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Conformational Transitions of Polypeptides in Ternary Solvent Systems

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ABSTRACT: Phase diagrams for conformational transitions of polypeptides in ternary organic solvent systems containing two active and one inert component are calculated for a model which takes into account possible interaction of the two active components with each other as well as with the polypeptide backbone. The results of experimental determinations of conformational transitions and isothermal phase boundaries for poly(β-benzyl L-aspartate) (PBA) in three-model solvent systems are in generally good accord with the theory. The solvent systems are: (a) 1-chloropropionic acid–dichloroacetic acid (DCA)–1,1,2,2-tetrachloroethane (TCE), (b) monochloroacetic acid–DCA–TCE, and (c) dimethyl sulfoxide–DCA–TCE.

Conformational transitions in organic solvent soluble polypeptides are commonly studied in binary solvent mixtures in which the solute–solvent interaction of one component is indifferent to the conformational state of the polymer, while the second component is to some extent capable of competitively hydrogen bonding with the peptide and thereby disrupting the helix or other ordered conformation. These solvent constituents are commonly referred to as the inert and active components, respectively. A few studies have been carried out in which both components are nominally in the active class;[1] in this case a more complicated description involving multiple competitive equilibria between the solute and the two active components and between the two solvents themselves would be necessary. The possible interaction of adjacent filled bonding sites on the macromolecule could also be taken account in a complete treatment and the presence of a third, inert, solvent which serves to modify the activities of the helix-disrupting constituents should be allowed for.

We wish to report here a theoretical treatment of conformational transitions of polypeptides in such ternary mixed solvents, together with the results of relevant experimental studies in which the isothermal helix–coil transition of poly(β-benzyl L-aspartate) (PBA) has been investigated in three different solvent systems. For each example we have chosen a strong and moderately weak active solvent together with an appropriate inert solvent. The latter was 1,1,2,2-tetrachloroethane (TCE), and the strongly helix disrupting solvent was dichloroacetic acid (DCA) throughout, while the weaker bonding solvents were (a) monochloroacetic acid (MCA), (b) 1-chloropropionic acid (CPA), and (c) dimethyl sulfoxide (DMSO), respectively.

Isothermal conformational phase equilibria involving ternary solvent systems can be most appropriately represented using an equilateral triangle, in which the apices represent the pure components, and the sides the mole fractional compositions of the respective binary solvent pairs. Any interior point is then simply related to the mole fractional composition of the ternary solvent mixture. The triangle itself constitutes an isothermal section of a right angular equilateral prism whose vertical coordinate represents temperature. We are considering here the phase boundary (or boundaries) separating domains containing helical and random-coil conformations, where the phase boundary is defined, as usual, as the locus of the points at which the average fractional helical content of the polypeptide is 0.5.

Theory

The equilibrium constant, s, describing the helix–coil equilibrium in the presence of a mole fraction x_A of an active solvent A in an active–inert (I) solvent mixture is given by[2]

$$s = K_1K_{2A}/(K_{2A} + x_A) \tag{1}$$

where K_1 and K_{2A} are the equilibrium constants, at a given temperature, relating to intramolecular (peptide–peptide) and intermolecular (peptide–solvent) interactions, respectively. In the presence of a mole fraction x_B of a second active solvent, B, eq 1 is modified:

$$s = \frac{K_1K_{2A}K_{2B}}{K_{2A}K_{2B} + x_A'K_{2B} + x_B'K_{2A}} \tag{2}$$

the constants K_{2A} and K_{2B} now referring to the individual interactions of A and B with the polypeptide. The quantities x_A' and x_B' are the effective mole fractions of free A and B available for this interaction, taking into account the possible formation of a mole fraction x_{AB}' of the complex AB. The

equilibrium A + B $\rightleftharpoons$ AB is characterized by a further constant K_3 where

$$K_3 = x_{AB}'/x_A'x_B' \tag{3}$$

We denote the solutions of eq 1 for the conditions $s = 1$, for the binary solvent pairs I–A and I–B, by x_A^0 and x_B^0, respectively (tantamount to stipulating K_{2A} and K_{2B} at the relevant temperature), and then rewrite eq 2:

$$s = \frac{K_1 x_A^0 x_B^0}{x_A^0 x_B^0 + (K_1 - 1)x_A' x_B^0 + (K_1 - 1)x_B' x_A^0} \tag{4}$$

