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A Helical Polymer with a Cooperative Response to Chiral Information

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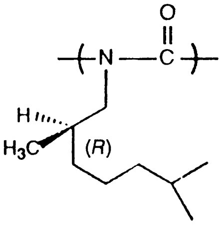
Polyisocyanates, long studied as theoretical models for wormlike chains in dilute solution and liquid crystals, differ from their biological helical analogs in the absence of a predetermined helical sense. These polymers have an unusual sensitivity to chiral effects that arises from a structure in which alternating right- and left-handed long helical blocks are separated by infrequent and mobile helical reversals. Statistical thermodynamic methods yield an exact description of the polymer and the cooperative nature of its chiral properties. Minute energies that favor one of the helical senses drive easily measurable conformational changes, even though such energies may be extremely difficult to calculate from structural theory. In addition, the chiral nature of the polymer can be used to test theoretical ideas concerned with cholesteric liquid crystals, one of which solves the problem of assigning the helical sense.

Polyisocyanates are stiff helical polymers that differ from helical biopolymers such as nucleic acids and proteins in the absence of chiral information in the building blocks (*1*, *2*). This means that both the left- and right-handed helical senses are equally probable and that in long polymers, intramolecular helical reversals may occur. The cooperative stereochemical phenomena that arise from this structure allow very small chiral biases in each monomer unit of the polymer to be magnified to control the helical sense of large portions of the chain. Study of the underlying detailed principles provides a new understanding of these complex macromolecular systems.

The polyisocyanate molecules are not perfect rods, and it was argued that the experimentally measured flexibility (relative to a perfectly stiff rod) was primarily due either to small torsional oscillations about the backbone rotational angles or to the existence of kinked helical reversals (*3*). One approach to resolving this question is the exclusion of the helical reversals from the chain by the incorporation of asymmetric pendant groups in the structure so that only one helical sense is formed. Large optical activities arising from the helical chromophore were then observed (*4*). One example of a substituent that produces such an effect is shown below.

A remarkable behavior was observed in other experiments designed to discriminate between the helical senses. Polymers formed from nearly equal proportions of mirror-image monomer units exhibited optical activity properties identical to those of polymers formed from enantiomerically pure monomers. Copolyisocyanates synthesized mainly from units with no preference for one helical sense but containing a small fraction of units with a helical sense preference showed relatively large optical activities arising from a large excess of one helical sense. Asymmetry in the side chains of the polyisocyanates arising from substitution of deuterium for hydrogen also forced large excesses of one helical sense over the other. Even dissolving the polyisocyanates in chiral solvents could cause an easily measurable excess of one helical sense.



These stereochemical phenomena are classic manifestations of cooperativity arising from the difficulty of forming the state that would reverse the helical sense, thereby forcing large numbers of the polymer chain units to act together in their response to the forces that direct chirality. Statistical mechanical studies of a three-state model (left- and right-handed helical states and the helical reversal state) reproduced the chain length and temperature dependence of the optically active polyisocyanates. This model allowed us to determine the energy of the helical reversal and therefore its population. Results of this study also led to the conclusion that the chain dimensions are controlled largely by an accumulation of local segmental motions rather than by specific breaks or kinks. This same method allowed determinations of chiral discrimination energies that were so small that the precision of structural theory was inadequate for their interpretation, a rare but not unparalleled situation arising from an equilibrium entirely due to the cooperative accumulation of minute energies.

Two classic routes to determine the relation of the sign of optical activity to the helical sense could not be used in the polyisocyanates. Crystals large enough to achieve atomic resolution in x-ray crystallography cannot be grown for polyisocyanates, as is the case generally for synthetic polymers. The chromophore of the helix, which is easily excited by ultraviolet (UV) light and shows substantial preferential selective absorption of circularly polarized light, cannot be described with the necessary theoretical precision to confidently assign the helical sense. Therefore, we designed another approach to determine the helical sense. The optically active polyisocyanates form cholesteric liquid crystals, and the pitch of the cholesteric liquid crystal can be connected to a theory concerned with the fitting of screws in a way that yields the helical sense of the polyisocyanate.

Optically Active Polyisocyanates

The helical chromophore in the polyisocyanates absorbs UV light, and if one sense of the helix predominates, there will be a differential absorption of left- and right-handed circularly polarized light that can be measured by circular dichroism (CD). This effect, as expected, is seen for the polyisocyanates constructed from monomer units that are asymmetric in the opposite mirror sense (Fig. 1). The CD spectra are of equal and opposite sign. What is surprising is the observation that a polyisocyanate constructed by the random placement of nearly equal numbers of these mirror-image related units exhibits the same CD spectrum as one constructed from only one kind of unit (*5*) (Fig. 1).

