarbon part of C10Cd thus represents a smectic liquid crystal with a structure similar to the interior of the bilayer lipid membranes. Let us therefore try to describe the two phase transitions in C10Cd by Landau-type expansion of the nonequilibrium free energy F in terms of order parameters ϑ, $S - S_c$, and p, used in the theory of smectic liquid crystals[17]:

$$F = \frac{1}{2}A\vartheta^2 + \frac{1}{4}B\vartheta^4 + \alpha(T - T_{c2})(S - S_c) - \frac{1}{2}c(S - S_c)^2 + \frac{1}{4}d(S - S_c)^4 + \frac{1}{2}ap^2 + \frac{1}{4}bp^4 - \frac{1}{2}A'\vartheta^2(S - S_c) - \frac{1}{2}a'p^2(S - S_c). \qquad (3)$$

Here S stands for the average nematic order parameter and A, B, α, c, d, a, b, A', and a' are all positive constants. The smectic-C order parameter ϑ gives the average tilt of the molecules with respect to the layer normal whereas p is the orientational order parameter for the 90° flipping of the chains and the terminal NH$_3$ groups between
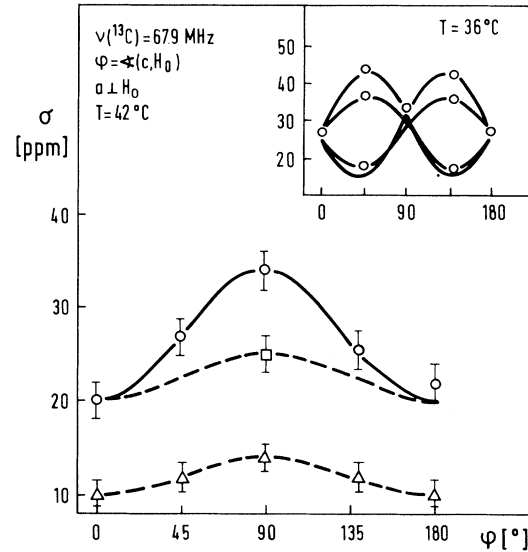
the two equilibrium orientations corresponding to $p = \pm 1$. S_c is the critical value of the average nematic order parameter S, i.e., the arithmetic average of S in the melted and rigid phase coexisting at the first-order transition temperature T_{c2} (Fig. 2).

In deriving expression (3) we assumed that the melting of the chains is the important driving mechanism which induces the transitions in S as well as in ϑ and p. It should be noted that S is — in contrast to the isotropic-nematic transition — here not a symmetry-breaking order parameter and is different from zero both above and below T_{c2}. Because of that the linear term in the expansion of F in powers of $S - S_c$ is not identically equal to zero as in the case of nematic liquid crystals[17] but has the same structure as the free energy at liquid-gas phase transitions.[18]

Minimizing the total free energy with respect to p, ϑ, and S, we obtain[19] for $-(c/d)^{1/2} < A/A' < (c/d)^{1/2} < a/a'$ the sequence of phases (Fig. 2) observed in C10Cd (for $T > T_{c2}$, $p = 0$, $\vartheta = 0$, and $S < S_c$; for $T_{c1} < T < T_{c2}$, $p = 0$, $\vartheta \neq 0$, and $S > S_c$; for $T < T_{c1}$, $p \neq 0$, $\vartheta \neq 0$, and $S > S_c$). The high-temperature transition at T_{c2} corresponds to a partial melting of the chains which simultaneously destroys the tilting of the molecules whereas the low-temperature transition at T_{c1} corresponds to an orientational transition and a disordering of the polar "heads."

The above free energy describes only one bilayer. To get the observed ordering of the whole crystal one has to introduce an interaction between the two neighboring hydrocarbon bilayers on the opposite sides of the same $CdCl_4$ layer. The additional term in the free energy can be written as

$$F' = -K\vartheta_1\vartheta_2, \tag{4}$$

where ϑ_1 and ϑ_2 are the smectic-C order parameters on the opposite sides of the $CdCl_4$ layer. For $K > 0$, one obtains $\vartheta_1 = \vartheta_2$ as observed in C10Cu; and, for $K > 0$, $\vartheta_1 = -\vartheta_2$ as observed in C10Cd (Fig. 1).

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