It can readily be shown that the mole fraction x_A' can be written as

$$x_A' = x_A(1 + x_{AB}') - x_{AB}' \tag{5}$$

with an analogous expression for x_B'. Phase boundaries are obtained from solutions of eq 4 for the condition $s = 1$. We denote such solvent compositions x_A^*, x_B^*, etc., and combining (4) and (5) find

$$\frac{x_A^*(1 + x_{AB}'^*) - x_{AB}'^*}{x_A^0} + \frac{x_B^*(1 + x_{AB}'^*) - x_{AB}'^*}{x_B^0} = 1 \tag{6}$$

The isothermal phase boundary is thus described by pairs of values of x_A^*, x_B^* which for a given parameter set K_3, x_A^0, and x_B^0 simultaneously satisfy eq 3, 5, and 6.

In arriving at eq 6 several assumptions have been made, including: (a) activities are replaced by mole fractions throughout, (b) complexes of A and B other than AB are ignored and it is assumed that AB is inert with respect to its interaction with the polypeptide, and (c) the polypeptide concentration is negligible. Adsorbate–adsorbate interaction on the polymer is not specifically taken into account but may be formally considered as being included in the association parameter K_3. In principle separate activity measurements of the two active solvents could distinguish between these factors. As in the earlier treatment of conformational transitions in the presence of a single active species,[2] possible equilibria among solvent molecules of the same species, e.g., dimerization, have not been explicitly considered. Where such equilibria exist they will change the reference state of the relevant solvent activities and hence the formal definitions of K_{2A}, etc., without changing the overall results. Similarly, in the present case, a consideration of the role of the complex AB vis-à-vis A_2 and/or B_2 dimers, for example, could be incorporated at the cost of introducing further equilibrium constants. There would seem to be no advantage however since again such effects can be accounted for by appropriate redefinition of the single arbitrary parameter K_3.

Typical phase boundaries calculated from eq 6 for given x_A^0, x_B^0 are shown as a function of K_3 in Figure 1. The limiting case $K_3 = 0$, indicative of no AB complex formation and a simple weighted additivity of activities of A and B, results in a linear solution for x_A^* as a function of x_B^*,

$$\frac{x_A^*}{x_A^0} + \frac{x_B^*}{x_B^0} = 1 \tag{6'}$$

and a rectilinear phase boundary in the ternary diagram. For finite K_3 the boundary becomes increasingly convex with respect to the apex corresponding to pure I. There will exist, therefore, the possibility of observing a double conformational transition, from coil to helix to coil, for example, by adding solvent B to a polypeptide in an A–I solvent mixture of initial composition y_1 (Figure 1). As K_3 increases further, a critical value will be reached at which the side AB becomes tangential to the phase boundary. For values of K_3 above this the solution of eq 6 therefore is degenerate and two branches of the phase boundary will be observed. The relatively strong tendency to form AB complex associated with this effect thus has the result of decreasing the net potential for helix disruption of an A–B mixture to the extent that, within a certain composition range, the ordered conformation can be regenerated from the random state. The maximum effect of this type is of course observed when $K_3 = \infty$. The latter condition implies that a given A–B solvent mixture will have in effect only free A or B, depending on which of these components is initially in stoichiometric excess. Equation 6 can then be written in closed form for the two branches of the phase boundary:

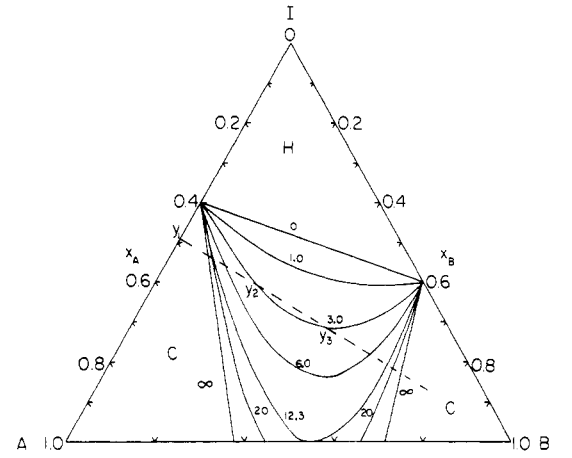

Figure 1. Calculated isothermal phase diagrams for conformational transition of polypeptide in ternary solvent systems as a function of solvent association constant K_3. Components A and B are active; I is inert. Parameters x_A^0 and x_B^0 arbitrarily assigned values of 0.4 and 0.6, respectively. Each phase boundary separates ordered, helical (H), and random-coil (C) domains as shown. For $K_3 > 12.3$ the phase boundary is split, with the helical domain located between the respective branches. The dashed line illustrates that the addition of solvent B to the polypeptide in an A–I mixture of initial composition y_1 induces a double conformational transition as the titration path intersects, for example, the phase boundary $K_3 = 3$ at compositions y_2 and y_3.

