This laboratory returns to the statistical mechanics of phase transitions as observed in the phase transition between the helix form and the random-coil form of a polypeptide, as will be described further below. Experimentally, the phase transition between these two states can be observed by monitoring the optical rotation in a solution of the polypeptide, since the two forms have differing optical rotary power. To understand the phase transition theoretically, two models will be used: one where the neighboring amino acid monomers are non-interacting (analogous to a non-interacting spin system), and then by an interacting model (analogous to the Ising model of interacting spins). The experimental measurements will be interpreted using the latter model to extract microscopic energy and energy parameters associated with the phase transition.

The lab is taken from and modified from “Experiments in Physical Chemistry” by Shoemaker et al., and differences in protocol are noted in the text below. Also, a description about polarimeters is appended to the lab description.

Introduction:

Polymer molecules in solution can be found in many different geometric conformations, and there exist a variety of experimental methods (e.g., viscosity, light scattering, optical rotation) for obtaining information about these conformations. In this experiment, the measurement of optical rotation will be used to study a special type of conformational change that occurs in many polypeptides.

The two important kinds of conformation of a polypeptide chain are the helix and the random coil. In the helical form the amide hydrogen of each “amide group,”
is internally hydrogen-bonded to the carbonyl oxygen of the third following amide group along the chain. Thus, this form involves a quite rigid, rodlike structure (see Fig. 1). Under different conditions, the polypeptide molecule may be in the form of a statistically random coil (see Exp. 28). The stable form of the polypeptide will depend on several factors—the nature of the peptide groups, the solvent, and the temperature. For example, poly-$\gamma$-benzyl-l-glutamate† (PBG),

\[
\begin{align*}
\text{O} \\
\text{\text{NH}} - \text{CH} - \text{C} \rightarrow_n \\
\text{CH}_2 \\
\text{CH}_2 \\
\text{CH}_2 \text{O} - \text{C} = \text{O}
\end{align*}
\]

†Note that the usual chemical description of polypeptides is in terms of amino acid residues, as in the PBG formula shown, rather than in terms of amide groups, which are more convenient for the present discussion.
has a helical conformation when dissolved in ethylene dichloride at 25°C, but it is in the random-coil form when dissolved in dichloroacetic acid at the same temperature. This difference is quite reasonable, since a hydrogen-bonding solvent such as dichloroacetic acid can form strong hydrogen bonds with the amide groups and thus disrupt the internal hydrogen bonds that are necessary for the helical form. For a mixed solvent of dichloroacetic acid and ethylene dichloride, PEG can be made to transform from the random coil to the helix by raising the temperature over a fairly narrow range. This rapid reversible transition will be investigated here.

As we shall see below, the solvent plays a crucial role in determining which form is stable at low temperatures. When the helix is stable at low temperatures, the transition to the random coil at high temperatures is called a “normal” transition. For the case in which the random coil is the more stable form at low temperatures, the transition is called an “inverted” transition.

THEORY

We wish to present here a very simplified and approximate statistical-mechanical theory of the helix–coil transition. The treatment is closely related to that given by Davidson. Let us consider the change

\[ \text{Coil} \cdot NS = \text{helix} + NS \]  

(1)

where the polymer molecules each consist of \(N\) segments (monomer units) and are dissolved in a solvent \(S\) that can hydrogen-bond to the amide groups when the chain is in the random-coil form. We shall (for convenience) artificially simplify the physical model of internal hydrogen bonding in the helix by assuming that the hydrogen bond formed by each amide group is with the next amide group along the chain (see Fig. 2) rather than the third following amide group as in the actual helix. We can then assume that the chain segments are independent of each other. Let \(q_1\) be the molecular partition function of a segment in the random-coil form with a solvent molecule \(S\) hydrogen-bonded to it, and \(q_2\) be the product of the partition function of a segment in the helical form times the partition function of a “free” solvent molecule \(S\). We will then define a parameter \(s\) by

\[ q_2 = sq_1 \]  

(2)

