

Electron microscopic study of supramolecular liquid crystalline polymers formed by molecular-recognition-directed self-assembly from complementary chiral components

(helical supramolecular structures/chiral liquid crystalline species/spontaneous racemate resolution)

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ABSTRACT Electron microscopic observation provides insight into the nature of the polymeric supramolecular liquid crystalline species $(TP_2, TU_2)_n$ formed by polyassociation of the complementary components TP_2 and TU_2 derived from D-, L-, or meso-tartaric acid (where T is any form of tartaric acid, D is the D species, and L is the L species) and from pyridine (P) and uracil (U) derivatives. Increasing the concentration of equimolar solutions of $(LP_2 + LU_2)$ mixtures leads to the progressive assembly of very long supramolecular-polymolecular entities. The process involves successively nucleation to give small nuclei, growth to filaments, and lateral association to tree-like species, strings, and fibers. The species formed are helical; their helicity is right-handed, induced by the chirality of the components and transferred to the larger entities. The data agree with the formulation of the primary filament as a triple-helical species formed by three helically wound supramolecular strands. $(DP_2 + DU_2)$ mixtures yield left-handed helical species. The helicity of the materials obtained from complementary components having different chirality is imposed by the U component, being, respectively, right- and left-handed for $(DP_2 + LU_2)$ and $(LP_2 + DU_2)$. No helicity is found for the meso compounds. The racemic mixture of all four L and D components yields long superhelices of opposite handedness that coexist in the same sample. This points to the occurrence of spontaneous racemate resolution by chiral selection of the components in the self-assembly of these supramolecular liquid crystalline species. The present results illustrate how extended supramolecular-polymolecular entities build up through molecular-recognition-directed polyassociation of complementary components. They also show that molecular chirality is translated into supramolecular helicity that is expressed at the level of the material on nanometric and micrometric scales.

Self-organization of molecular species into a well-defined supramolecular architecture may be induced by selective association of complementary components following a plan based on molecular recognition events. The information necessary for the process to take place must be stored in the components and the algorithm that it follows operates via selective molecular interactions (1, 2). If the organized entities thus produced lead to changes in the properties of the material, the process amounts to a macroscopic expression, at the level of the material, of molecular recognition occurring on the molecular scale. This laboratory has shown (3) that the association of complementary molecular components that are not mesogenic by themselves may yield supramolecular liquid crystalline phases. Extension to the complementary chiral components TP_2 and TU_2 (where T is tartaric acid, P is 2,6-diaminopyridine, and U is uracil), each

containing two identical recognition groups, led to the molecular-recognition-directed self-assembly of supramolecular liquid crystalline polymers (4). Association of TP_2 and TU_2 is expected to occur through formation of three hydrogen bonds between the recognition units derived from P and U and linked via a T spacer that may be L (L), D (D), or meso (M). The resulting supramolecular species is represented by $(TP_2, TU_2)_n$ (Fig. 1). It was found that, whereas the individual species $LP_2, LU_2, DP_2, DU_2, MP_2,$ and MU_2 were solids, the mixtures $(LP_2 + LU_2), (DP_2 + LU_2),$ and $(MP_2 + MU_2)$ gave thermotropic mesophases presenting an exceptionally wide domain of liquid crystallinity (from $<25^\circ\text{C}$ to $220\text{--}250^\circ\text{C}$) and a hexagonal columnar structure, with a total column diameter of $37\text{--}38 \text{ \AA}$. The data obtained led to the assignment to the $(LP_2, LU_2)_n$ species of a triple helix superstructure made of three strands resulting from the polyassociation via base pairing of the two complementary components LP_2 and LU_2 in a polymeric fashion, with the PU base pairs located at stacking distance (probably in parallel planes almost perpendicular to the axis of the column) and the aliphatic chains sticking out of the column (Fig. 2). The same structure is of course expected for the $(DP_2, DU_2)_n$ species. On the other hand for the meso form $(MP_2, MU_2)_n$, the data fitted a model built on three polymeric strands in a zig-zag arrangement (4). The special nature of these materials as molecular-recognition-induced self-assembled supramolecular liquid crystalline polymers and the effect of chirality on their structural features led us to investigate further the nature of the species present in these mesophases at a scale that could reveal their microscopic appearance.