$$\frac{x_A^* - x_B^*}{x_A^0(1 - x_B^*)} = 1 \qquad (x_A^* > x_B^*) \tag{6a''}$$

and

$$\frac{x_B^* - x_A^*}{x_B^0(1 - x_A^*)} = 1 \qquad (x_B^* > x_A^*) \tag{6b''}$$

Extension to other situations, for example, the case $1 \leq x_A^0$ or $x_B^0 \leq \infty$, is straightforward.

Experimental Section

The results predicted on the basis of the theory above were tested by experimental determinations of conformational transitions and phase boundaries for poly(β-benzyl L-aspartate) in the three solvent systems detailed in the introductory section. The phase boundaries were all determined at 67 °C to facilitate solubilization of the polypeptide in the solvent systems used and thereby permit an intercomparison of the results.

The helix-coil transition of PBA in DCA–TCE mixtures has been studied over a wide range of temperatures and solvent compositions, thus it was established that $x_{DCA}^0 = 0.064$ at 67 °C.[3] Considerable data are available for the transition of PBA in DMSO-containing mixtures, though in this case the inert constituent was dioxane rather than TCE. Therefore, because critical compositions are known in certain cases to be somewhat dependent on the nature of the inert solvent used (contrary to the strict predictions of theory), the parameter x_{DMSO}^0 in DMSO–TCE mixtures could only be estimated to be around 0.4 at 67 °C.

No previous data are available for the conformational transition of PBA in either monochloroacetic or 1-chloropropionic acids; from elementary structural considerations it was presumed that both solvents would be substantially weaker in their helix-disrupting capabilities at a given temperature than is DCA, and this was found to be the case.

Materials. PBA (Pilot Chemicals, $M_v = 1.8 \times 10^5$) and DMSO (Matheson, "Spectroquality") were used as received. The chlorinated

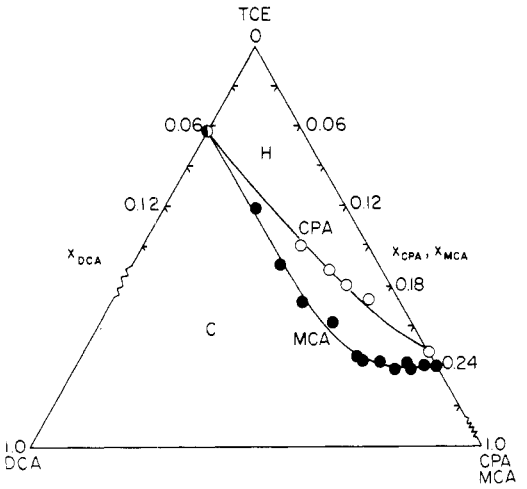

Figure 2. Phase diagram for PBA in the DCA–MCA–TCE and the DCA–CPA–TCE ternary solvent systems at 67 °C; the phase boundaries are labeled MCA and CPA, respectively. Full lines represent theoretical best fits of data (see text). The boundaries separate the helical (H) and random-coil (C) domains as shown.

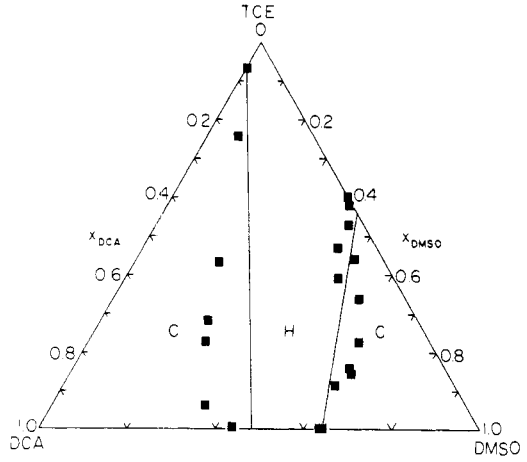

Figure 3. Phase diagram for PBA in the DCA–DMSO–TCE ternary solvent system at 67 °C. Full lines represent calculated phase boundaries using $K_3 = \infty$ (see text). The two branches of the boundary separate the helical (H) and two random-coil (C) domains as shown.

acids (Eastman Reagent Grade) were distilled once under vacuum before use.