If the helical reversals between left- and right-handed helical segments of the polymer are difficult to attain, this surprising

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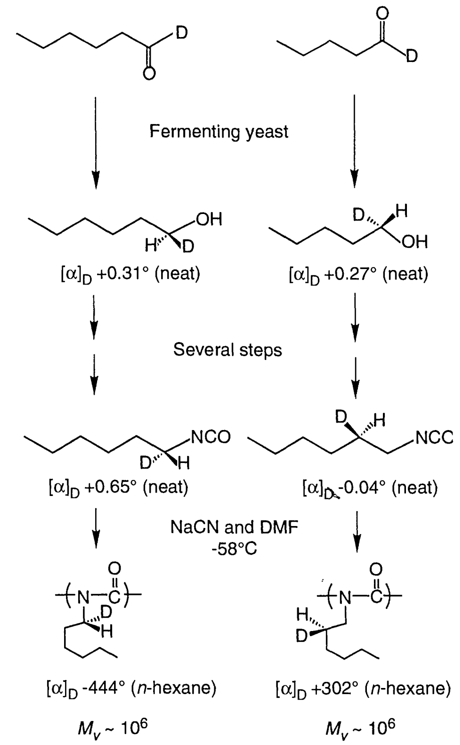
observation could arise as the side-chain mirror-image monomer units are all forced to take the same backbone helical sense. In this single helical sense extending over many units of the chain, there would be an averaging of the unit energies favoring the two helical senses, and the driving force for choosing the helical sense of the majority units would grow larger as the cooperating units grew in number. The lower energy of the helical sense of the majority units would lead to fewer helical reversals as the number of monomer units with a preference for the minority helical sense decreased. From the data in Fig. 1, the helical reversals are negligible in the copolymer of a ratio of 56/44 of one mirror image unit to the other but become significant with a ratio of 51/49. It is, however, remarkable in this copolymer that the intensity of the CD spectrum is about one-third of that in the homopolymer, indicating that twice as many segments adopt the helical sense preferred by the majority monomers. The ability of the majority units to impose their preferred helical sense becomes more prominent as the helical reversal becomes more difficult to attain (*6, 7*).

This cooperative picture is supported by the finding that a series of polyisocyanates synthesized with even very small proportions of units favoring one helical sense randomly dispersed among large numbers with no preference exhibit CD spectra that are identical in form to those in Fig. 1 and whose intensities correspond to helical sense excesses out of proportion to the fraction of the chiral units. This behavior reaches a remarkable limit in a copolymer with only one chiral unit in a thousand parts, which shows an optical activity nearly 20% of the value for a polyisocyanate consisting of only these chiral units (*8*).

The optical activities in these experiments depend strongly and reversibly on temperature, so that the helical reversal states that both enforce and limit the cooperativity are not locked-in by structural changes made during synthesis. Instead, as discussed below, the helical reversal population changes sharply with temperature.

The question of optical activity arising from isotopic substitution intrigued investigators for many years after the discovery of deuterium (*9*). The problem, as seen in the α and β deuterio-*n*-hexyl isocyanates and their precursors (Scheme 1), was the very small values measured. The very large values for the derived polyisocyanates (Scheme 1) do not arise directly from the isotopic substitution but from the helical chromophore. The CD spectra of both deuterated polyisocyanates (Fig. 2) take the same form as seen in Fig. 1 (*10*). In the polyisocyanates with the stereospecifically deuterated side chains, the mirror-image relation of the left- and right-handed helical states is broken. However small this effect is expected to be per monomer unit, the results show that the cooperativity multiplies these effects so that the difference in the energy of the right- and left-handed helices becomes sufficient to lead to a large excess of one helical sense.

The sensitivity of this polymer can extend to chiral information provided by the solvent environment. To exhibit such a response, a molecule must respond to chiral information by easily interconverting between mirror-image forms (*11*). When unperturbed by chirality, both forms coexist equally and stereomutate at equal rates across the mirror boundary that separates them. The left- and right-handed helical conformations in polyisocyanates constructed of achiral monomers should provide such an example. Although this phenomenon, termed by Kuhn (*11*) as an asymmetric transformation of the first kind, has potential importance in revealing details of the interactions between molecules, there are only a few examples.



Scheme 1: Key intermediates and optical activities at the sodium D line for the syntheses of (left) poly[(*R*)-1-deuterio-*n*-hexyl isocyanate] and (right) poly[(*R*)-2-deuterio-1-hexyl isocyanate]; DMF, dimethylformamide; M_v, viscosity average molecular weight.

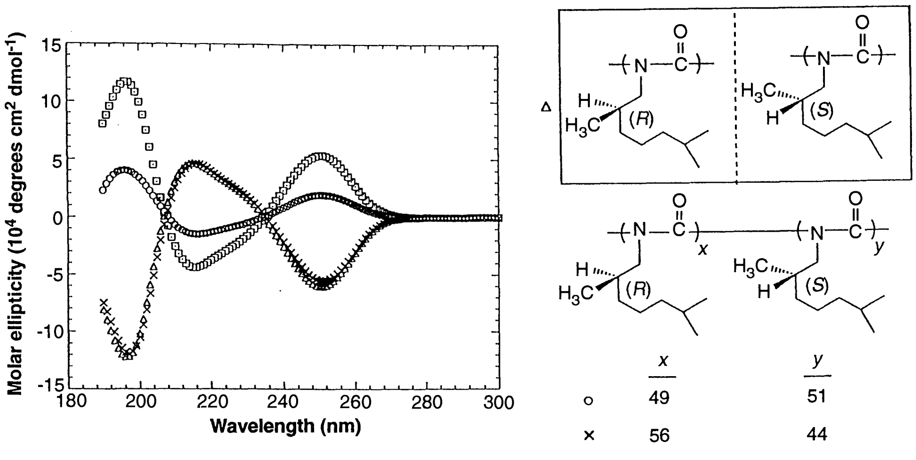


Fig. 1. Circular dichroism spectra in hexane solution at room temperature of the homopolymers and copolymers of (*R*)- and (*S*)-2,6-dimethylheptyl isocyanates plotted as molar ellipticity against wavelength. [Reprinted from (*5*) with permission, American Chemical Society]

When poly(*n*-hexyl isocyanate) is dissolved in a series of optically active chlorinated hydrocarbons (*12*), the familiar CD spectrum reveals the inequality of the helical senses, demonstrating the asymmetric transformation. Moreover, changes in the structures of the chiral solvents that would not be expected to play a large role make a large difference in the magnitude of the effect and can even switch the preferred helical sense of the dissolved polyisocyanate (Fig. 3).