Note that \(s\) is the ratio of partition functions and many of the contributions to \(q_1\) and \(q_2\) (e.g., vibrational terms) will cancel out. The principal contributions to \(s\) will involve

FIGURE 2

Hypothetical model of an internally hydrogen-bonded chain, with the simplification that adjacent “amide groups” are connected by hydrogen bonds. Note the distinction between the amino acid residue and the “amide group”; the latter is the more convenient unit of structure for the present discussion.
differences between the helix and random-coil form, and we may guess that \( s \) can be represented in the general form:

\[
s = s_0 e^{-\varepsilon kT}
\]

(3)

where \( \varepsilon = \varepsilon_h - \varepsilon_c \) is the energy change per segment and \( s_0 \) is related to the entropy change per segment for the change in state of Eq. (1).

**First-Order Transition Model.** To simplify the model even further, let us assume for the time being that a given polymer molecule is either completely in the helical form or completely in the random-coil form. This assumption will lead to a first-order transition between these two forms. The partition function for a random-coil molecule \( q_c \) is then given by

\[
q_c = q_1^N
\]

(4)

while that for a helical molecule \( q_h \) will be

\[
q_h = q_2^N = s^N q_1^N
\]

(5)

if one neglects end effects (e.g., the fact that at the beginning of the chain the first carbonyl oxygen is not involved in an internal hydrogen bond).

At constant pressure, the two forms will be in equilibrium with each other at some temperature \( T^* \) at which \( \Delta G = 0 \) for the change in state (1). Since \( \Delta V \) will be quite small for change (1), we can take \( \Delta A = 0 \) and \( \tilde{A}_c = \tilde{A}_h \) at \( T^* \), where \( \tilde{A} \) is the Helmholtz free energy per mole of polymer. For independent polymer molecules, we have:

\[
\tilde{A} = -kT \ln Q = -kT \ln q^{N_0} = -RT \ln q
\]

(6)

where \( N_0 \) is Avogadro's number, \( q \) is the partition function for a single polymer molecule, and \( Q \) is the (canonical) partition function for a mole of molecules. Thus, one finds that \( q_h = q_c \) (or \( s = 1 \)) at \( T^* \). With the use of Eq. (3), the value of \( T^* \) can be related to the parameters \( \varepsilon \) and \( s_0 \):

\[
\frac{\varepsilon}{kT^*} = \ln s_0
\]

(7)

For an inert (non-hydrogen-bonding) solvent, one observes experimentally a "normal" transition: the helix, which is stable at low temperatures, is transformed at higher temperatures into the random coil. This case is represented by our model when \( \varepsilon < 0 \) and \( s_0 < 1 \). Since the solvent is inert, random-coil segments are essentially unbound, whereas helical segments have internal hydrogen bonds and thus a lower energy (\( \varepsilon_h - \varepsilon_c < 0 \)). In the random-coil polymer molecule, considerable rotation can occur about the single bonds in the chain skeleton, but the helical form has a rather rigid structure. Therefore, there is a decrease in the entropy per segment on changing from the relatively free rotational configurations of the flexible random coil to the more rotationally restricted helix (and thus \( s_0 < 1 \)). From Eqs. (3) to (6), it follows that \( s > 1 \) at low temperatures (\( T < T^* \)) and thus \( \tilde{A}_h < \tilde{A}_c \) and the helix is the more stable form. At high temperatures (\( T > T^* \)), \( s \equiv s_0 < 1 \) and the random coil is the more stable form.

For an active (hydrogen-bonding) solvent, one observes experimentally an "inverted" transition: the random coil is stable at low temperatures and changes into the helix when the solution is heated. This case is represented by our model when \( \varepsilon > 0 \) and \( s_0 > 1 \). Here the solvent plays a dominant role, since a solvent molecule is strongly hydrogen-bonded to each random-coil segment. In terms of energy, there is little difference between polymers with random-coil segments and those with helical segments, since there is comparable hydrogen bonding in both forms. It is now almost impossible to predict the sign of
but it is certainly quite reasonable to find that the energy change associated with (1) may be positive \((e_h - e_c > 0)\). Although a helical segment will still have a lower entropy than a random-coil segment owing to the rigidity of the helix, the solvated random coil is now less flexible than previously. More important, the free solvent molecules will have a higher entropy than solvent molecules that are bonded to the random coil. Thus the entropy change associated with (1) is positive because of the release of S molecules (and therefore \(s_0 > 1\)). From Eqs. (3) to (6), we see that at low temperatures \(s < 1\) and the random coil will be stable in this inverted case.