Procedure. The PBA was dissolved (0.1% w/v) in the appropriate active solvent or solvent mixture and the second active solvent (or mixture) containing the same concentration of PBA was added in suitable aliquots. The optical rotation at 436 mμ of the solution was followed through each isothermal titration using a Perkin-Elmer 141 M spectropolarimeter with a thermostated cell held at 67 °C. Typically sigmoidal curves of optical rotation vs. solvent composition were obtained in the vicinity of the phase transitions, and the solvent compositions corresponding to the presence of equal amounts of helix and coil, that is to a point on the phase boundary, could be readily determined. After a preliminary estimate of the position of a given phase boundary had been made, subsequent solvent titration paths were arranged so that the latter and the boundary were approximately orthogonal, thereby maximizing the sharpness of the transition. Thus the critical solvent compositions x_A^* and x_B^* could be readily determined to within 0.01 mol fractional units or better in most cases.

A few points on each boundary were checked by observing the thermally induced conformational transition of PBA in the appropriate solvent mixture. For all three solvent systems the thermal transitions in PBA at 67 °C were found to be in the "normal", helix-to-coil direction, regardless of solvent composition.

Results and Discussion

Phase boundaries for the PBA transition in the three solvent systems are shown in Figures 2 and 3. It will be seen first

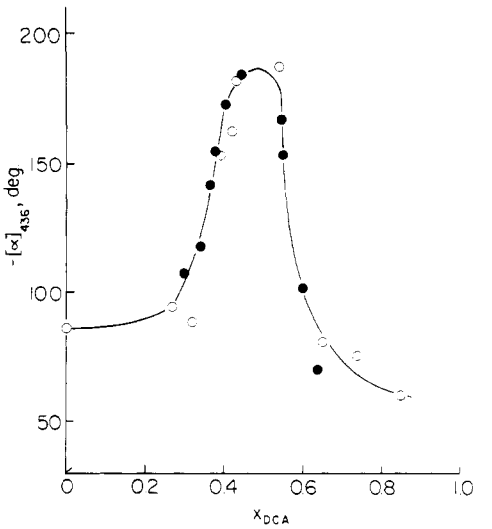

Figure 4. Specific optical rotation (436 nm) of PBA in DCA–DMSO mixtures at 67 °C as a function of solvent composition expressed as mole fraction DCA, x_{DCA}. Open circles: change in $[\alpha]_{436}$ as DCA is added to PBA in DMSO through the helical domain, approximately $0.36 < x_{DCA} < 0.56$. Filled circles: results of the solvent titration in the opposite direction, again showing coil–helix–coil progression (see text).

that the effective reduction of activity in the DCA–CPA mixture is relatively modest, with an almost rectilinear phase boundary joining the coordinates x_{DCA}^0 (=0.064) and x_{CPA}^0 (=0.23). The best fit of eq 5 was obtained with a value for K_3 of 2.1 ± 0.3.

Monochloroacetic acid (MCA) is only slightly weaker than is CPA in this context ($x_{MCA}^0 = 0.24$) but its net interaction with DCA is much more pronounced. Thus a distinctly convex, asymmetrical phase boundary was observed which could be best fitted with an association equilibrium constant K_3 of 8.4 ± 0.5 (Figure 2).

The phase diagram for the DCA–DMSO–TCE solvent system, Figure 3 ($x_{DMSO}^0 = 0.44$), is qualitatively different from the two cases discussed above. DMSO is known to react with strong mineral acids to yield a variety of products. With DCA the reaction is apparently limited to the formation of a complex[5] which, according to the measured phase diagram, appears to be very nearly inert. The result is that addition of DMSO to a solution of PBA in pure DCA will induce the formation of the PBA helix from the coil; further addition of DMSO produces a reversion to the disordered conformation. It is important to note that this sequence of transitions is reversible, as may be seen from Figure 4. In a test of this point, the mole fraction of DCA, x_{DCA}, in a solution of PBA in pure DMSO was increased from 0 to 0.85; a portion of the solution with the latter solvent composition was then titrated back to about 0.30 mol fraction DCA by the addition of further DMSO. The optical rotation during the double isothermal titration (coil–helix–coil) was followed with the results shown. The midpoints of the transition curves shown correspond to equal helix and coil content in the polypeptide, i.e., to $s = 1$, and the corresponding values of x_{DCA} thus obtained are those shown on the binary DCA–DMSO co-ordinate in Figure 3.

