The pre-melt phase of \( n \)-alkanes: Crystallographic evidence for a kinked chain structure

(phase transition/paraffin chain melting/lipid \( \alpha \)-phase/kinked chain/electron diffraction)

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ABSTRACT Electron diffraction measurements on epitaxially grown crystals of orthorhombic \( n \)-hexatriacontane, \( n \)-C\(_{36}H\(_{74}\)\( \), give evidence for the chain-defect mechanism for linear chain melting. The derived structural model is also in accord with recent spectroscopic studies of odd-chain \( n \)-alkanes, and the diffraction data specifically exclude models based on helices or rigid chain rotors. Stability of a kinked chain structure, moreover, is indicated by an observed hysteresis effect that gives different pretransition temperatures for solution-grown and annealed crystals.

The hexagonal methylene subcell packing of alkane chains in the ‘gel-phase’ or \( \alpha \)-polymorphic form of numerous long chain amphiphiles has been found to be an important structural entity in the lyotropic and thermotropic phase behavior of these materials (1), and it might indicate the molecular mechanism for long chain melting, if the structure were well-characterized. Because of its relevance to the physical behavior of many biological lipids (2), the gel-phase structure has been extensively studied by various spectroscopic and diffraction techniques and has been the subject of numerous theoretical calculations. From these studies three distinct molecular models have emerged: (i) a rigid rotor made up of the entire alkyl chain (3); (ii) a chain helix much like the one found in polytetrafluoroethylene (4); and (iii) a kinked chain structure, composed of either a random distribution of point defects (5) or isolated regions with discrete blocks of extended zigzag chains (6).

Diffraction techniques have not been very helpful for discriminating among these three models. The best data from the hexagonal subcell have come from electron diffraction experiments (7–10); however, in the projection down the molecular chain axes, all three models degenerate into the methylene rotor, which has been found (7) to account for the observed \( h0l \) intensities. Thus, one cannot distinguish among these models in this projection. Attempts at a single-crystal x-ray determination only served to establish the relative shift of adjacent lamellae (11). Low-angle x-ray measurements (12) give indirect evidence for the kinked chain model, accounting for changes in peak shapes and long spacings by the increased interlamellar void volume imposed by the introduction of chain point defects.

The most convincing evidence for kinked-chain structures in the transition from the orthorhombic to the hexagonal methylene subcells comes from infrared and Raman spectroscopic measurements on pure \( n \)-alkanes (5, 13–15). Two kinked chain conformations were found to predominate: the “end-gauche” conformation at the chain end and the gauche-trans-gauche conformation in the body of the chain. In this paper, we present crystallographic evidence for a chain defect model using electron diffraction data from heat-treated \( n \)-alkane single crystals that had been appropriately oriented by epitaxial crystallization.

MATERIALS AND METHODS

The even-chain \( \alpha \)-paraffin, \( n \)-hexatriacontane (98% pure), purchased from Aldrich, as well as numerous alkane derivatives (16), can be epitaxially crystallized by cooling dilute solutions in molten aromatic or aliphatic ring molecular solvents such as naphthalene, trioxane, or benzoic acid, as described in detail elsewhere (17–20). Unlike the solution-grown crystals of these materials, in which the long molecular chain axes are more or less perpendicular to the best-developed crystal face because of optimized van der Waals interactions (21), the chain axes in epitaxially grown crystals are now parallel to the largest crystal face because of nucleation on the substrate crystal (17). Thus, electron diffraction patterns from projections containing the molecular long chains can be obtained from these epitaxially grown crystals giving intensity data well-fit by the known crystal structure and therefore useful for quantitative crystal structural analysis (unpublished data). There are also some crystals on any electron microscope grid surface that are oriented in the crystal habit given by solution growth so that both crystalline projections can be examined in the same diffraction experiment. For the experiment described here, the paraffin was epitaxially crystallized from cooled molten benzoic acid solution.

Samples of a phospholipid, 1,2-distearoyl-sn-glycero-phosphocholine, were epitaxially crystallized on carbon-covered 400-mesh copper electron microscope grids from molten naphthalene solution. This latter compound (purchased from Sigma) was included in this study because it has been found to have a stable \( \alpha \)-polymorphic form with chains packing in the hexagonal methylene subcell (unpublished data).