Recalling that \(\Delta V\) is small so that \(\Delta G \approx \Delta A\), we can make use of Eqs. (3) to (6) to obtain

\[
\Delta S = -\left(\frac{\partial \Delta G}{\partial T}\right)_p = R \left(\frac{\partial}{\partial T}\left(T \ln \frac{q_h}{q_c}\right)\right) = NR \ln s_0
\]

(8)

The entropy change per mole of monomer, \(\Delta S_m\), is then given by

\[
\Delta S_m = \frac{\Delta S}{N} = R \ln s_0
\]

(9)

Since \(\Delta G = 0\) for the first-order transition at \(p\) and \(T^*\), we have for the enthalpy change per mole of monomer,

\[
\Delta H_m = T^* \Delta S_m = RT^* \ln s_0 = N_0 e
\]

(10)

The first-order theory presented above is related to the treatment of Baur and Nosanow,\(^4\) who used a similar but perhaps physically less realistic model to derive the same results [that is, Eqs. (7), (9), and (10)].\(^\dagger\)

**Cooperative Transition Model.** It is an experimental fact that the helix–coil transition is not a first-order transition. In order to account for this, one must adopt a more realistic model by eliminating the assumption that an entire polymer molecule is all in a given form. Indeed, a polymer molecule can have some sections that are helical and others that are randomly coiled. The formation of a helical region will be a cooperative process. Since segment (i.e., amide group) \(n\) is hydrogen-bonded to segment \(n + 3, n + 1\) to \(n + 4, n + 2\) to \(n + 5\), etc., it is difficult to initiate this ordered internal bonding; but once a single hydrogen-bond link is made, the next ones along the chain are much easier to achieve (a sort of “zipper” effect).

No attempt will be made here to develop the details of such a cooperative model. Readers with a sufficient background in statistical mechanics should refer to Davidson’s presentation\(^3\) or to the original papers in the literature.\(^5\) The crucial idea is to introduce a ‘‘nucleation’’ parameter \(\sigma\) which is independent of temperature and very small in magnitude. It is then assumed that the partition function \(q_2\) is changed to the value \(\sigma q_2\) for the initial segment of a helical section (i.e., the internally bonded segment directly adjacent to a solvent-bonded segment). Physically, this means that it is difficult to initiate a new helical section. For such a model, it can be shown\(^2,5\) that \(X_h\), the mole fraction of the segments in helical sections, will undergo a very rapid but continuous change from \(X_h = 0\)

\(\dagger\)In addition, Baur and Nosanow showed for the inverted case that the helical form will be stable only between \(T^*\) another, much higher transition temperature \(T'\), where the helix reverts to the random-coil form. (For PBG, it is estimated\(^6\) that \(T' = 700\) K.) This is physically reasonable in terms of our model: at very high temperatures, all hydrogen bonds (internal or with solvent) will be broken and the random coil will predominate owing to its higher entropy.
when \( s < 1 \) to \( X_h \approx 1 \) when \( s > 1 \). For \( \sigma \leq 10^{-2} \), \( X_h \) is very well represented by the approximation

\[
X_h = \begin{cases} 
  sF(s) & \text{for } s < 1 \\
  \frac{1}{2} & \text{for } s = 1 \\
  F(s) & \text{for } s > 1 
\end{cases}
\]  

(11)

where the function \( F(s) \) is given by

\[
F(s) = \frac{1}{2} \left( 1 + \frac{(s-1) + 2\sigma}{[(s-1)^2 + 4\sigma s]^{1/2}} \right) 
\]  

(12)