The best fit of the experimental results in terms of eq 3 and 6 for the DCA–DMSO–TCE system is with $K_3 = \infty$. While this produces reasonable agreement with experiment, deviation is observed in solvent compositions containing excess DCA. These may be attributed to the formation of DCA–DMSO complexes of other than 1:1 stoichiometry which would have the effect of further decreasing the availability of DCA to enter into competitive bonding with the polypeptide, and hence expand the helical domain in the phase diagram as is observed.

The above results show that isothermal polypeptide conformational transitions in the presence of two interacting solvents and an inert solvent can be satisfactorily accounted for with effectively a single parameter model at least in situations where the solvent–solvent interaction is not overwhelming in comparison with the solvent–solute interaction. In certain cases it may also be feasible to determine the respective solvent activities in ternary systems experimentally. This would yield a zero-parameter prediction of polypeptide stabilities in such a system, provided that other factors entering into K_3 (e.g., adsorbate–adsorbate interaction) were unimportant. Additional information concerning, for example, transition enthalpies and fractions of bonded sites at equilibrium require the extension of the above treatment to include the effect of temperature on the phase boundaries. This will be done in a future report.

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Monte Carlo Calculations on Polypeptide Chains. IX. A Study of the Effect of Long-Range Interactions on the Helix–Coil Transition

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ABSTRACT: A Monte Carlo statistical mechanical study of the helix–coil transition for a hard-sphere model of poly(L-alanine) has been conducted based on the theory of Lifson and Roig but including the effects of long-range interactions. A stochastic model of the kinetics of the helix–coil transition is presented, and a Monte Carlo simulation of the kinetics based on this model was used to generate equilibrium chain samples, each chain of which consisted of Lifson–Roig weighted sequences of helix and coil residues. Each of the chains in this sample was then used many times by assigning at random specific sterically allowed coil states from a hard-sphere Ramachandran dipeptide map. Unperturbed properties were then calculated using this sample and perturbed properties by using only the non-self-conflicting subset. The properties calculated were the average degree of hydrogen bonding, the average length of a helical sequence, the mean-square end-to-end distance, the mean-square radius of gyration, and the distribution functions for the end-to-end distance and radius of gyration. This study was conducted at chain lengths 10, 34, and 85 residues. Helix–coil transition theory was fit to the perturbed transition curves in an attempt to ascertain if theory could then predict the perturbed values of the dimensions. For the hard-sphere model used in these calculations, it was found that current helix–coil transition theory does not predict the correct perturbed dimensions.

I. Introduction

Since 1951 when Pauling, Corey, and Branson[1] first proposed the α helix as an important conformation for polypeptide chains, there has been a continuing interest in the forces which stabilize this conformation. Schellman[2,3] provided the first theoretical treatments of the helix–coil transition, and his work was followed shortly by various statistical mechanical theories such as those by Gibbs and DiMarzio,[4] Hill,[5] Zimm and Bragg,[6] Peller,[7] and Lifson and Roig.[8] Poland and Scheraga[9,10] and DeVoe[11] have provided recent reviews of this subject. While these theories all differ in their details, they each assign statistical weight parameters to the random coil and helical sequences and then apply standard mathematical techniques to calculate the partition function and the various average properties, such as the fractional hydrogen bonding parameter θ and the average length of helical sequences $\bar{l}$. In their formulation all of these theories take account of the fact that it is more difficult for helical sequences to nucleate than to propagate once they are formed. This causes the helix–coil transition to be highly cooperative and to increase in sharpness with increasing chain length. As a function of chain length, the transition can be divided into three regions. For very short chains the transition is of the all or none type, there being only completely helical and completely random coil molecules present to any appreciable extent at equilibrium. For intermediate chain lengths the transition is of the single helix type, the helix melting from both ends. For long chain lengths the transition proceeds through internal breaks in the helix so that a given molecule has more than one helical sequence.

The general objective of helix–coil transition theory is to explain the dependence of the average properties on chain length, temperature and solvent composition. This is accomplished by treating the various statistical weights as adjustable parameters. Lifson and Roig,[8] for example, introduced the three parameters u, v, and w. u is the statistical weight of a chain unit in the randomly coiled state, i.e., the composite of all configuration space involving internal rotation about a pair of φ and ψ rotational angles for respectively the backbone N–C$^\alpha$ and C$^\alpha$–C′ single bonds, except for a small region designated as the α-helical region. v is the statistical weight of a helical unit which has one or both of its neighboring units in the coil state, and w is that for a helical unit when both of its neighbors are also in the helical state. Since only the