All electron diffraction experiments were carried out on a JEOL JEM-100B electron microscope taking care that incident illumination was low enough and the photographic emulsion (Kodak NS-5T screenless x-ray film) sensitive enough to ensure the recording of diffraction patterns well before the onset of appreciable radiation damage to the specimen (22). All transmission experiments were done at 100 kV. Reflection diffraction measurements were made on the phospholipid at 40 kV with thin film samples crystallized from ethanol onto a polished aluminum stub. Heating experiments were carried out during the transmission diffraction work using an EM-SCH liquid nitrogen cooling holder with an added resistive heater (modification made by M. Naruse, JEOL USA). The heater is powered by a Lambda model LL-901-OV regulated dc power supply (Lambda Electronics, Melville, NY) and is controlled by an Omega model 800MT on-off potentiometric controller (Omega Engineering, Stanford, CT) that senses the specimen temperature measured by a proximate copper/constantan thermocouple. With this apparatus, we have been able to hold temperatures between

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-145°C and 90°C with at least ±1°C accuracy. Displayed temperature was calibrated at 0°C, room temperature, and 100°C with water at 1 atm.

Because there are slight changes in the irregular elastic bend contours of these epitaxially grown crystals and/or slight movements induced by specimen heating, we have not attempted a quantitative crystal structure analysis. Rather we concentrate here on a qualitative description of the changes in the total diffraction pattern based on the known crystal structure of n-hexatriacontane (23). Measurements of relative changes in diffraction intensity were made by scanning the diffraction films with a Joyce–Loebl MKIIIC flatbed microdensitometer.

Structure factor calculations were made using Doyle–Turner (24) electron scattering factors. Only carbon atom positions were used in the model calculations. Continuous diffuse scattering calculations were based on a theoretical model given by Amorós and Amorós (25) as carried out earlier (26–28) to explain diffuse electron diffraction intensity from other long-chain compounds.

RESULTS AND DISCUSSION

According to Vand (29), the hexagonal form of n-hexatriacontane, which corresponds to the gel form or α-phase of alkane-chain lipids, appears two degrees below the melting point—i.e., at 73.5°C—and causes an abrupt lowering of crystal density. Solution-grown crystals of the paraffin heated to produce this α-phase give hexagonal electron diffraction patterns (Fig. 1) characteristic of alkyl chains perpendicular to the crystal surface and not the 19.5° chain axis tilt to the surface normal reported by other workers for this material (32). Samples heated to this pretransition and then rapidly cooled ca. 30°C exhibit, on subsequent reheating, a hysteresis effect with the pretransition to the hexagonal chain packing occurring 3–5°C below the initially observed pretransition temperature. This is in accord with previous observations on odd-chain alkanes (5).

Results of heating experiments on epitaxially grown crystals are shown in Fig. 2 and may be summarized as follows: When these crystals are heated to 67–69°C (i.e., at least 6°C below the pretransition temperature), there is an obvious falloff in the low-angle (00l) intensities, with an attendant slight increase in the long unit-cell spacing similar in magnitude to that found for an odd-chain paraffin (32). However, except for an attenuation similar to that expected from an increased overall temperature factor, the strong reflections due to the polyethylene chain scattering are not changed by this heating. Continuous, diffuse scattering is more apparent in the diffraction patterns at elevated temperature. Rapid cooling by ca. 30°C increases the resolution of the low-angle 00l reflections but not to the extent noted before heating. That is, as found with solution-grown crystals, there is a hysteresis effect. Cooling of the specimens with liquid nitrogen dramatically reduces the diffuse scattering but does not eliminate it.

These observations allow an evaluation of various theoretical models (see also Table 1).

(i) General increase of overall temperature factor. Use of larger isotropic or anisotropic (e.g., with largest average displacement along long-chain axes) temperature factors for structure factor calculations would explain only the general falloff of all intensities with increasing sin θ/λ. This does not explain the intensity reduction of lower order 00l reflections and the retention of, e.g., relatively intense (0,0,74) and (0,0,76) reflections.