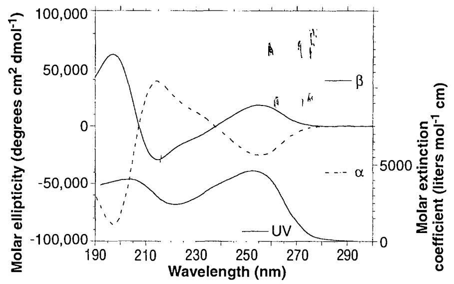


Fig. 2. Circular dichroism and UV spectra for poly[(*R*)-1-deuterio-1-hexyl isocyanate] (α) and poly[(*R*)-2-deuterio-1-hexyl isocyanate] (β) in hexane plotted as wavelength against molar ellipticity and molar extinction coefficient, respectively.

Statistical Thermodynamic Model for Polyisocyanate Cooperativity

All structural evidence in poly(alkyl isocyanate) is consistent with a helical conformation that is variable only in the mirror-image sense. The population of helical reversals must be low to be consistent with the extended chain dimensions, otherwise the chain would bend or kink. This property implies long sequences with the same helical sense with the length depending in long chains on the frequency of the reversals and in short chains on the degree of polymerization. The degree of polymerization N will be the dominant parameter when the number of units between reversals, as determined by the reversal energy G_r and the temperature T, is far larger than N. In that case, most chains will be of a single helical sense, that is, the polymer sample in the absence of a chiral bias will be racemic in the conventional sense with equal numbers of right- and left-handed macromolecules.

If we consider the above picture modified by a chiral perturbation, such as deuterium substitution (Scheme 1 and Fig. 2) or chiral solvation (Fig. 3), that is uniformly distributed over all units, the enforced same helix sense of many units is the source of the cooperativity. Each unit must act in concert with many neighboring units, which causes the averaging and amplification properties responsible for the unusual experimental results. The larger the number of cooperating units, the larger the aggregate effect of the chiral bias favoring one helical sense, and therefore, the larger the optical activity that is directly related to the ratio of the helical senses. This ratio depends on two energies: The very small free energy difference per monomer residue between the left- and right-handed helical senses, G_h, and the much larger free energy difference between the helical and the helical reversal state, G_r (*13*).

Statistical mechanical analysis leading to Eqs. 1 and 2 expresses the quantitative characteristics of the cooperative picture. In short chains (Eq. 1), where N is much smaller than the cooperative length $l = \exp(G_r/RT)$ (R is the gas constant), the number of cooperating units depends directly on N, and the effect of T on the helix sense ratio, as measured by the optical activity, is similar to that of other equilibria. In long chains (Eq. 2), where l is small compared to N and the population of reversals rises sharply with T, the number of cooperating units depends strongly on T and not on chain length. This effect appears as an unusually steep dependence of the optical activity on T.

$$\ln\left(\frac{[\alpha]_m + [\alpha]}{[\alpha]_m - [\alpha]}\right) = \frac{2G_hN}{RT} \qquad (1)$$

$$[\alpha] = [\alpha]_m \frac{\dfrac{lG_h}{RT}}{\left[\left(\dfrac{lG_h}{RT}\right)^2 + 1\right]^{1/2}} \qquad (2)$$

In Eqs. 1 and 2, the terms $[\alpha]$ and $[\alpha]_m$ are the observed and the single-helical-sense maximum specific rotations, respectively. Consistent with the qualitative model for the cooperativity, G_r does not appear in Eq. 1 and N does not appear in Eq. 2.

The numerical analytical methods necessary for precise experimental verification of the predictions of the partition function for the polyisocyanates (*13*) required a large number of polymer samples with narrowly defined degrees of polymerization ranging from very short to very long chains. Fortunately, the methods of synthesis used to produce the deuterated polyisocyanates (*14*) yield mixtures with a wide variety of molecular weights. After broadening the distribution even further (*15*), we subjected these mixtures to separation by a chromatographic method that involves passing a dilute solution of the polymers through a column of porous beads that retard the flow of the shorter chains and quickly pass the longer chains, depending on their size (*16, 17*). The amounts collected of these samples were so small that they were handled in solution, and their amounts and N values were determined by comparison to the UV absorption and light-scattering properties of standard samples.

A numerical analysis of the temperature dependence of the optical activities in this broad range of samples allowed only a narrow range of values of G_h and G_r to fit the data. Figure 4 shows the fit between experiment and theory for the polyisocyanate stereospecifically deuterated at the β position with precisely the predicted strong dependence of the optical activity on N at low molecular weights moving to a strong dependence on T at high molecular weights (*17*). The α-deuterated polyisocyanate fit equally well, as did the experimental temperature and molecular weight dependence of the optical activities of poly(n-hexyl isocyanate) dissolved in one of the chiral solvents, (R)-2-chlorobutane (*12*).

