Thermodynamic Properties of Solutions of Helical Polypeptides

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Partial pressures of mixtures of pyridine and of 1,2-dichloroethane with poly-γ-benzyl-L-glutamate have been determined at 25° for volume fractions of solvent from $\varphi_1 = 0$ to ca. 0.4. Similar experiments have been carried out on mixtures of chloroform with poly-β-benzyl-L-aspartate. In all cases the solvent activities increase gradually and smoothly with solvent concentration; they are insensitive to the molecular weight of the polymer. The Henry's law slope at $\varphi_1 = 0$ is very much less than would be required by ideal solution theory, which should apply to a system of impenetrable rod-like particles at high concentration. The observed behavior is readily explicable in terms of solvent mixing with side chains of α-helical solute particles. The driving force for this mixing is primarily entropic. It dominates the concentrated solution thermodynamic behavior of these systems and appears to be a requisite for solubility of polypeptides in the form of intact helices. The solvent activities are devoid of evidence of phase transitions. The occurrence of conformational changes with dilution is rendered extremely unlikely by the results presented. The side chains of the undiluted polypeptide evidently are disordered.

Introduction

Consider the dissolution of a system of macromolecules in rigid, rod-like conformation initially packed in crystalline array. The process usually can be separated, hypothetically at least, into two steps.[1,2] One involves randomization of the chain configuration, the rigid, rod-like (or helical) conformation being replaced by the so-called random coil. The other consists in the actual dilution, or mixing, of solute with solvent. The order in which these two processes are considered to occur is unimportant; in actual situations the two usually take place simultaneously.

Mixing with solvent without randomization of the chain conformation of the crystalline state, though the rare exception rather than the rule among polymers, finds abundant illustration in those synthetic polypeptides which choose the α-helix of Pauling and Corey as their most stable conformation in the crystalline (bulk solid) state according to X-ray diffraction and other evidence.[3] As is well known,[4] such polypeptides in a number of instances can be dissolved in suitable solvents in the form of highly asymmetric, helicoidal particles. That the polypeptide molecules so dispersed retain the conformation of the α-helix characteristic of the bulk state has been challenged recently by Luzatti[5] and by Benoît[6] and their co-workers, who find evidence for somewhat more elongated particles in dilute solution. The dimensions they find approximate those expected for a $^3/_{10}$ helix. The marginal difference in axis ratio for helices of the two kinds (this difference being only about 30%) is unimportant insofar as the present discussion is concerned. Entrance into the controversy is therefore unnecessary at this point. It suffices to note that the polypeptide molecules in the solutions under consideration occur as structured, highly asymmetric particles having axis ratios of the order of 100 or more. The question to be considered is the following: wherein may the thermodynamics of mixing of rod-like molecules with solvent be expected to differ from the mixing of the same, or comparable, macromolecules to form solutions of random coils?

Theory indicates that the two processes differentiated above may be treated as mutually independent, in first approximation at least. That is, statistical mechanical treatments[1,2] invariably lead to a partition function which is separable, approximately if not exactly, into two factors which relate respectively to the intramolecular configuration and to the intermolecular mixing. Only the latter depends on composition. It follows that the configuration should be independent of the composition (in this approximation) and that the thermodynamic functions for mixtures should be little affected by the configuration, be it rod-like or random coil. The latter inference of theory is the one of particular importance here. If it were not subject to an important qualification in the case of highly anisometric particles, it would imply that the solubility and other thermodynamic properties of solutions of polypeptide helices should fit the same pattern as solutions of random coils. High asymmetry of the dissolved species does in fact introduce a feature which drastically alters the character of its solutions as the concentration is increased.[7]

In dilute solutions such particles are free to adopt random orientations. As the concentration is increased, however, a point is eventually reached beyond which randomness of orientation of rods of high axis ratio is no longer compatible with confinement to the space at their disposal. The solute molecules are then forced to assume orientations locally correlated to one another, *i.e.*, semiparallel. More detailed considerations[7,8] show that phase separation must invariably intervene before this limiting concentration is reached. Of the two coexisting phases, the more dilute is isotropic, and the more concentrated one is anisotropic,

(1) P. J. Flory, *Proc. Roy. Soc.* (London), **A234**, 60 (1956).
(2) P. J. Flory, *J. Polymer Sci.*, **49**, 105 (1961).
(3) A. Elliott, E. M. Bradbury, A. R. Downie, and W. E. Hanby, "Polyamino Acids, Polypeptides and Proteins," M. A. Stahman, Ed., University of Wisconsin Press, Madison, Wis., 1962, pp. 255–269.
(4) P. Doty and W. B. Gratzer, ref. 3, pp. 111–118.
(5) V. Luzatti, M. Cesari, G. Spach, F. Masson, and J. M. Vincent, *J. Mol. Biol.*, **3**, 566 (1961).
(6) G. Spach, L. Freund, M. Daune, and H. Benoît, *ibid.*, **7**, 468 (1963); J. Marchal and C. Lapp, *J. chim. phys.*, **61**, 999 (1964).
(7) P. J. Flory, *Proc. Roy. Soc.* (London), **A234**, 73 (1956).
(8) L. Onsager, *Ann. N. Y. Acad. Sci.*, **51**, 627 (1949).

or tactoidal, even in absence of stress. The concentrations of the phases do not differ greatly, usually being in the ratio of about 1.5 to 1.0. They depend on the axis ratio, but are little affected by solvent–solute interaction. Typically, they may be in the vicinity of 10% for polypeptide molecular weights on the order of 10^5.