The resulting variation of \( X_h \) with the parameter \( s \) is shown in Fig. 3, which implies that a rapid cooperative transition occurs in the vicinity of a critical temperature \( T^* \) (the value of \( T \) for which \( s = 1 \)). It is the fact that \( \sigma \) is very small that ensures a sharp transition. If \( \sigma \) were taken to be 1 (which corresponds to ignoring the nucleation problem), this model would yield \( X_h = s/(1 + s) \), which corresponds to a very gradual transition. For high-molecular-weight PGB, a value of \( \sigma = 2 \times 10^{-4} \) seems to be appropriate to obtain a fairly good quantitative fit to the experimental data.\(^2\) It should be noted that the transition is sharpest, and the model is most successful, when polypeptides with \( N \approx 1000 \) are used.

**METHOD**

Since the helical form of a polypeptide is rigid and rodlike, its solutions are more viscous than corresponding solutions of the random-coil form, and the transition can be detected by measuring the intrinsic viscosity (see Exp. 28). However, the highly ordered structure of the helical form causes it to have an optical-rotatory power markedly different from that of a random coil. Indeed, use of optical rotation for following the transition gives better results than does intrinsic viscosity, since the optical rotation is related directly to the fraction \( X_h \) of segments in the helical form, whereas the hydrodynamic properties of the polymer molecule change substantially at the first appearance of random-coil regions in the helix (in effect, causing the otherwise straight rod to bend randomly in one or several places).

**FIGURE 3**

Percentage of polypeptide in helix form, as a function of the parameter \( s \) (on a log scale), for \( \sigma = 10^{-4} \) and \( \sigma = 10^{-2} \).
The polarimeter and its operation are described in Chapter XX. Although not stressed there, it is obviously necessary to use a polarimeter in which the temperature of the sample tube can be well controlled. The specific rotation $[\alpha]_D$ as determined with the sodium D doublet at $t^\circ$C is defined by Eq. (XX-9), and we note here that for a solution containing two solutes (helix and random coil) one can show that

$$[\alpha]_D = X_h[\alpha]_h^* + X_c[\alpha]_c^*$$

where $[\alpha]_h^*$ and $[\alpha]_c^*$ are the specific rotations of helix and random-coil form. In a narrow range of temperature near $T^*$, $[\alpha]_h^*$ and $[\alpha]_c^*$ will be essentially constant. Thus measurements of $[\alpha]_D$ will determine the mole fraction $X_h$ as a function of $T$.

**EXPERIMENTAL**

A solution of poly-$\gamma$-benzyl-$l$-glutamate (PBG) in a mixed solvent of dichloroacetic acid (DCA) and ethylene dichloride, 76 volume percent in DCA, will be used. The dichloroacetic acid should be purified by vacuum distillation; the ethylene dichloride may be purified by conventional distillation in air. Mix the proper volumes of the two liquids to make about 50 mL of the desired solvent. In order to prepare approximately 25 mL of a 2.5 weight percent polymer solution, weigh accurately a sample of about 1 g of PBG and dissolve it in about 38 g of solvent (also weighed accurately). The resulting solution is very viscous; it must be mixed well and then allowed to stand for two or three days until any small undissolved particles have settled and the solution is clear. During this period, the solution should be placed in a polyethylene bottle and stored in a refrigerator. The remaining solvent should also be stored for future density determinations.

Since the solution is difficult to prepare, it is recommended that it be made available to the student. **Warning: The solvent is extremely corrosive.**

Detailed instructions for using the polarimeter and the temperature-regulating bath will be provided in the laboratory. After determining the path lengths of the cells to be used, fill a cell with pure solvent and zero-adjust the instrument. Next, rinse and fill a cell with the polymer solution.†

After mounting the solution cell in the polarimeter, it should not be necessary to move this cell during the rest of the experiment. Provisions must be made for measuring the temperature of the sample without obscuring the light path. Begin measurements with the temperature at about $10^\circ$C and read the optical rotation $\alpha$ at reasonably spaced temperature intervals until about $50^\circ$C is reached. The exact temperature intervals to be used are at your discretion, but should be close enough to follow the essential features of the variation of optical rotation; small intervals (perhaps 1 or 2°C) should be used when the optical rotation is changing rapidly with temperature (near $30^\circ$C), and larger intervals may be used when the changes with temperature are small. Be sure to allow sufficient time for thermal equilibration between readings.