One may also point out that the $(LP_2, LU_2)_n$ species is a triple-helix analogue of the double-helical DNA, resulting from the hydrogen-bonded base pairing between two complementary heterocyclic bases and the chirality of the T residue. These structural elements recall, respectively, the base pairs and the ribose unit in double-helical nucleic acids. In addition, the triple-helical feature bears relation to that of collagen (5) and triple-stranded nucleic acids (6).

We describe here the results of a detailed study of various mixtures of the $LP_2, LU_2, DP_2, DU_2, MP_2,$ and MU_2 substances by electron microscopy. Of particular interest was the question of how the chirality of the components would affect the structure of the $(TU_2, TP_2)_n$ species and especially whether or not chiral recognition might occur at the supramolecular level.

MATERIALS AND METHODS

The synthesis of the monomeric molecules $LP_2, LU_2, DP_2, DU_2, MP_2,$ and MU_2 used in this work has been reported (4).

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Abbreviations: T, tartaric acid; D, D-tartaric acid; L, L-tartaric acid; M, meso-tartaric acid; P, 2,6-diaminopyridine.

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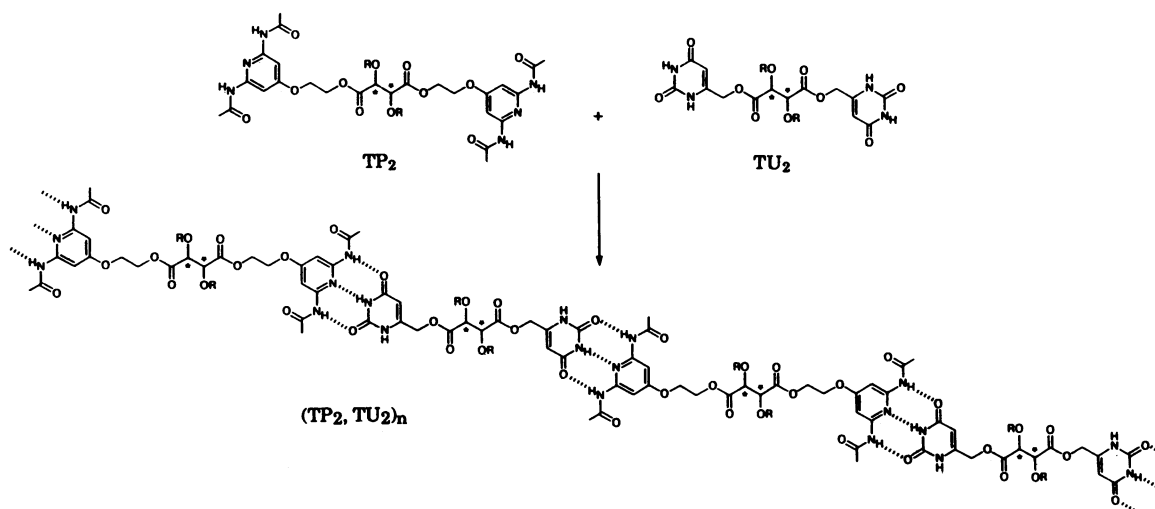


FIG. 1. Self-assembly of the polymolecular supramolecular species $(TP_2, TU_2)_n$ from the chiral components TP_2 and TU_2 via hydrogen bonding. T represents L-, D-, or *meso* (M)-tartaric acid; R is $C_{12}H_{25}$.

Each compound was dissolved in chloroform at various concentrations. The solutions of complementary molecules were obtained by mixing the same volumes of equimolar chloroformic solutions of individual components. They were then deposited, as very thin layers, on carbon-coated electron microscope grids, which, after evaporation of the solvent, were shadowed with platinum-carbon in a Balzers 301 freeze-etching unit and observed with a Philips 301 electron microscope.