These predictions of theory[7,8] are well substantiated[2] by experiments on various solutions of helical polypeptides.[9-11] Thus, these solutes exhibit the behavior to be expected of rod-like particles in solution.

The foregoing observations naturally invite consideration of the thermodynamic properties of such solutions at higher concentrations. It is here that high orientation must prevail, and consequently large departures from the behavior of random coil solutions might be expected.

According to the theory referred to,[7] the activity a_1 of solvent in a binary solution of impenetrable, rod-like solute particles at a concentration such that partial order prevails at equilibrium is given by

$$a_1 = \varphi_1 \exp\{[(y - 1)/r]\varphi_2 + 2/y + \chi_1\varphi_2^2\} \qquad (1)$$
$$1 < y \leq r$$

where φ_1 and φ_2 are the volume fractions of solvent and of solute, respectively, r is the ratio of the molar volumes of solute and solvent,[12] χ_1 is the usual thermodynamic interaction parameter, and y indexes the degree of disorientation. In the limit of perfect parallel alignment of the solute particles, $y = 1$, a condition which obtains at high concentrations. At sufficiently low concentration, where complete disorder prevails, $y = r$. The disorientation index for states of equilibrium at intermediate concentrations is given by solutions of the equation[7]

$$\varphi_2 = [r/(r - y)][1 - \exp(-2/y)] \qquad (2)$$

At high concentrations where eq. 2 nominally yields $y \leq 1$, the entropy of mixing reduces to the expression for an ideal (or regular) solution; hence

$$a_1 = x_1 \exp(\chi_1\varphi_2^2) \qquad (3)$$

where x_1 is the mole fraction of solvent. That mixing should be ideal in this limit is directly apparent from the one-dimensional nature of mixing of perfectly aligned, long rods with small molecules. Thus, random mixing along a single coordinate should be ideal irrespective of the comparative sizes of the component molecules.

If solutions of polypeptide helices do indeed behave as simple rod-like structures at all concentrations, then marked departures from random-coil solution thermodynamics should be readily apparent at higher concentrations. Specifically, the activity should approach the *mole fraction* x_1 in the limit $\varphi_2 = 1$, whereas for ordinary polymer solutions it approximates $e\varphi_1$ in this limit, where φ_1 is the *volume fraction*, and e is the base of natural logarithms. For solutes of high molecular weight the difference is very large.

The present investigation was undertaken with the object of examining the activities of solutions of synthetic polypeptides in the helical form at concentrations in the range where tactoidal order prevails. Poly-γ-benzyl-L-glutamate (PBLG) and poly-β-benzyl-L-aspartate (PBLA) were the polypeptides chosen for study. The selection of solvents (pyridine, chloroform, and ethylene dichloride) was limited to those whose vapor pressures are adequate to permit conduct of the experiments at 25°.

Experimental

Polypeptides were purchased from Pilot Chemicals, Inc., and Schwarz Bioresearch, Inc. They were dried *in vacuo* at 65° for 5 days prior to use. Solvents were dried, fractionally distilled, and thoroughly degassed immediately before each study.

The gravimetric sorption apparatus used is essentially the same as that employed by Mandelkern, Prager, and Long.[13] The measurements were carried out as follows. A weighed sample (20 to 100 mg.) of the dried polypeptide was placed on a quartz grid or pan and suspended from a calibrated helical quartz spring (sensitivity 1 mg./mm.). The assembly was mounted in a chamber which was subsequently evacuated to 10^{-5} mm. A stopcock regulated the amount of solvent vapor allowed to enter the chamber, and the resulting pressure was read on a U-tube manometer. All parts of the system accessible to solvent vapor equilibrating with the polypeptide, including the manometer, were immersed in a constant-temperature bath (25.00 ± 0.02°). The increase in weight owing to sorption of solvent by the polypeptide was obtained from the extension of the quartz spring as measured with a cathetometer reading to ±0.03 mm. Sorption equilibrium was assumed to have been attained when no further change in weight was detectable over an extended period of time. This point was usually reached in less than 6 hr., except at solvent concentrations where the solution began to flow; a day was generally found necessary for equilibration in the latter range. The volume fraction of solvent in the mixture was calculated from the weight fractions and specific volumes of the pure components. Values of the latter quantity, 0.787 cm.^{3} g.$^{-1}$ for PBLG and 0.765 cm.^{3} g.$^{-1}$ for PBLA, were taken from the respective partial specific volumes in dilute solutions as reported in the literature.[5,14,15] These results are insensitive to the solvent[5,15]; the value for PBLG was confirmed by direct pycnometric measurement on the pure polymer.