In a critical test of the theory and the experimental fit, the excess energy of the helical reversal, G_r, is independent of the source of the chiral discrimination in both polar and nonpolar solvents. This conformational cooperative picture of the polyisocyanates passes all tests. Let us now see how the values of the various free energies may be used.

Conformational Properties of the Polyisocyanates

We used the value of G_r in hexane, 3900 cal/mol, to define the torsional potential about the recurring backbone amide bonds in an otherwise unchanged empirical force field (EFF) and were able to ask detailed questions about the conformational properties of the polyisocyanates (*18*). The EFF indicated an unusual property of the polyisocyanates. Both x-ray measurements on oriented films (*19*) and the EFF show the helix to arise from alternating approximately cis and trans arrangements around the amide bonds of the backbone. When the torsional

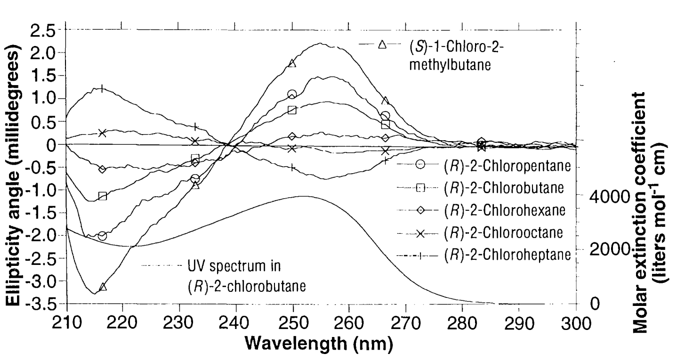


Fig. 3. Circular dichroism spectra plotted as ellipticity angle against wavelength for poly(n-hexyl isocyanate) dissolved in various optically active solvents. Experimental data are the continuous lines with the symbols designating the solvent. The UV spectrum is plotted as wavelength against molar extinction coefficient for poly(n-hexyl isocyanate) dissolved in (R)-2-chlorobutane. [Reprinted from (*12*) with permission, American Chemical Society]

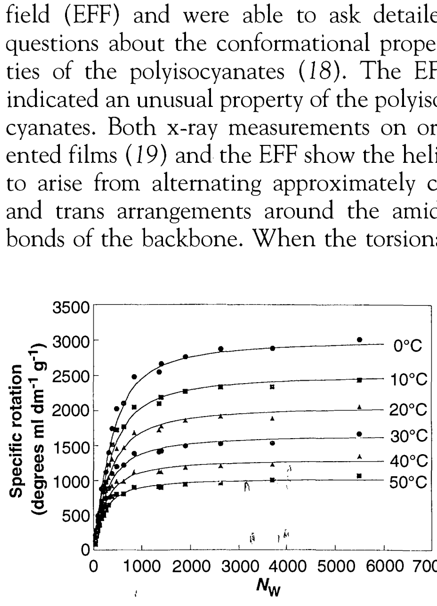


Fig. 4. Fitting of the temperature and degree of polymerization (N_W) dependence of the specific rotation at a wavelength of 300 nm for poly[(R)-2-deuterio-1-hexyl isocyanate] in 1-chlorobutane against the statistical thermodynamic theory. Symbols, experimental data; curves, theoretical values calculated with G_h = 0.36 cal/mol and G_r = 3900 cal/mol at 30°C. [Reprinted from (*17*) with permission, American Chemical Society]

angles about these bonds move collectively, that is, as one increases and the other decreases, the number of monomer units per helical turn changes over a large range but with a remarkably small energetic cost and with only a small change in the displacement along the backbone per monomer unit (*18*). This correlated twisting is therefore perfectly consistent with the stiff character of the polymer. Its prerequisite is a helicity entirely based on the structural characteristics of the backbone. Other synthetic helical polymers may also exhibit this motion. For comparison, such a motion in an α-helical polypeptide or in DNA would exact a high cost by interfering with the hydrogen bonding networks.

On the contrary, the energetically costly helical reversal arises when there is a large torsion of either of the alternating approximately cis or trans bonds in the backbone that is not compensated by a correlated torsion as above. Figure 5 shows this force-field-energy–minimized helical reversal along with short sections of the helices of opposite sense.

While the force field calculation shows the helical reversal to cause a bend in the chain of about 50° (Fig. 5), the excess energy of the reversal in hexane translates at ambient temperatures to one reversal about every 150 nm along the backbone. Poly(*n*-hexyl isocyanate) is experimentally characterized in hexane by a persistence length q of 40 nm, which corresponds to a correlated direction for over 200 monomer residues. This characteristic of stiff chains is defined by the equation $\langle \cos\theta \rangle = \exp(-L/q)$, where $\langle \cos\theta \rangle$ is the average cosine of the angle made by tangents at the ends of a chain of contour length L. The reversals are simply too far apart at 150 nm and cause too small a deviation in chain direction (50°) to make an important contribution to the chain dimensions. In this quantitative evaluation of the role of the helical reversals on the global dimensions of the polyisocyanates, a long-standing unsolved problem in polymer physics (*3*) yields to a stereochemical approach (*20*). Thermal fluctuations must therefore be the main feature controlling the persistence length, that is, the less-than-rodlike characteristics of poly(alkyl isocyanates); this interpretation is supported by calculations. Very small independent fluctuations about the preferred energy minima in bond torsion and angle can reproduce the experimental radius of gyration and persistence length (*21*).