In order to calculate $[\alpha]_D$, the density of the solutions must be known at each temperature. The density of the solution is close enough to that of the solvent so that they can be assumed to be identical. The density of the solvent should be determined with a pycnometer for at least three different temperatures in the range 10 to 50°C. The volume of the pycnometer should first be determined by weighing it empty and also filled with distilled water at a known temperature. Since the density of water as a function of temperature is

†A difficulty in filling these cells is the problem of avoiding any air bubbles that might obscure part of the light path. Since the solution is viscous, this can be a tedious job and may require some time. If possible, it is wise to fill the cells about an hour before use and allow them to stand.
well known, the volume of the pycnometer can be determined. This procedure (see Exp. 9) is then repeated using the solvent, and since the volume of the pycnometer is now known, the density can be calculated. The solvent density should be plotted against temperature and the best straight line drawn through the points obtained.

Record the composition of the solution and the molecular weight (or $N$ value) of the polymer used.

**CALCULATIONS**

For each data point, calculate the specific rotation at temperature $t$ from the expression

$$[\alpha]^t = 100 \frac{\alpha}{Lpp}$$

(14)

where $\alpha$ is the observed rotation in degrees, $L$ is the path length of the cell in decimeters, $p$ is the weight percent of solute in the solution, and $\rho$ is the density of the solution in g cm$^{-3}$. These density values are interpolated from the $\rho$-versus-$T$ plot for the solvent.

Construct a plot of $[\alpha]^t$ versus temperature, and determine the limiting (asymptotic) values of the rotation: $[\alpha]_h$ from the high-temperature plateau, $[\alpha]_c$ from the low-temperature plateau. If it is assumed that these represent temperature-independent specific rotations for the helix and random-coil forms, respectively, Eq. (13) gives for $X_h$:

$$X_h = \frac{[\alpha]^t - [\alpha]_c}{[\alpha]_h - [\alpha]_c}$$

(15)

Using this equation, you can add to your plot a scale of $X_h$ values so that the plot will also represent $X_h$ versus $T$. Determine and report the "transition" temperature $T^*$ at which $X_h = 0.5$.

**DISCUSSION**

If one arbitrarily defined an equilibrium constant by $K = X_h/X_c$ and used the data in this experiment together with the Van't Hoff equation $d \ln K/d(1/T) = \Delta H^\circ/R$ to determine the value of $\Delta H^\circ$, to what (if anything) would such an enthalpy change refer?

**SAFETY ISSUES**

Many chlorinated hydrocarbons are toxic to some degree. Thus the mixed solvent of dichloroacetic acid and ethylene dichloride should be handled with care. This solvent is also very corrosive. If it is necessary to prepare the PBG solutions, gloves should be worn.

**APPARATUS**

Polarimeter, with one or more optical cells (complete with windows, caps, and Teflon or neoprene washers); constant-temperature control for range 10 to 50°C; pycnometer; 25-mL pipette for filling the pycnometer and a small pipette for filling the cell; rubber
bulb for pipetting; 0 to 50°C thermometer; 250-mL beaker; gum-rubber tubing; lint-free tissues for wiping cell windows; 50-mL polyethylene bottles for PBG solution and excess solvent.

Poly-γ-benzyl-l-glutamate, M.W. 300 000 (1 g of solid or 25 mL of 2.5 weight percent solution); ethylene dichloride–dichloroacetic acid solvent (76 volume percent DCA) stored in polyethylene bottle; wash acetone. The polypeptide can be obtained from Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178. Check the current Chem. Sources for other suppliers.