In the course of this work it was recognized that the initial solvent added to the crystalline polypeptide might conceivably locate at interstitial sites of the solid without greatly altering the relative positions of the polypeptide molecules. Sorption in this manner would occur without commensurate change in volume. Experimental measurement of the specific volumes of

(9) C. Robinson, *Trans. Faraday Soc.*, **52**, 571 (1956).
(10) C. Robinson, J. C. Ward, and R. B. Beevers, *Discussions Faraday Soc.*, **25**, 29 (1958).
(11) J. Hermans, Jr., *J. Colloid Sci.*, **17**, 638 (1962).
(12) An idealization adopted in the model used for theoretical derivation stipulates that the solvent molecule shall be of such diameter as to be replaceable by a segment of the helical, or rod-like, solute. This condition is not fulfilled in general; hence, we adopt the definition of r (previously designated x) given above in order to permit application to actual mixtures. By thus defining r, the activity at the extremes of concentration is of acceptable form.
(13) L. Mandelkern and F. A. Long, *J. Polymer Sci.*, **6**, 457 (1951); S. Prager and F. A. Long, *J. Am. Chem. Soc.*, **73**, 4072 (1951).
(14) E. Shechter, G. Spach, and H. Benoît, *J. chim. phys.*, **59**, 1179 (1962).
(15) C. DeLoze, P. Saludjian, and A. J. Kovacs, *Biopolymers*, **2**, 43 (1964).

several mixtures was therefore undertaken. A calibrated Weld-type pycnometer containing a weighed sample of dry polypeptide was placed inside a vessel, designed for the purpose, which was connected to the vacuum system and immersed in the constant-temperature bath at 25°. The vessel was fitted with a small tube leading to the interior of the pycnometer and connected by a stopcock to a reservoir of polypeptide-inert filling fluid. General Electric SF-100 silicone fluid having a density d^{25} 0.9644 (±0.0002) g. cc.$^{-1}$ at 25° was chosen for this purpose. After equilibration of the sample with solvent vapor at a chosen pressure, the fluid, also at 25°, was transferred to the pycnometer containing the swollen sample. The filled pycnometer was removed from the system, and the contents were weighed within 5 min. after filling. Speed in this operation was necessary owing to the possibility of solvent diffusion from the polypeptide to the silicone fluid.

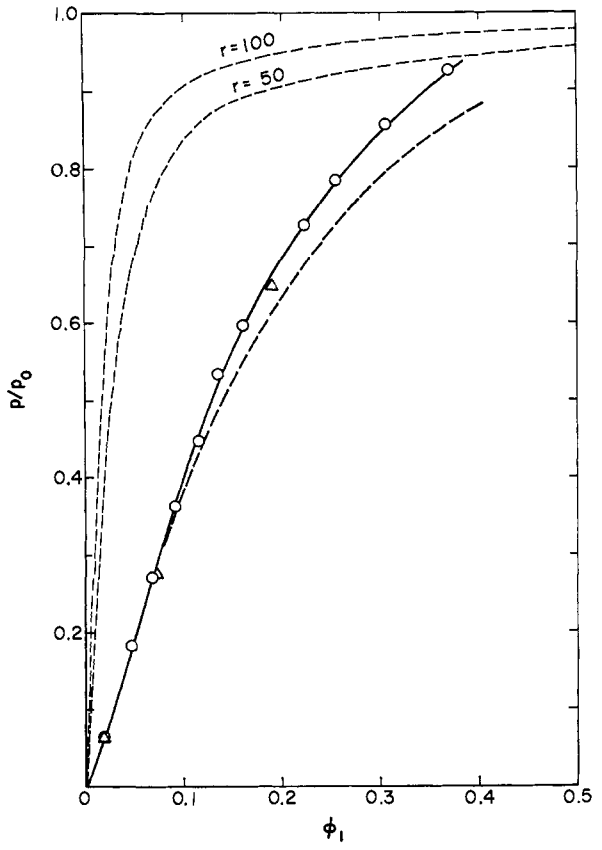

Figure 1. Reduced vapor pressure *vs.* volume fraction of pyridine mixed with high molecular weight PBLG (M = 2.5 × 10^5) at 25°: ascending pressures, O; descending pressures, △. Upper dashed curves calculated using eq. 1 and 2 with r = 50 and 100 as indicated; lower dashed curve calculated according to eq. 4 with χ_1 = 0.35.