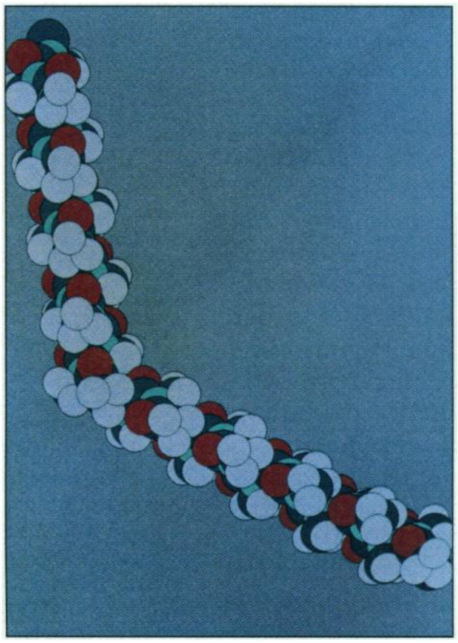


Fig. 5. Conformational representation of the helical reversal in poly(ethyl isocyanate) calculated in (*18*).

The importance of local fluctuations offers a clue to another long-standing question in the poly(alkyl isocyanates), the large reduction of polymer dimension when dissolved in polar solvents (*22*). In the presence of a nonpolar solvent or in a vacuum, the energy dependence of the torsional motions about the bonds within the helical structure may be expected to have a shape that is determined by the competition between the conjugative stabilization of planarity and the steric repulsions of planarity. As solvent polarity is increased, there is an additional polarization interaction between the solvent and the chain backbone stabilizing more planar local geometries. This arises because the planar state of an amide, cis or trans, can support increased charge separation (*23*). This results in a slight broadening of the torsional energy well and a corresponding increase in the fluctuation amplitude. Solvent-dependent nuclear magnetic resonance linewidth measurements support this conclusion (*24*). The polymer dimension is exceptionally sensitive to the magnitude of these fluctuations, so that their increase will decrease the global chain dimensions (*22*).

Although the experimental information on the helical reversal state has led, as seen above, to a windfall of conformational insights, much less can be made from the chiral discriminations. Their magnitude is the problem. These energies, which are in the range of 1 cal per mole of monomer units (*13*, *17*), are, considering their large conformational effect, remarkably small. They may be compared to other somewhat larger structural isotope effects determined in noncooperative systems where changes in conformational equilibria associated with deuterium substitution were measured. These are known to arise from bond motional differences of the substituted hydrogens (*25*, *26*). This means that slight differences in vibrational frequency for the stereochemically distinct diastereotopic hydrogens on the α and β methylene groups of poly(*n*-hexyl isocyanate) are the source of the discrimination between the left- and right-handed helical senses upon deuterium substitution. Because deuterium substitution for the higher rather than the lower frequency bond yields the lowest energy state (*26*), we can easily assign relative frequency to the diastereotopic hydrogens on the concerned methylene groups in poly(*n*-hexyl isocyanate) as a function of the helical sense of the backbone. As the reversals move and the helical sense changes, the hydrogens on the affected methylene groups exchange frequency positions. Although a correlation with theory to predict these frequencies is possible in principle, it would be very difficult considering the magnitude of the effects.

This difficulty of relating these effects to molecular structure arises from the insufficient accuracy of present theoretical tools. This finding is reinforced in the chiral solvation experiments because the chiral bias favoring one helical sense when poly(*n*-hexyl isocyanate) is dissolved in (*R*)-2-chlorobutane is minuscule compared to even the structural isotope effects, 0.04 cal per mole of monomer residues (*12*).

The prerequisite for the experimental determination of such minute energies is not just a cooperative system but one in which the equilibrium is determined by the accumulation of these minute energies (*27*–*29*). In crystals and proteins, two other examples of cooperative structures, very weak interactions exist but are overlaid on much larger forces that dominate in controlling the structure.

The Lyotropic Cholesteric State of Polyisocyanates

As an isotropic solution of elongated stiff objects increases in concentration, the disorderly arrangement is associated with a decrease in translational entropy related to the excluded volume of the solute particles. A phase boundary is reached at higher solution concentrations in which the objects order themselves. This birefringent liquid-crystal state causes a large decrease in orientational entropy with a compensating large increase in translational entropy. In this way, Onsager (*30*) was able to account for the behavior of aqueous solutions of tobacco mosaic virus (TMV) without invoking energetic interactions between the charged viral particles.

The nematic state of TMV is an interesting exception to the rule that chiral mesogenic solutes form the twisted variant of the nematic state, the cholesteric liquid crystal. Following Onsager's approach, Straley (*31*) was able to connect the fact that helices take up less space when packed in a helical arrangement compared to a parallel arrangement to a larger excluded volume for the parallel arrangement and therefore a lower translational entropy, that is, a lower stability for such helices in a nematic over a cholesteric state. Figure 6 represents this idea with right-handed screw bits. The entropic source of the twisting does not, however, act alone (*32*) because various energetic interactions of chiral objects also lead to higher stability in the twisted state.