(ii) Rigidly rotating chain. If heating is accompanied by the onset of a rigid-chain rotation as has been suggested (3), there should be a marked reduction and eventual disappearance of 0kl intensities with k odd due to the transition to a C-centered unit cell. Observations of strong (0,1,34), (0,1,36),

Fig. 1. Data from orthorhombic solution-grown crystals of n-hexatriacontane (space group Pca21). (A) Transmission hkl electron diffraction pattern at room temperature, characteristic of the so-called O1 methylene chain “subcell” packing (30); a = 7.42 Å, b = 4.96 Å. (B) Electron diffraction pattern for specimen in pretransition phase (75°C). The pattern is characteristic of hexagonal methylene chain packing (7); a = 4.80 Å. (C) Heating curves for n-hexatriacontane, plotted as an axial ratio a/b of an orthorhombic chain packing (31). The first heating (×) of solution-grown crystals shows the expected pre-melt hexagonal phase between 72 and 74°C. After cooling and reheating (○), this transition appears 3–5°C lower, presumably due to the slow relaxation time for the kinked chains.
(0,1,38), (0,3,36), and (0,3,38) reflections after heating refute this model, and so does its prediction of unattenuated low-angle (00l) intensities with chain rotation.

(iii) Helical chain. If a 132-helix of the type found for polytetrafluoroethylene (33) is used as a model for the heated chain, then the low-angle (00l) reflections are predicted to remain unchanged. Moreover, there are predicted changes in 0kl intensities that are not observed in the electron diffraction patterns—i.e., although the basic transform of the polyethylene chain is preserved, intensity ratios—e.g., for strong 01l and 03l reflections—are predicted that would be distinctly different from those of the straight zigzag chain. Such changes are not seen in the experimental data.

(iv) Kink model. There are various suggestions for kink models incorporating various kinds of point defects in the paraffin chains (5, 14, 34, 35). Following recent spectroscopic studies (5, 14, 15), we use the gauche-trans-gauche kink conformation as the major defect component, however specifically using experimentally determined crystallographic coordinates provided by the crystal structure of ethylene di(11-bromoundecanoate) (36, 37), which incorporates such a defect in its diester region into an O₂ methylene subcell packing. Although end kinks (14) have also been observed in crystal structures (38, 39), they are not specifically considered here.

A population of 12 n-hexatriacontane chains was used to construct the starting model. Each chain was divided into nine sectors and the presence or absence of a kink in each sector was established by the roll of a die to give the supercell representation shown in Fig. 3. The population of defects was averaged for these chains including an average over the molecular center of symmetry. As shown by Fig. 3, the resultant voids at either end of the kinked molecule were generated by starting z/c coordinates for adjacent chains at alternate methyl end planes (e.g., z/c of C1 or C36, respectively, near 0 or 0.5). The average unit-cell model, conforming to an unaltered zonal symmetry, therefore, had a fraction of chain atoms (ca. 60% for most of the structure) with unaltered coordinates and a fraction with atoms in the kink. The population of each type is nearly constant along the chain as originally proposed (13). Later calculations incorporated a larger fractional void volume following an observation (14) that the defect concentration may be higher at chain ends. A large anisotropic thermal motion (B₁₁ = 20 Å²) was also applied to defect atoms to simulate a distribution of their location around ideal sites along the chain.

Although there is considerable freedom for adjusting the thermal and positional parameters discussed above, we use defect concentrations in the model discussed here that agree with earlier work (5). The basic defect model conforms very well to the observed change in diffraction pattern. The lower

"Correcting a typographical error in ref. 37, we find that the atomic coordinates in the local orthorhombic axial system for a kinked chain region are as given below:

<table>
<thead>
<tr>
<th></th>
<th>x, Å</th>
<th>y, Å</th>
<th>z, Å</th>
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<td>C-6</td>
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Since the structure factor calculations were made in projection, no correction for somewhat short distances originally due to C-O bonds was made. In the original diester structure, atoms C-2 and C-5 are linkage oxygens.

This leaves chain end carbons at chain positions (m or n), where n = 37 - m with unaltered coordinates at the following populations: C(3,36), 10%; C(2,35), 21%; C(3,34), 40%; C(4,33), 40%; C(5,32), ~60%.