By this means the volume of a sample containing a weighed amount of polymer and equilibrated with vapor at a specified pressure was directly measured; the weight of diluent present was calculated from the gravimetric sorption experiments. The data obtained at three different PBLG–pyridine compositions are given in the third column of Table I. Shown for comparison are the specific volumes of the mixtures

Table I. Specific Volumes of PBLG–Pyridine Mixtures at 25°

Reduced vap. press.	Wt. fraction of pyridine	Obsd. sp. vol., ml. g.$^{-1}$	Calcd. sp. vol., ml. g.$^{-1}$
0.532	0.104	0.812	0.812
0.627	0.133	0.819	0.819
0.836	0.234	0.832	0.842

calculated from the weight fractions and specific volumes of the separate pure components assuming mixing without volume change. The agreement between the calculated and observed results is excellent for the two samples having the lower solvent weight fractions. The small difference of approximately 1% for the most dilute sample is within the limits of experimental error.

These data serve to justify the computation of volume fractions for all mixtures on the assumption of additivity of volumes.

Results

Vapor pressure ratios p_1/p_1^0 for mixtures of pyridine with PBLG, having a molecular weight M = 2.5 × 10^5, are plotted against the volume fraction composition in Figure 1. All experiments were at 25°. Corresponding results for mixtures of pyridine with the lower molecular weight PBLG, M = 1.0 × 10^5, are presented in Figure 2. The concentration ranges were restricted to those within which the mixtures were homogenous, the single phase being anisotropic, or tactoidal. Extension to lower concentrations would have required a differential method suitable for p_1/p_1^0 values approaching unity.

The experiments were conducted at a series of pressures taken first in increasing and subsequently in decreasing order. Close agreement between results for ascending and descending sequences of measurements attests to the attainment of equilibrium over most of the concentration range covered. Hysteresis is apparent in the case of the lower molecular weight PBLG (Figure 2) at relatively high solvent compositions, but was not in evidence for the higher molecular weight material. It was noted that the lower molecular weight PBLG underwent viscous flow beginning approximately at the concentration for onset of hysteresis. In contrast, the higher molecular weight PBLG maintained its geometric form at all concentrations over which measurements were carried out.

The results appear to be independent of the polymer molecular weight over the concentration range covered, provided however that the pressure-ascending curve in Figure 2 is assumed to be representative of this sample at higher concentrations of pyridine. The upper dashed curves in Figure 1 represent the activity a_1 calculated according to eq. 1 and 2 with r = 100 and 50, respectively. The former value of r approximates the axis ratio for a PBLG α-helix of molecular weight 2.5 × 10^5; the latter corresponds roughly to the axis ratio for the lower molecular weight PBLG. If r were interpreted as a ratio of molar volumes, somewhat larger values would be required. In any case, the divergence between experiment and the foregoing theory for a solution of coherent, impenetrable

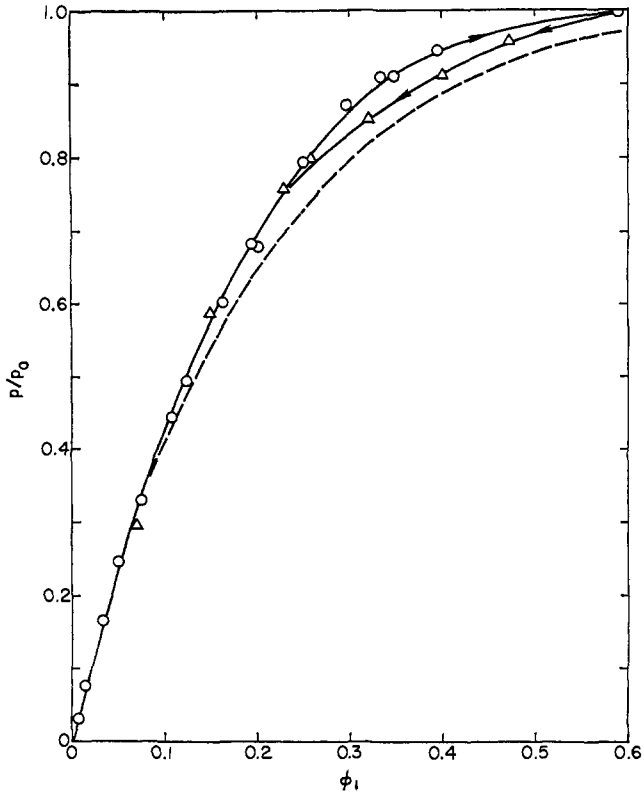

Figure 2. Reduced vapor pressure *vs.* volume fraction of pyridine mixed with lower molecular weight PBLG ($M = 1.0 \times 10^5$) at 25°: ascending pressures, O; descending pressures, △. Dashed curve calculated according to eq. 4 with $\chi_1 = 0.35$.