RESULTS

$(LP_2 + LU_2)$ Mixtures. The most extensively studied materials were the equimolar $(LP_2 + LU_2)$ mixtures. Depending on the initial concentration of the components, different

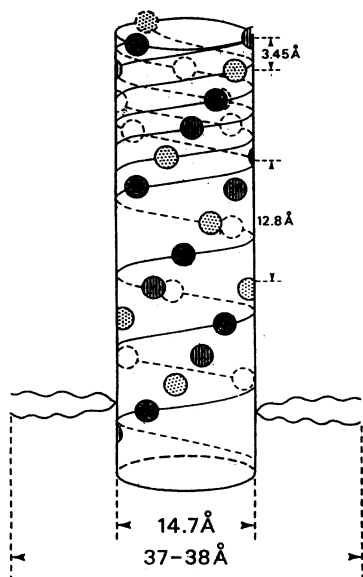


FIG. 2. Schematic representation of the columnar superstructure suggested by the x-ray data for $(LP_2, LU_2)_n$; each spot represents a PU or UP base pair; spots of the same type belong to the same helical strand of the triple helix; the dimensions indicated are compatible with an arrangement of the PTP and UTU components along the strands; the aliphatic chains stick out of the cylinder, more or less perpendicularly to its axis. For $(LP_2, LU_2)_n$, a single helical strand and the full triple helix are, respectively, represented on the bottom and at the top of the column (4).

polymeric associations were observed, after evaporation of the solvent (Fig. 3).

For highly diluted solutions ($<0.5 \mu\text{g/ml}$), only very small particles were observed (Fig. 3A). For a 10 times higher concentration ($5 \mu\text{g/ml}$), short linear entities were formed (Fig. 3B). Their width was measured on filaments oriented in the direction of shadowing; their height was calculated from the measured length of shadow and the knowledge of the angle of shadowing, for species oriented perpendicularly to the direction of shadowing. The values of the mean width and heights were found to be very close ($\approx 45 \pm 5 \text{ \AA}$), indicating cylindrical symmetry.

Increasing the initial concentration of the components to $>50 \mu\text{g/ml}$ led to the appearance of two-dimensional associations (Fig. 3C and E). The distance between the polymeric molecules, which can be precisely measured by optical diffraction on the partially oriented samples, is of the order of the transverse dimension of separated individual filaments (40 \AA , as compared to $45 \pm 5 \text{ \AA}$). Very long ($>2 \mu\text{m}$) helical strings were also observed (Fig. 3D). In some places, more complex structures appeared on the first layer of two-dimensional associations (Fig. 3F). These latter structures, which always developed in the direction parallel to the orientation of the polymeric molecules, displayed the same right-handed helicity everywhere.

$(DP_2 + DU_2)$ Mixtures. The concentration-dependent behavior of D-D mixtures (Fig. 4) was similar to that of L-L mixtures; small particles, linear individual molecules and their two-dimensional associations, and complex superhelical structures were observed. The only, but fundamental, difference concerned the handedness of the superhelical structures that, as expected, was left-handed—i.e., opposite to that of the L-L mixtures.

$(DP_2 + LU_2)$ Mixtures. Again the concentration dependence of these mixtures was similar to that of the L-L mixtures. The large majority of superhelical structures displayed right-handed L-L type helicity (Fig. 5A and B). In some places, somewhat different morphologies were found, but nowhere could D-D type left-handedness be detected.

$(LP_2 + DU_2)$ Mixtures. These mixtures gave superhelical structures displaying D-D type left-handedness with morphologies resulting from merging of fibers (Fig. 5C). In some places a different morphology was present (Fig. 5D), but nowhere could L-L type right-handedness be detected.