In poly(γ-benzyl-L-glutamate), the helical sense is known by correlation of the optical activity properties with those of the α-helical sections of sperm whale myoglobin, where x-ray crystallography yields the sense of the helices (*33*). This polypeptide has been the subject of extensive research related to theory in the area of liquid crystals (*34*). Such an x-ray analysis with the necessary atomic resolution is, however, not possible to solve the problem of helical-sense assignment for synthetic polymers such as the optically active polyisocyanates because crystals cannot be grown large enough. However, polyisocyanates form lyotropic liquid crystals (*35*), and Straley's (*31*) screw model may be used to assign the helical sense of an optically active polyisocyanate. Figure 6 demonstrates that the right-handed screw bits fall into a counterclockwise arrangement among the screws, predicting a left-handed cholesteric state.

Extrapolation of the T^{-1} dependence of the cholesteric pitch wave number (2π/pitch) to zero allows the isolation of the entropic term discussed by Straley (*31*). This extrapolation has been carried out for a triple-helical polysaccharide in water, for poly(γ-benzyl-L-glutamate) in several solvents, and for poly-(*R*)-(2,6-dimethylheptyl isocyanate) in two solvents (*36*) (Fig. 7). The extrapolated twist state of the cholesteric arrangement is counterclockwise for the former polymers in all of the solvents and clockwise for the polyisocyanate. Following Straley's model (Fig. 6), this corresponds to a right-handed helix for the polysaccharide and the polypeptide (*36*), a result consistent with other evidence, whereas in the polyisocyanate, an EFF calculation (*18*) predicts the left-handed helix, again in agreement with the result obtained from the liquid-crystal behavior (Fig. 7). However, this approach may not be universally applicable because right-handed screws with large diameters and a small pitch pack most closely clockwise rather than counterclockwise, as in Straley's model.

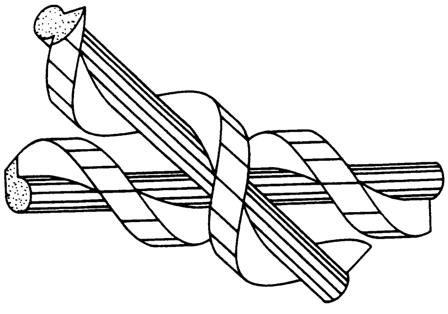


Fig. 6. Fitting of two right-handed screws. [Figure 3 of (*31*) reprinted with the permission of the author and the American Institute of Physics]

Let us change now to another observation in the polyisocyanates that may be associated with predictions of the effect of liquid-crystal organization on the conformational properties of the mesogenic entities that form this phase state. This is a subject of much interest (*37*). All optically active polyisocyanates made up predominantly of hexyl isocyanate monomer units whose chains contain some helical sense reversals show steep and sudden changes in optical activity associated with thermally reversible gel formation in dilute solutions of a wide variety of hydrocarbon solvents (*38*) (Fig. 8). Light and neutron scattering and viscosity and UV spectral changes accompany these events, consistent with the formation of reversible cross-linkable aggregates with a parallel arrangement of the chains (*38, 39*).

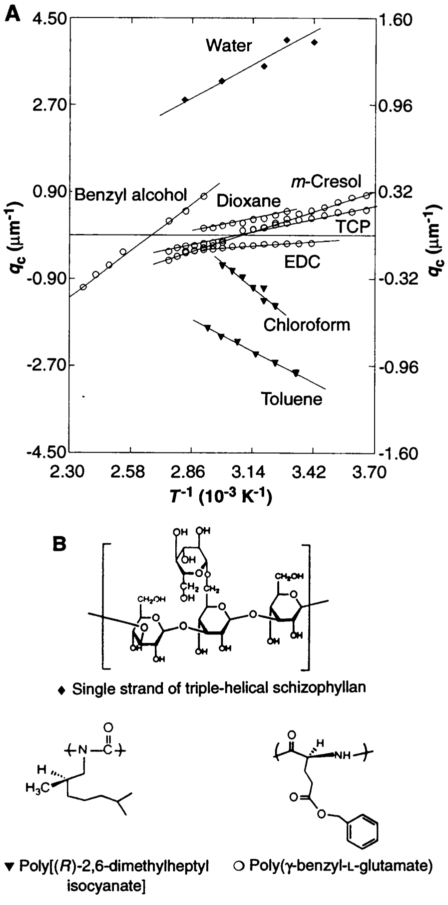


Fig. 7. (**A**) Plots of the inverse absolute temperature ($1/T$) versus the pitch wave number (q_c) for the three lyotropic cholesteric liquid crystals designated in (**B**) {left ordinate for (■) schizophyllan and (▲) poly[(*R*)-2,6-dimethylheptyl isocyanate]; right ordinate for (○) poly(γ-benzyl-L-glutamate)}. The positive and negative values correspond to right- and left-handed cholesteric states, respectively. TCP, trichloropropane; EDC, ethylene dichloride. [Reprinted from (*36*) with permission, American Chemical Society]