Fig. 2. Data from epitaxially grown crystals of n-hexatriacontane. (A) Transmission 0kl electron diffraction at room temperature; c = 95.14 Å (the strongest high-angle reflections on c are (0,0,74) and (0,0,76)). (B) Electron diffraction pattern from the same crystal heated to somewhat below the transition to the hexagonal form (69°C). Note the dramatic disappearance of inner order 00l reflections but retention of strong (kkl) intensities due to the polyethylene chain packing [e.g., (0,0,74), (0,0,76), (0,1,36), (0,2,74), (0,3,36), and (0,3,38)]. (However, below the α-transition, the Pca2₁ unit-cell symmetry is preserved.) On heating, the axial spacing increases ca. 2% in accord with measurements on odd-chain alkanes (32). Also note the presence of strong continuous diffuse scattering. (C) Near disappearance of diffuse electron scattering in a specimen cooled to -145°C.
angle (001) reflections, which bespeak the perfection of the lamellar structure, are modulated by a trapezoidal atomic population function due to the average density of kinks along the chain and, more importantly, to the random voids at the chain ends. Since the alignment of the chain carbon atoms in the unit cell is slightly disturbed, the transform of the polyethylene packing is also modulated by an overall gaussian term similar to a temperature factor. An attendant general falloff of intensity is also observed. [It is apparent from Table 1, however, that the kink population in this calculation, which models the concentration above the α-transition, is somewhat too large to explain the structure just below this transition. This also agrees with the spectroscopic observation that chain-end defects are more important at lower temperatures (5), and these are principally the end gauche type.] This single crystal diffraction data thus supports a kink structure for the pretransition phase of alkane chains (Fig. 4).

Although the kink defects are clearly the major determinant in the long-chain α-transition, the presence of an overall helical twist to the chain as evidenced by the crystal structures of some long-chain compounds (40) is not precluded. Even though such helices do not have so short a repeat period as polytetrafluoroethylene, such twisting will assist in imparting an overall cylindrical cross-section to the chains in the hexagonal methylene subcell. In this subcell packing, increased chain motion of these chain cylinders is expected to result in slips along the chain axes that eventually account for the screw dislocations seen in high-temperature crystal forms of linear waxes (41) and paraffins (42) and also the pretransition form of phospholipid (43). Sharp diffraction bands in our reflection electron diffraction patterns from distearoyl-sn-glycerophosphocholine, however, indicate that the alignment of methane units can be preserved in this hexagonal subcell and, thus, the chain translational jumps would involve at least increments of ½ C₀, where C₀ is the zigzag chain repeat.

An intriguing aspect of the pretransition is the observed hysteresis phenomenon, which makes it apparent that long-range lamellar order is not quickly restored when the crystals are cooled. This indicates stability of the kinked chain structure. Such a slow relaxation rate for the molecular chain uncoiling, for example, may be important for the crystallization of polymers from quenched melts as distinguished from solution (44). The persistence of continuous diffuse scattering at low temperature (Fig. 2C) may also indicate the stable inclusion or freezing of kinks into the crystalline lattice. Although this diffuse scattering is well explained by a thermal model (25) based on the known n-hexatriacontane crystal structure (23), recent experience with other molecular crystal structures (45) indicates that additional experiments must be done to distinguish thermal diffuse scattering from defect diffuse scattering.

Table 1. Structure factor magnitudes for several paraffin models

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<th>Orthorhombic unit cell</th>
<th>Rotor model, ( B = 6 , \text{Å}^2 )</th>
<th>13\text{α}-Helix, ( B = 6 , \text{Å}^2 )</th>
<th>Kink model</th>
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Fig. 3. Supercell model of \( \text{n-hexatriacontane} \) constructed to evaluate the average contribution of undistorted and kinked chain segments to the total diffraction patterns. The gauche-trans-gauche kink segments are based on the ethylene diester region of ethylene di(11-bromoundecanoate) (36, 37).
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38. Pangborn, W. A. (1973) Dissertation (Univ. of Maryland School of Medicine, Baltimore, MD).