$(MP_2 + MU_2)$ Mixtures. The concentration-dependent behavior of these mixtures was very different from all of the mixtures of chiral components described above. No individ-

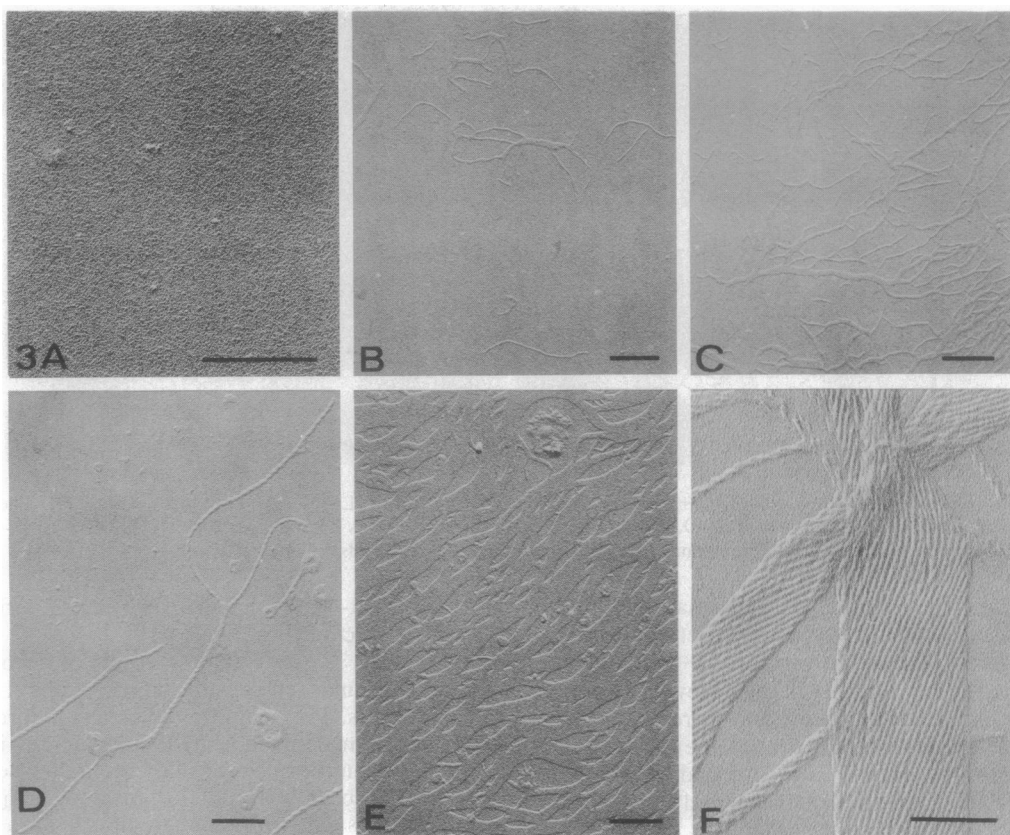


FIG. 3. Air-dried very thin layers of $(LP_2 + LU_2)$ mixtures on carbon-coated electron microscope grids. The concentrations of the chloroform solutions of the components were $0.5 \mu\text{g/ml}$ (A), $5 \mu\text{g/ml}$ (B), $50 \mu\text{g/ml}$ (C), and $500 \mu\text{g/ml}$ (D–F). Only small particles are present in A, elongated molecules are in B, elongated molecules and some two-dimensional associations are in C, long superhelical strings are in D, two-dimensional sheets of polymeric molecules are in E, and superhelical structures developing on the two-dimensional sheets are in F. (Bars = $0.2 \mu\text{m}$.)

ual or superhelical structures were observed. Only thin layers of material having ill-defined structure were formed after evaporation of the solvent (Fig. 6).

$(LP_2 + LU_2 + DP_2 + DU_2)$ Racemic Mixtures. Racemic preparations were obtained by mixing equimolar amounts of the solutions of all four individual components. After evaporation of the solvent, some superhelical structures were formed. Most of them displayed L–L type handedness, some were D–D type, and others were both types within the same domains (arrows in Fig. 7).

DISCUSSION

The choice of the method used in this work to follow the self-assembly of supramolecular polymeric species was based on the assumption that the evaporation of the solvent

from very thin layers of solutions containing different concentrations of the components would stop the self-assembly at different stages of development. A large domain of concentrations was explored, starting from solutions allowing the coverage of only a very small part of the accessible surfaces up to solutions resulting in the formation of many layers of the dry products. Hydrophobic surfaces (carbon-coated electron microscope grids) were used to ensure the uniform spreading of chloroform solutions leading to its uniform evaporation and, consequently, to uniformly distributed materials.