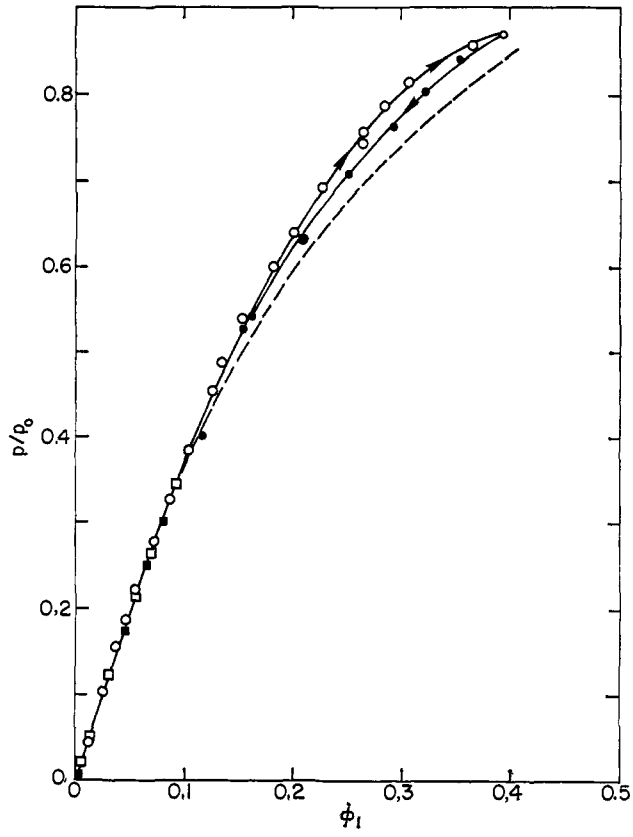

Figure 3. Reduced vapor pressure *vs.* volume fraction of ethylene dichloride mixed with PBLG at 25°. $M = 1 \times 10^5$: ascending pressures, O; descending pressures, ●. $M = 2.5 \times 10^5$: ascending pressures, □; descending pressures, ■. Dashed curve calculated according to eq. 4 with $\chi_1 = 0.20$.

rod-like particles, is beyond all possibility of reconciliation by reasonable adjustments of r and of the interaction parameter χ_1. As will be apparent by comparison of Figure 1 and 2, results for the lower molecular weight PBLG are about equally discordant with eq. 1 and 2.

Vapor pressure measurements for the 1,2-dichloroethane–PBLG system, shown in Figure 3, display a similar dependence on composition. Again no effect of molecular weight is discernible. Reduced vapor pressures for mixtures of chloroform with PBLA are presented in Figure 4. These results bear close resemblance to those shown in Figures 1, 2, and 3 for PBLG. The dependence of the activity on composition obviously is insensitive to details of the polypeptide structure and to the solvent, provided of course that the solvent is one in which the polypeptide helices are dispersed intact.

In view of the striking discrepancy between experiment and theory as represented by the upper dashed curves in Figure 1, serious consideration was given to the possibility that sorption of solvent might be interstitial, *i.e.*, in voids between the helices. Careful measurements were therefore undertaken for the express purpose of observing the volume changes associated with sorption of solvent. As reported in the Experimental section, these measurements show the volumes of the two components to be quite accurately additive. Hence, the possibility that solvent enters voids between coherent helical macromolecules may safely be dismissed on the basis of direct experimental observations.

Discussion

Departure of the experimental results from the theory for coherent, impenetrable, rod-like particles is most marked in the limit $\varphi_1 = 0$ where, according to eq. 3, the solvent activity should be given by $a_1 = x_1 \exp(\chi_1) = (\varphi_1 r) \exp(\chi_1)$. The observed Henry's law constant is lower than the value thus calculated for any reasonable value of χ_1 by nearly two orders of magnitude. Moreover, the activity, represented by p_1/p_1^0 if correction for vapor nonideality is ignored, does not depend perceptibly on the molecular weight at a given *volume* fraction. This feature also is at variance with eq. 3.

Hydrodynamic and light-scattering studies have provided compelling evidence that the polypeptides here considered disperse as virtual rods in dilute solution.[14,16] The tactoidal (*i.e.*, anisotropic) properties manifested by these solutions[9-11] beginning at concentrations of *ca.* 10% confirm the persistence of this molecular conformation in concentrated solutions as well. There is, of course, the likelihood of appreciable departures from the rigid helical form for very long chains. Small fluctuations in bond angles and distances intrinsic to the α-helix may, for example, impart a gentle curvature to the helix axis. At high concentrations parallel alignment is enforced by geometric requirements, even if the helices should depart somewhat from the rigid, rod-like form normally

(16) P. Doty, J. H. Bradbury, and A. M. Holtzer, *J. Am. Chem. Soc.*, 78, 947 (1956).