One theoretical study directly applicable to the polyisocyanates predicts that defects present in isolated chains of a stiff helical polymer would be excluded when these chains are in the liquid-crystal state (*40*). The steep increase in the optical activity exhibited in Fig. 8 signals a reduction in the helical reversal population consistent with the theory. The polymer responds conformationally to enhance parallel packing. Al-

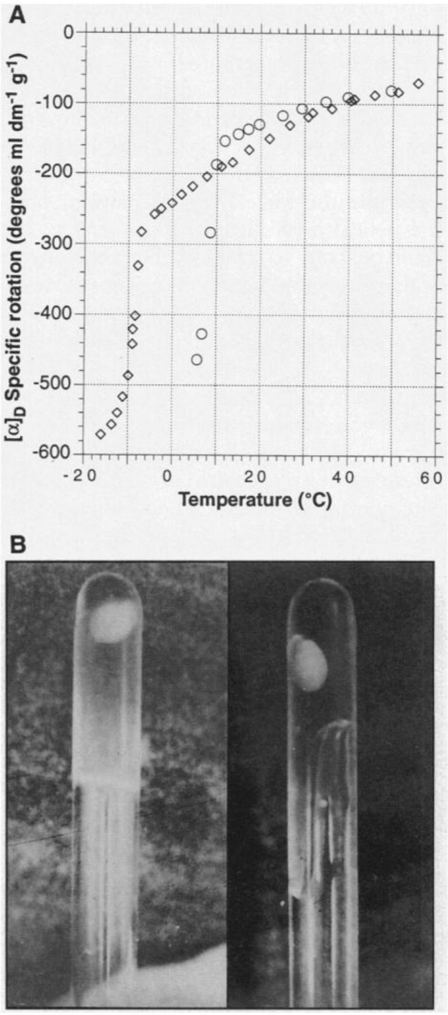


Fig. 8. (**A**) Dependence of the specific rotation at the sodium D line (589 nm) on temperature for a copolymer of 99.5% hexyl isocyanate and 0.5% (*R*)-2,6-dimethylheptyl isocyanate dissolved in (◇) *n*-hexane (4.24×10^{-4} g ml^{-1}) and (○) *n*-octane (1.5×10^{-4} g ml^{-1}). (**B**) Photographs of the (left) gel and (right) sol states formed from 10 mg of poly(*n*-hexyl isocyanate) in 2 ml of *n*-hexane as described in (*38*). The white object is a magnetic stirrer. [Reprinted from (*38*) with permission, American Chemical Society]

though the liquid-crystal forming poly(γ-benzyl-L-glutamate) also forms thermally reversible gels, the precise structure of the parallel aggregates in the polypeptide and in the polyisocyanate is uncertain (*41*), leaving this confirmation of liquid-crystal theory (*40*) open for now.

It would be best to test these ideas directly in the microscopically observed liquid-crystal phase, and the polyisocyanates offer this opportunity because of the helical reversal, an approach not available for polypeptides, which are constrained by the chirality of their amino acid residues to a single helical sense. Following from the properties of cholesteric liquid crystals (*36*), in short chains without helical reversals, both the optical activity in dilute solution and the pitch in the cholesteric state will depend on the ratio of right- to left-handed helices. By contrast, in long chains, where the optical activity in dilute solution reflects the ratio of monomer units in right- and left-handed helical segments of the chain, the elimination of helical reversals in the cholesteric state will lead to the same helical sense in all chains. The pitch of the cholesteric state therefore will be independent of the optical activity in dilute solution. Such a result would have interesting consequences: In the liquid crystal, the accumulation of the chiral bias per monomer unit would be limited only by N, which can be much larger than the number of monomer units between reversals at accessible temperatures.

Concluding Remarks

The thermally reversible gels discussed above (*38*) (Fig. 8) also form in octanes and kerosene at ambient temperatures, suggesting potential in the control of fuel flammability and spill (*42*). However, very little is now known about the details of the responsible phase boundaries or the kinetic and structural characteristics of the gel.

The nonlinear response of helical-sense excess, and therefore optical activity, to the enantiomeric excess in the polyisocyanates (*5*) (Fig. 1) may help in the development of photoresolution for the creation of an optical switch. The low enantiomeric excesses produced in photoresolutions that have hindered this approach may still be high enough to affect large excesses of one helical sense when pendant to the polyisocyanates (*43*). For this approach, more must be known about the speed of the movement of the helical reversal through the chain.

The successful formation of blends, that is, solid solutions of certain functionalized polyisocyanates with random coil polymers, has allowed the measurement of the optical activities of the polyisocyanate component in amorphous glassy and rubbery states. The helical reversal then becomes an interesting length scale probe of the glass transition at temperature T_g because the kinked reversal may be expected to be frozen in place only below T_g. Experimental observations support this picture (*44*).

When a polyisocyanate without a preference for one helical sense is dissolved in a mixture of a nonracemic chiral solvent and an achiral solvent, the induced optical activity of the polymer (*11*, *12*) (Fig. 3) must depend on the ratio of the two cosolvents in the vicinity of the polymer chains. This principle has been used as a novel method for the quantitative determination of preferential solvation (*45*).

Finally, the helical sense ratio of the polyisocyanates may be sensitive to cryptochiral molecules, which show no evidence of chirality by spectroscopic means (*46*).