The modifications of the material obtained from $(LP_2 + LU_2)$ mixtures as a function of the concentration of the solutions agree with the progressive assembly of polymolecular species of supramolecular nature, by multiple linear association of the chiral complementary components as de-

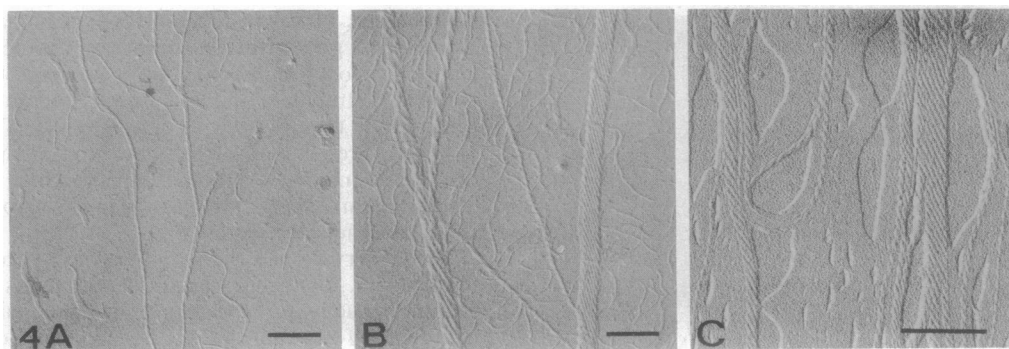


FIG. 4. Air-dried chloroform solutions of $(DP_2 + DU_2)$ mixtures. The concentration of the solutions was $5 \mu\text{g/ml}$ in A, $50 \mu\text{g/ml}$ in B, and $500 \mu\text{g/ml}$ in C. Notice the presence of superhelical structures with handedness opposite to that found for the L–L mixtures. (Bars = $0.2 \mu\text{m}$.)

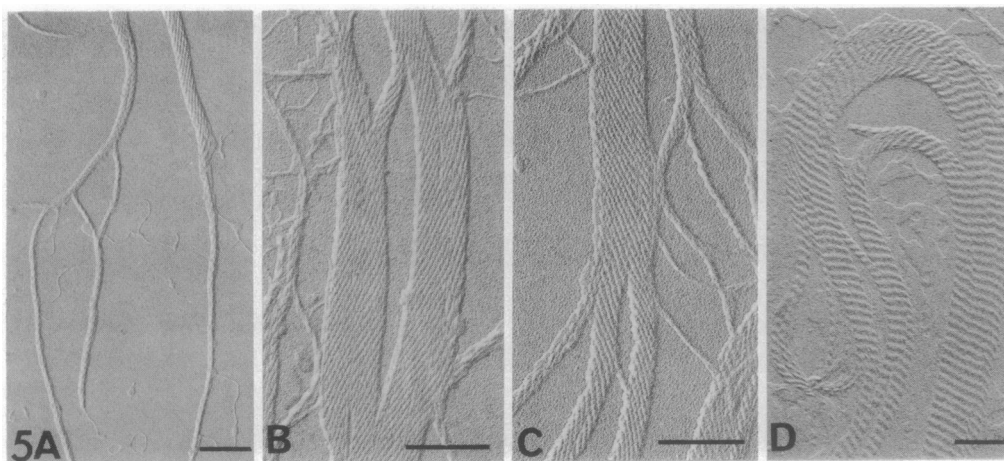


FIG. 5. Air-dried chloroform solutions of $(DP_2 + LU_2)$ mixtures (A and B) and of $(LP_2 + DU_2)$ mixtures (C and D). The concentration of solutions was $50 \mu\text{g/ml}$ in A and $500 \mu\text{g/ml}$ in B–D. Notice the superhelical structures having L–L type (A and B) and D–D type (C and D) handedness and the regions showing two-dimensional packing of parallel helical strands. (Bars = $0.2 \mu\text{m}$.)

pictured by structure $(TP_2, TU_2)_n$ (Fig. 1). One may analyze the process in three stages and five types of structural features: at low concentration, nucleation takes place yielding very small particles or nuclei (Fig. 3A); then growth by linear expansion forms filaments (Fig. 3B); and thereafter, lateral association, probably by entanglement of the dodecyl side chains gives tree-like species (Fig. 3C) and superhelical strings (Fig. 3D) that evolve toward thicker and thicker fibers as more filaments interact laterally (Fig. 3F).