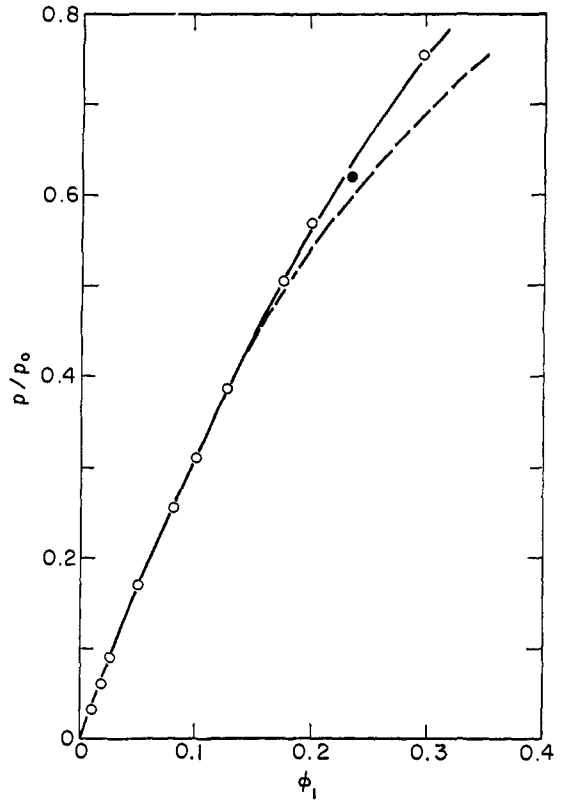

Figure 4. Reduced vapor pressure *vs.* volume fraction of chloroform in solution with PBLA ($M = 2.2 \times 10^5$) at 25°. Ascending pressures, ○; descending pressure, ●. Dashed curve calculated using eq. 4 with $\chi_1 = 0$.

ascribed to them. Parallelity, rather than rigid rectilinearity, is the essential criterion for adherence to eq. 3.

As we have implied above, the theory leading to eq. 1–3 treats the solute particles as coherent, impenetrable rods whose internal structures are unaltered by the dilution process. While there is sound evidence that the basic structural array of the helix and the hydrogen bonds supporting it are essentially unaffected by dissolution, the disposition of the side chains is another matter. In particular, if they are relatively large and possess bonds susceptible to rotation, molecules of the diluent may mix with the side chains. The entropy associated with dispersion of solvent in this manner may easily dominate the entropy attributable to mixing of solvent with impervious rods.

The rather large side chains of PBLG, comprising nearly three-fourths of the polymer by weight, have the capacity to assume numerous configurations through rotations about the six single bonds of the skeleton of each of them. Many of these configurations are sterically forbidden, of course. Examination of the right-handed α-helix constructed from Courtaulds molecular models clearly shows a large number of configurations to be acceptable for the side chains. The corresponding model for the left-handed (stable form) α-helix of PBLA, having one less methylene group in the side chain, was similarly examined. Its bond rotational possibilities are fewer, but numerous nevertheless. A polypeptide such as poly-L-alanine, having only a small pendent substituent (*i.e.*, CH_3), is incapable of side-chain conformational isomerism. Hence, mixing of side chains with solvent does not present itself as a possible mechanism for solvent sorption.

With these thoughts in mind, we are led to suggest that the solution process in the region of small volume fractions of solvent will be largely dependent on the nature and size of the side chains attached to the main chain backbone. The side chains of the benzyl glutamate and of the benzyl aspartate polymers form a "soft" shell of considerable thickness around the helical core, and it is this outer layer that accommodates the first quantities of solvent absorbed by the polymer. Solvent thus absorbed expands the side-chain domains. The side chains being limited in length, this expansion process must reach saturation as more solvent is absorbed. At the conclusion of this first stage of the dilution process, the helices remain parallel to one another; the system accordingly is quasicrystalline. Further acquisition of solvent must necessarily be interhelical. In this second stage the rods with swollen outer shells gradually gain angular freedom, each rod eventually acting as a kinetic unit. Disorder of the initial alignment progresses until the concentration is reduced to the point where separation of a second phase occurs, the latter being isotropic and somewhat more dilute than the anisotropic (tactoidal) coexisting phase.

This description of the second stage of the dilution process is a restatement of the concepts underlying the statistical mechanical treatment of solutions of rod-like molecules.[7] The thermodynamic properties should fall within the domain of that theory. To be sure, the two stages will not be sharply differentiated as the foregoing account might suggest. Before mixing of solvent with side chains reaches its ultimate extent, intermolecular dispersion of solvent will commence, and incipient disordering of neighboring helix axes will occur.