In summary, the essential characteristics of a stiff helical structure in which the helical senses are both equally probable and easily interconvertible are the prerequisites for the unusual stereochemical properties described above. There is an increasing synthetic interest in both molecular and supramolecular helical structures (*47*). The work described here may encourage the search for parallel properties among these materials.

REFERENCES AND NOTES

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RESEARCH ARTICLE

Crystal Structure of DNA Photolyase from *Escherichia coli*

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Photolyase repairs ultraviolet (UV) damage to DNA by splitting the cyclobutane ring of the major UV photoproduct, the *cis,syn*-cyclobutane pyrimidine dimer (Pyr<>Pyr). The reaction is initiated by blue light and proceeds through long-range energy transfer, single electron transfer, and enzyme catalysis by a radical mechanism. The three-dimensional crystallographic structure of DNA photolyase from *Escherichia coli* is presented and the atomic model was refined to an *R* value of 0.172 at 2.3 Å resolution. The polypeptide chain of 471 amino acids is folded into an amino-terminal α/β domain resembling dinucleotide binding domains and a carboxyl-terminal helical domain; a loop of 72 residues connects the domains. The light-harvesting cofactor 5,10-methenyltetrahydrofolylpolyglutamate (MTHF) binds in a cleft between the two domains. Energy transfer from MTHF to the catalytic cofactor flavin adenine dinucleotide (FAD) occurs over a distance of 16.8 Å. The FAD adopts a U-shaped conformation between two helix clusters in the center of the helical domain and is accessible through a hole in the surface of this domain. Dimensions and polarity of the hole match those of a Pyr<>Pyr dinucleotide, suggesting that the Pyr<>Pyr "flips out" of the helix to fit into this hole, and that electron transfer between the flavin and the Pyr<>Pyr occurs over van der Waals contact distance.

The ultraviolet component of sunlight leads to the formation of cyclobutadipyrimidines (pyrimidine dimers, Pyr<>Pyr) in DNA. These lesions block replication and transcription and thus have cytotoxic and mutagenic effects (*1*). With the gradual depletion of the stratospheric ozone layer, a higher flux of UV is reaching the surface of the Earth and hence more Pyr<>Pyr are being formed in the biosphere with potentially serious consequences (*2*). Cells survive the harmful effects of Pyr<>Pyr by excision repair or by photoreactivation. In photoreactivation, the enzyme DNA photolyase (*3*) binds to Pyr<>Pyr and, upon excitation by blue light, splits the cyclobutane ring and restores the intact bases (*4*).

$$\mathrm{E} + \mathrm{Pyr{<}{>}Pyr} \xrightarrow{\text{Light (400 nm)}} \mathrm{E} + \mathrm{Pyr\text{-}Pyr}$$

Photolyase has been found in bacteria, archaebacteria, and eukaryotes including goldfish, rattlesnake, and marsupials (*1*). The enzyme consists of a single polypeptide chain of 454 to 614 amino acids (*5*) and two noncovalently attached prosthetic groups. One of these is the catalytic cofactor $FADH^-$ (*6*); the other one is a light-harvesting cofactor. Photolyases are classified into two groups according to the type of light-harvesting cofactors: The folate class enzymes bind 5,10-methenyltetrahydrofolylpolyglutamate (MTHF), and the deazaflavin class enzymes contain 7,8-didemethyl-8-hydroxy-5-deazariboflavin.

A blue-light photoreceptor with strong sequence similarity to microbial photolyases but with no photolyase activity has been discovered in plants (*7*). Conversely, animal photolyases have only weak sequence similarity to microbial photolyases (*5*). Both the blue-light photoreceptor and the animal photolyases have FAD and MTHF as cofactors (*8*) suggesting functional similarities with the *Escherichia coli* photolyase described below.

As apparent from the reaction scheme, binding of the enzyme and catalysis are distinct both temporally and mechanistically. Binding is independent of light, catalysis is light-initiated. Photolyase is one of the rare specific DNA binding proteins that bind to its target in both double- and single-stranded DNA with comparable affinities (*9*). The binding constants are $K_D = 10^{-9}$ M and $K_D = 10^{-8}$ M, respectively for double- and single-stranded DNA containing a thymine dimer (T<>T) (*4*, *9*). Photolyase also binds to T<>T in the dinucleotide form, albeit with considerably lower affinity [$K_D = 10^{-4}$ M (*10*)]. Thus, both the dimer and the backbone deformation caused by the dimer contribute to binding.

Catalysis is initiated by absorption of a blue-light photon and leads to splitting of two C-C bonds. Five steps can be distinguished in the overall reaction. First, the light-harvesting cofactor absorbs a photon; second, the excitation energy is transferred to the catalytic cofactor; third, an electron is transferred to the Pyr<>Pyr in the substrate; fourth, the C5-C5 and C6-C6 sigma bonds of the cyclobutane ring are broken; fifth, the electron is transferred back to the flavin and the now intact DNA dissociates from the enzyme (*4*).

In addition to energy and electron transfer during the repair reaction, two other photoinduced electron transfer processes have been observed in DNA photolyase from *E. coli*. (i) During purification, the catalytic cofactor of the enzyme is oxidized to the catalytically inert neutral radical form, $FADH^{\bullet}$ (*11*). This form can be reactivated by irradiation with white light, which causes electron transfer from Trp^{306}

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