The species formed present helical chirality and the diameter of the individual filaments ($45 \pm 5 \text{ \AA}$) is very close to that of the columnar superstructures ($37\text{--}38 \text{ \AA}$, Fig. 2) deduced from x-ray diffraction study of oriented gel phases. This agrees with the triple-helical nature of the filaments constituted by three strands of polyassociated complementary components (4). Helical wrapping of the primary triple-helix filaments around each other yields thicker and very long ($>2 \mu\text{m}$) string-like helical species (Fig. 3D). At higher concentration, two-dimensional sheets are obtained on top of which still more complex superhelical structures may develop displaying textures of a sort of supramolecular macramé (Fig. 3F)! For a given pair of complementary chiral components (LP_2, LU_2) or (DP_2, DU_2) , these helical and superhelical structures uniformly show the same handedness, respec-

tively, right- or left-handed for species derived from L- or D-tartaric acid (Figs. 3 and 4).

All these features are the result of recognition-induced supramolecular self-organization from chiral complementary components. They confirm the helical nature of the primary species formed and indicate that its helicity is transferred to the larger entities that it builds up. Self-assembly of helical fibers has been reported for instance in gels of guanine nucleosides (7), in micellar fibers of a phospholipid-nucleoside conjugate (8) and of *N*-alkylaldonamides (9), and in lithium stearate grease (10, 11).

In mixtures of complementary components having different chirality, the helicity handedness is found to be imposed by the U component (Fig. 5). Thus, $(DP_2 + LU_2)$ and $(LP_2 + DU_2)$ display, respectively, L–L type and D–D type handedness. The origin of this phenomenon is not clear. It could result from stronger stereochemical preference of the TU_2 unit relatively to TP_2 . It points to a twisting of a phase by one of its components. It will be of interest to determine the composition of the (DP_2, DU_2, LU_2) mixture in which LU_2 is able to induce its handedness in the supramolecular entities. One may note that this would amount to a signal amplification, since a minor component ($<50\%$) would impose its features at the supramolecular level and on a nanometric scale.

The absence of helical features in the materials formed by the meso components (Fig. 6) is not unexpected and agrees with the nonhelical triple-strand zig-zag superstructure proposed on the basis of the x-ray diffraction data (4).

Of particular interest is the behavior of the racemic solutions containing all four chiral components $LP_2, LU_2, DP_2,$ and DU_2 (Fig. 7). The formation of long L–L or D–D type superhelices of opposite handedness indicates that, once started, the growth of these supermolecules continues by addition of only stereochemically compatible components. When two strands of opposite helicity merge laterally, chevron type of textures are formed (Fig. 7). The presence of less-structured domains may be due to too rapid evaporation of the solvent, hindering the correct choice of the complementary components and allowing only the formation of very short superhelical species.

The observation of superhelices points to an important feature—i.e., the occurrence of spontaneous racemate resolution through *chiral selection* in molecular-recognition-directed self-assembly of supramolecular liquid crystalline polymers. However, in view of the results obtained for $(DP_2 + LU_2)$ and $(LP_2 + DU_2)$ mixtures, the composition of the superhelical structures may be either pure (LP_2, LU_2) and

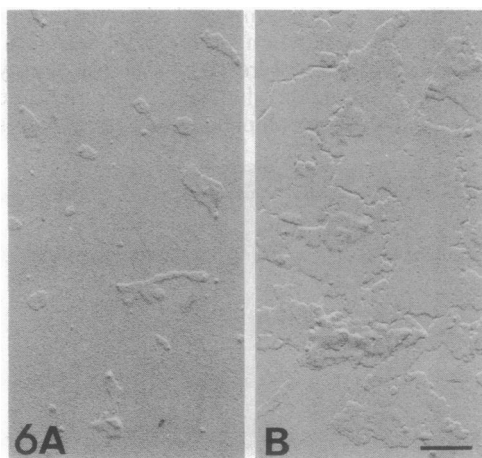


FIG. 6. Air-dried chloroform solutions of $(MP_2 + MU_2)$ mixtures. The concentration of solutions was $50 \mu\text{g/ml}$ in A and $500 \mu\text{g/ml}$ in B. Only structureless thin layers of material are deposited as single or multiple layers. (Bars = $0.2 \mu\text{m}$.)