The thermodynamics of the postulated side-chain mixing with solvent requires separate treatment. A partition function for a system of flexible, randomly oriented chains having one end immobilized is readily derived through the use of a lattice model or by any of the other schemes appropriate for mixtures of chain molecules. The treatment follows essentially the same course as that of polymer solutions,[17] except that the first segment of each chain is taken to be part of the helical core, and hence alternative locations are not offered to it. Each succeeding segment of the chain can then locate on a site neighboring the preceding segment if the site is vacant; the probability of a vacancy is approximated by the volume fraction of solvent in the side-chain domains. From the configurational partition function the total entropy of mixing is established, and, as for polymer solutions, an enthalpy term in the usual van Laar form may be incorporated to yield the total mixing free energy. Choosing the standard states to be pure solvent and flexible disoriented chains, the free energy of mixing is given by

$$\Delta G_M = RT[n_1 \ln \varphi_1' + \chi_1 n_1 \varphi_2'] \qquad (4)$$

where n_1 is the number of solvent molecules in the mixture, and $\varphi_1' = 1 - \varphi_2'$ is the volume fraction of

(17) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953.

solvent calculated on the basis of mixing being confined to the side chains. Thus, ascribing the same density to side chains as to the polypeptide as a whole, we have

$$\varphi_2'/\varphi_1' = (M_s/M_u)(\varphi_2/\varphi_1) \quad (5)$$

where M_s and M_u are, respectively, the molecular weights of the side chain and of the entire peptide unit.

The activity of the solvent according to eq. 4 is

$$a_1 = \varphi_1' \exp(\varphi_2' + \chi_1\varphi_2'^2) \quad (6)$$

The lower dashed curves in Figures 1–4 have been calculated from eq. 6 using eq. 5 for the adjusted volume fraction compositions. The values of χ_1 chosen to fit the experimental curves in the limit $\varphi_1 = 0$ are given in the legends to the figures. They are reasonable, but of doubtful significance in the light of the somewhat arbitrary assignment of the entire side chain to the soft shell available for mixing with solvent. A concentration gradient is inevitable, with more solvent occurring in the outer reaches of the side chains than near the helix core. Refinement to take account of this circumstance seems unwarranted without a proper basis for representing the distribution of solvent within the side chain domain. Obviously, revision in this direction would effectively decrease M_s and hence affect the relationship of φ' to φ. This, in turn, would lead to different values of χ_1.

Compliance with eq. 6 must, in any event, be limited to high polypeptide concentrations where mixing of solvent occurs exclusively within the side-chain domains. The concentration at which saturation of the side-chain domain is reached may be estimated crudely from geometrical considerations, on the assumption that the solvent is uniformly dispersed in this domain. If solvent were considered to permeate the side chains up to the β-carbon and if the effective maximum swollen radius of the side-chain domain were identified with the position of the benzylic carbon when the side chain is *fully extended*, then the radius of the side chain at saturation with solvent would be 9.4 Å. measured from the helix axis. If all solvent in the mixture were assigned to the side-chain domain, this point of saturation would be reached at $\varphi_1 = 0.37$. The estimated figure is obviously sensitive to the maximum radius assumed. The side chains will not, of course, be fully extended at saturation; on the other hand, the phenyl group, ignored above, will participate in the mixing process. Whatever the estimate of the side-chain domain at saturation may be, deviation from eq. 6 may be expected to commence well in advance of "saturation," owing to incidence of intermolecular mixing of solvent, whereby solvent enters interhelical regions, before completion of mixing with side chains. The direction of the departures observed (Figures 1–3) supports this interpretation. The approximate independence of the isotherms on the molecular weight of the polymer is also consistent with interpretation according to eq. 6 and the ideas expressed above.

The fact that the activity increases smoothly with the concentration of solvent, starting from $a_1 = 0$ at $\varphi_1 = 0$ in all cases examined, indicates that the side chains of the pure polypeptide are disordered. If at $\varphi_1 = 0$ these were in a regular array, a finite threshold activity a_1* would be required for the first increment of diluent, the situation being analogous in this respect to the mixing of a solvent with a crystalline solid. Absence of any intimation of sigmoidal shape in the experimental curves near $\varphi_1 = 0$ is unequivocal evidence for prevalence of a state of disorder in the polymer, or in that portion of it which engages in mixing with solvent; this disorder obtains *from the outset of the mixing process.* It must follow also that the disordered side chains of neighboring helices intermingle in the pure polypeptide as well as in mixtures with small quantities of diluent. In this connection it is significant that X-ray diffraction fails to reveal the positions of the side chains in the solid polymer.[3]

The simple form of the sorption isotherms, and in particular the uninterrupted increase of the activity a_1 with φ_1, is clear evidence for absence of phase transitions within the ranges of our measurements. Our results therefore stand at variance with inferences drawn by Luzatti and co-workers[5] from the X-ray diffraction of PBLG solutions in several solvents over corresponding ranges of concentration. They reported discontinuous changes in diffraction spacings with concentration, and these were attributed to different phases. For the solvents pyridine and dimethylformamide, lattice dimensions of a "complex phase" alleged to exist at high concentrations were reported to be independent of concentration. The constituent responsible for the reported X-ray reflections was therefore concluded to be a phase of fixed composition. If such a phase were formed in mixtures of PBLG with pyridine, the activity of the solvent would necessarily remain constant over the interval of its occurrence. Coexistence of phases of different (and definite) composition would certainly be reflected in the activity isotherms, contrary to observation.

