mined are given in Table I. In many instances no good fit to the data could be obtained.

With the three ((BzlGlu)$_n$, (CbzOrn)$_n$, (CbzLys)$_n$) dimethylformamide-soluble polymers only the solvent–side-chain mixing and the BET models give a good fit to the experimental data of the models tried. The BET c parameter is a measure of the ratio of the equilibrium constants for the strong and the weak binding sites. As c is less than unity for (BzlGlu)$_n$ the BET isotherm appears to have no physical meaning in this case. The c values for (CbzLys)$_n$ and (CbzOrn)$_n$ are greater than unity, though not large. If the literal interpretation of the BET model is used, *i.e.*, the weak binding sites correspond to solvent condensation with a heat of sorption equal to the heat of condensation, then the strong binding site would be expected to have a heat of sorption in excess of the heat of dimethylformamide condensation. The fact that no observable heat is detected for (CbzOrn)$_n$ suggests that although the BET isotherm fits the data, it is not the model of choice.

With the (MeGlu)$_n$ the data can be fit to either a BET or the Langmuir + impenetrable rod models. The BET analysis yields values for the number of strong binding sites which is independent of temperature, and a temperature-dependent c parameter which corresponds to an enthalpy of 3 ± 0.5 kcal/mol of sorbed solvent. The model assuming binding sites for isolated sorption plus solvent-impenetrable rod mixing gives a sensible though not so good a fit, whereas the model assuming strong binding sites plus solvent–side-chain mixing does not fit the data. Taken literally one must postulate that when dimethylformamide is sorbed onto (MeGlu)$_n$, the side chains stay ordered. Additional information, presented in the accompanying paper, must be utilized to decide between the two models which fit the data. The absence of a significant temperature dependence in the binding constant does suggest, however, that the Langmuir + impenetrable rod model has less physical significance. Analysis of the (BzlAsp)$_n$ data suggests a conclusion analogous to (MeGlu)$_n$.

The analysis of the (Ala)$_n$ data is particularly interesting, in that (Ala)$_n$ has no flexible side chain. Although the BET equation gives statistically a much better fit, several of the models can be sensibly fit to the data. The strong, independent sorption sites + solvent–side-chain mixing model makes no physical sense. The three remaining models which can fit the data, L, BET, and L + IR, could each be considered to be physically meaningful. The number of strong binding sites are, however, considerably different when analyzed by the different model equations. Thus, auxiliary information is needed in order to distinguish among the models.

Structurally (Phe)$_n$ is similar to (Ala)$_n$ in that it has very little possible sidechain flexibility. The conclusions are analogous to those for (Ala)$_n$. We were, however, able to measure isotherms at more than one temperature, thus allowing the temperature dependence of the BET c parameter to be estimated. The temperature variation in c corresponds to an enthalpy of 4 ± 1 kcal, and is similar to that estimated for (MeGlu)$_n$.

The analysis of the vapor sorption isotherms can now be summarized. In terms of goodness of fit the Flory–Leonard solvent–side-chain mixing model adequately describes the sorption onto the three dimethylformamide-soluble polymers (BzlGlu)$_n$, (CbzLys)$_n$, and (CbzOrn)$_n$, whereas the finite layer BET model best describes sorption onto the non-dimethylformamide-soluble polymers (BzlAsp)$_n$, (MeGlu)$_n$, (Phe)$_n$, and (Ala)$_n$. Analysis of the temperature dependence of the BET c parameter indicates an enthalpy of condensation of dimethylformamide onto (Phe)$_n$ and (MeGlu)$_n$ which is 3–4 kcal in excess of the heat of dimethylformamide gas–liquid condensation enthalpy. Without additional information a unique isothermal model cannot be chosen for the four nonsoluble polymers from the various models considered. Finally the sorption data strongly indicate that (MeGlu)$_n$ and (BzlAsp)$_n$ have side chains which are ordered, and remain ordered during vapor sorption of dimethylformamide.

Effect of Side-Chain Substituents on the Thermodynamics and Dynamics of Helical Polypeptides. II. Nuclear Magnetic Resonance Studies

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ABSTRACT: The solvent dependence of polymer nmr line widths has been determined for a series of helical polymers using dimethylformamide-d_7. The side chains of poly(benzyl glutamate) and poly(carbobenzoxyornithine) were found to become increasingly flexible as solvent was added. By contrast, the flexibility of sidechains of poly(phenylalanine), poly(benzyl aspartate) and poly(methyl glutamate) were found to be little effected by solvent sorption. These results were consistent with sorption isotherm data. Strong interactions of solvent and polymer can be detected, and correlate well with thermodynamic data.

In previous papers[1,2] we have reported the results of vapor sorption studies of dimethylformamide onto a number of polypeptides, poly(ϵ-carbobenzoxy-L-lysine) ((CbzLys)$_n$), poly(δ-carbobenzoxy-L-ornithine) ((CbzOrn)$_n$), poly(γ-benzyl L-glutamate) ((BzlGlu)$_n$), poly(γ-methyl L-glutamate) ((MeGlu)$_n$), poly(β-benzyl L-aspartate) ((BzlAsp)$_n$), poly(L-phenylalanine) ((Phe)$_n$), and poly(L-alanine) ((Ala)$_n$). In this paper we present the results of proton magnetic resonance studies of the interaction between dimethylformamide and the polypeptides. The mobility of the dimethylformamide molecules sorbed by the solid polymers and the effect of the sorbed dimethylformamide molecules on the mobility of polymer side chains were also investigated.