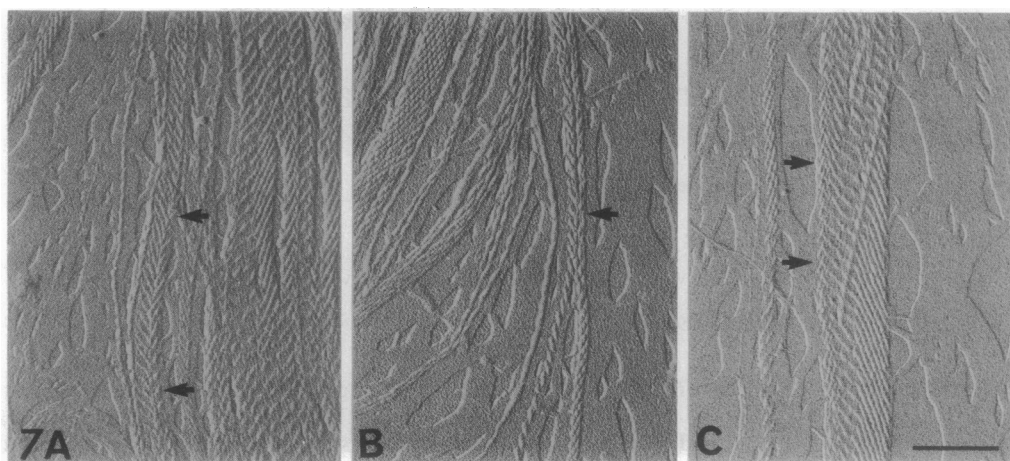


FIG. 7. Air-dried chloroform solutions of racemic mixtures of LP₂, LU₂, DP₂, and DU₂. Notice the presence of superhelical structures showing L-L and D-D type of handedness and of extended regions of mixed handedness (arrows). (Bars = 0.2 μm.)

(DP₂, DU₂) or contain also DP₂ and LP₂, respectively, since the mixed species adopt the chirality imposed by the U component, so that the composition of each superhelical species could be (DP₂, LP₂, LU₂) and (DP₂, LP₂, DU₂) for right- and left-handed helices, respectively. Chain-length-induced racemate resolution has been reported in the formation of right-handed and left-handed helices by D- and L-glucanamides bearing, respectively, *N*-octyl and *N*-dodecyl chains (11). Such chiral selection features of self-organized entities are of general significance in connection with the questions of spontaneous resolution and of chirality amplification.

The present data have been obtained by air drying chloroform solutions containing complementary components on hydrophobic surfaces. We do not know whether the observed structures were formed in the solutions during the evaporation of the solvent and then "deposited" on the surface or whether they form exclusively on these hydrophobic surfaces. A preliminary answer was recently obtained by freeze drying of the diluted solutions that indicated the presence of almost identical structures in air-dried and freeze-dried preparations. More direct information will probably come from the study by cryoelectron microscopy of ultrarapidly frozen solutions of the same mixtures of components.

CONCLUSION

The present results provide evidence and illustration through direct observation for the self-organization of helical supramolecular entities through molecular-recognition-directed polyassociation of chiral complementary components. Molecular chirality is thus translated into supramolecular helicity that is expressed at the level of the material

at nanometric and micrometric dimensions. Molecular recognition based on the molecular information stored in the components should make possible the spontaneous but designed generation of supramolecular shapes from complementary units. Such recognition-directed automorphogenesis (1) (see for instance ref. 12) provides access to the controlled self-organization of well-defined polymolecular architectures and patterns at the supramolecular level in gels, micelles, molecular layers, films, and molecular solids.

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