Extending the arguments presented above, we arrive at the conclusion that the helical structure occurring in the pure polypeptide, *i.e.*, the α-helix, must persist throughout the range of our measurements. From the fact that the activities at greatest dilutions included are in the neighborhood of 0.9, it is apparent also that solute–solvent interactions are largely dissipated over this range. The environment of a solute particle will change little with further dilution. Marked alteration in the intramolecular conformation by dilution beyond the range of our measurements could conceivably occur only through the fortuity that the free energies of the competing conformations happen to be nearly coincident—a circumstance which would be of exceedingly rare occurrence. Luzatti[5] and others[6] contend however that PBLG undergoes transformation from the α-helix to the $^3/_{10}$ helix at dilutions exceeding $\varphi_1 = 0.6$ in three different solvents at ordinary temperature. Their conclusions, based on X-ray scattering and extrapolations of hydrodynamic data, would seem to elude rational explanation.

The entropy gain from mixing of solvent with the side chains at high concentrations has important implications for the solubility of helical polypeptides in general. The considerable reduction in activity (see Figures 1–4) obviously enhances solubility. The mixing of side chains with solvent produces, in effect, a repulsion between the solute particles. In this respect, the action

of the side chains finds parallel in the stabilization of colloidal particles of carbon black suspended in organic media by adsorption of aromatic compounds bearing long aliphatic side chains.[18] (Aromatic compounds with short side chains are ineffective.) When two particles thus stabilized approach one another, the entropy decrease resulting from "desolvation" (*i.e.*, unmixing) of these pendant chains introduces a repulsion between the particles. This explanation has been developed in detail by Mackor.[19]

The solubility of PBLG and PBLA in a variety of solvents appears to be conditioned by their bulky, flexible side chains. In contrast, other helical polypeptides such as poly-L-alanine, poly-L-phenylalanine, and poly-L-leucine are for the most part insoluble except in those solvents which cause partial or total disruption of the helical structure.[20] Their smaller, less flexible substituents offer little opportunity for side chain, mixing; hence, the repulsions generated by mixing of solvent with long side chains are absent, or much reduced, in these polypeptides. We suggest it is on this account that their tendency to disperse in solvents as intact helices is so limited.

The molecules of poly-L-alanine, etc., having shorter side chains, may be presumed to conform more nearly to the characteristics expected of impervious rod-like particles. The forces operative between two such particles in dense array are inevitably large owing to their size. In the absence of an electric double layer or other source of repulsion, their solubility is consequently very small. The predictions of theory for concentrated solutions of impervious rod-like particles with very small attractive interactions may therefore be beyond reach of experiment.

The previous theory[2,7] of solutions of rod-like particles appears to be in substantial agreement with experiment on PBLG and PBLA polypeptides throughout the low concentration range and including, especially, the region of the tactoidal phase separation. Here the solvent within the domain of the side chains may properly be regarded as a part of the solute particle. Eventual removal of solvent from these domains at high concentrations introduces a feature not accounted for in that theory. Consequently, functions derived previously for the chemical potentials require revision when applied to the helical polypeptides in this range of concentration. In particular, the coexistence of a phase of *high* concentration in equilibrium with a dilute phase, predicted theoretically[7] for a system of coherent, rod-like solute particles in a solvent in which the interaction is unfavorable to mixing (corresponding to a net attraction between solute particles), cannot be expected to occur among solutions of the soluble α-helical polypeptides.

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(20) The effects of highly polar substituents involve factors going beyond those considered in the present discussion.

The Viscoelastic Relaxation Mechanism of Inorganic Polymers. Amorphous Selenium[1]

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Amorphous selenium, a representative inorganic polymer which consists of a mixture of rings and chains, may relax in response to stress in one of several ways, the most probable being bond interchange and molecular slippage. In this work, which was undertaken in an attempt to elucidate the relaxation behavior of this material, the viscoelastic properties of selenium were studied by the technique of stress relaxation. Since the molecular weight dependence of the stress relaxation plots is quite analogous to that observed in the organic polymers relaxing by simple molecular slippage, it is concluded that this is also the mechanism encountered here.

I. Introduction

In a recent publication dealing with the viscoelastic properties of amorphous selenium,[2a] it was found that the material is very strongly reminiscent of the normal organic polymers like polystyrene or poly(methyl methacrylate). Thus, the mechanism of stress relaxation may well be mechanical flow. However, in the melt, selenium is known to reorganize to yield an equilibrium mixture of rings and chains,[2b] indicating that the process of bond interchange is proceeding at an appreciable rate, a process which is exactly analogous to the one occurring in the relaxation of the polysulfide rubbers.[3] In the polysulfide rubbers, flow occurs far

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