REFERENCES


GENERAL READING


Polarimeters

The polarimeter (Fig. 8) is an instrument for measuring the optical rotation produced by a liquid or solution. The specific rotation \([\alpha]_D\) of a solute in solution at a given wavelength \(\lambda\) and Celsius temperature \(t\) is given by

\[
[\alpha]_D = \frac{100\alpha}{Lc} = \frac{100\alpha}{Lpp}
\] (9)

where \(\alpha\) is the angle in degrees through which the electric vector is rotated, \(L\) is the path length in decimeters, \(c\) is the concentration of solute in grams per 100 cm\(^3\) of solution, \(p\)

**FIGURE 8**

Schematic drawing of a polarimeter.
is the weight percent of solute in the solution, and \( \rho \) is the density of the solution in g cm\(^{-3}\). The angle \( \alpha \) is considered positive if the rotation of the electric vector as the light proceeds through the solution is in the sense of a left-hand screw or negative if it is in the sense of a right-hand screw.

Usually the optical rotation is measured with the sodium D yellow line (a doublet, 589.0 and 589.6 nm). For more precise work the 546.1-nm green mercury line may be used.

Light from the source (a sodium-vapor arc lamp or a mercury-vapor lamp with appropriate filter) is polarized by a Nicol prism, termed the polarizer, which consists of two prisms of calcite cemented together with Canada balsam so that one of the two rays produced in double refraction (the "ordinary ray") is totally reflected at the interface and lost, while the other (the "extraordinary ray") is transmitted. The polarized light passes through the solution and then through a second Nicol prism, termed the analyzer, which can be rotated around the instrument axis. The normal position (zero rotation) is one in which the two Nicol prisms are at 90° to each other, and no light passes through. When an optically rotating medium is introduced between the two Nicol prisms, light is transmitted; the observed rotation \( \alpha \) is the angle in degrees through which the dial must be turned (clockwise with respect to the observer if \( \alpha \) is positive, counterclockwise if negative) in order to restore the field to complete darkness.

As the dial is turned, the intensity of emergent light is proportional to \( \sin^2(\alpha - \alpha_d) \), where \( \alpha_d \) is the setting for complete darkness. Since this quantity behaves approximately quadratically near the dark position, the setting is of limited sensitivity if only a single polarizer and a single analyzer are used. In many instruments the field of view is divided into two equal parts with polarization angles differing by a few degrees. This is done either by use of a composite polarizer (two Nicol prisms cemented together side by side with their planes of polarization at a small angle) or by use of an added Nicol prism covering half of the field of the polarizer. In use, the analyzer is adjusted so that the two fields appear equally bright.

Although laboratory polarimeters generally use Nicol prisms as polarizers and analyzers, dichroic crystals (such as tourmaline) or dichroic sheet polarizers (such as Polaroid) may be used in the construction of special apparatus.†

The polarimeter is commonly used in organic and analytical chemistry as an aid in identification of optically active compounds (especially natural products) and in estimation of their purity and freedom from contamination by their optical enantiomers. The polarimeter has occasional application to chemical kinetics as a means of following the course of a chemical reaction in which optically active species are involved. Since the rotation \( \alpha \) is a linear function of concentration, the polarimeter can be used (in the same way that a dilatometer might be used) in studying the acid-catalyzed hydrolysis of an optically active ester, acetal, glycoside, etc.

In modern organic chemistry optical-rotatory dispersion,\(^{16}\) the variation with wavelength of optical-rotatory power (and certain related properties), is used in molecular-structure investigation as a means of identifying and characterizing chromophore groups. Automatic polarimetric spectrophotometers of high complexity have been developed for this purpose.

†In the phenomenon known as dichroism, the optical absorption depends strongly on the orientation of the plane of polarization with respect to the crystallographic axes (or axis of preferred orientation). Commercial sheet polarizers are made from acicular dichroic crystals, herapathite (iodoquinine) in the case of Polaroid, suspended in a viscous or plastic medium and aligned by extrusion or stretching. Dichroic polarizers and analyzers are inferior to Nicol prisms for use in polarimeters, because the transmission is considerably less than unity when the planes of polarization are parallel and the absorption is not quite complete when they are perpendicular.