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[2] J. H. Rai and W. G. Miller, *Macromolecules*, **6**, 257 (1973).

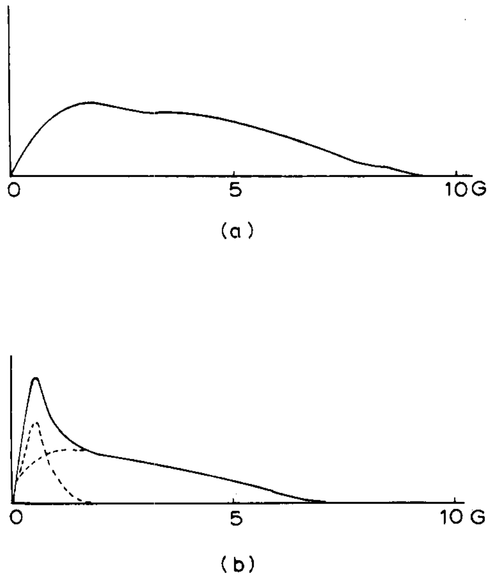

Figure 1. Nuclear magnetic resonance broad line spectrum, derivative curve: (a) solid polymer $(MeGlu)_n$; (b) $(MeGlu)_n$ + 16.1 wt % dimethylformamide-d_7.

Experimental Section

The source and handling of the polypeptides and solvent used in the present work have been described previously.[1,2] A Varian DP-60 spectrometer was used for the proton wide-line nmr measurements, which were carried out at 28°. For dimethylformamide line-width measurements, an A-60D spectrometer was used at 28°. Both $(CbzOrn)_n$-dimethylformamide and $(BzlGlu)_n$-dimethylformamide solutions were prepared by the vapor sorption method. A weighed amount of $(BzlGlu)_n$ (or $(CbzOrn)_n$) was placed in a 5-mm nmr tube. The tube and a beaker containing dimethylformamide were placed in a desiccator. The desiccator was evacuated and sealed, and the polymer was allowed to absorb dimethylformamide until the desired composition was obtained. With $(MeGlu)_n$, $(BzlAsp)_n$, $(Phe)_n$, and $(Ala)_n$, about 0.5 ml of dimethylformamide was added to the polymer, and excess dimethylformamide was removed slowly under low pressure until the desired composition was obtained. Deuterated dimethylformamide (d_7, 99.0% M.I.P.) was obtained from Wilmad Glass Co., Inc., New Jersey.

Results

Proton magnetic resonance spectra of solid $(BzlGlu)_n$, $(BzlAsp)_n$, $(MeGlu)_n$, $(Phe)_n$, and $(CbzOrn)_n$, and the mixtures of the polymers with dimethylformamide were taken experimentally in the first derivative mode at ambient temperature (~28°). The spectra were taken at a modulation frequency (40 Hz) which was substantially less than the line width, and also at small modulation amplitudes to eliminate modulation broadening. The most significant effect of adding dimethylformamide to the polymers is the appearance of a narrow line superimposed on the broadline of the polymer. This behavior is observed with each of the five polymers. As shown in Figure 1, the narrow line does not arise entirely from the dimethylformamide protons, as similar behavior was observed when deuterated dimethylformamide was sorbed onto the polymers. The nmr second moment[3] determined as a function of dimethylformamide-d_7 composition is shown in Figure 2. It is apparent that the effect of dimethylformamide-d_7 on both the $(BzlGlu)_n$ and $(CbzOrn)_n$ second moments is significantly different from its effect on the second moments of $(Phe)_n$, $(BzlAsp)_n$, and $(MeGlu)_n$. The second moment of both $(CbzOrn)_n$ and $(BzlGlu)_n$ decreases smoothly and continuously with increase in dimethylformamide-d_7. The second moments of $(Phe)_n$, $(BzlAsp)_n$, and $(MeGlu)_n$, however, are seen to decrease upon adding small amounts of dimethylformamide-d_7 with little further decrease when the amount added exceeds ~10 wt %. The range of solvent composition that can be studied is rather limited with $(Phe)_n$, $(BzlAsp)_n$, and $(MeGlu)_n$ if a single-phase system is to be maintained. The nmr second moments of solid $(Phe)_n$, $(BzlGlu)_n$, $(BzlAsp)_n$, and $(MeGlu)_n$ have been determined.[4-7] Our values are in reasonable agreement.

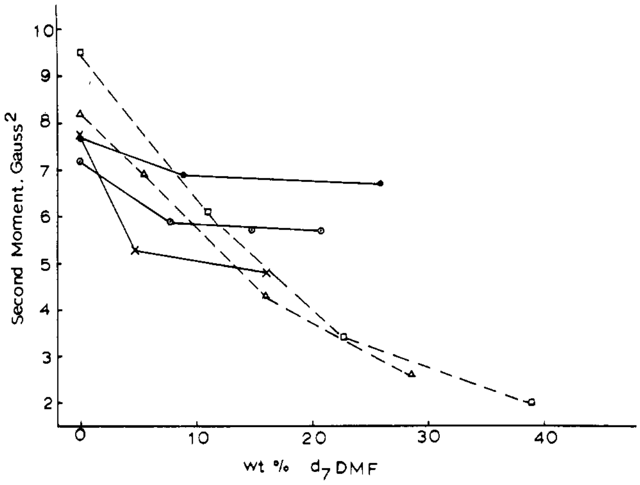

Figure 2. Nuclear magnetic resonance second moment as a function of dimethylformamide-d_7 content: (●) $(Phe)_n$, (□) $(CbzOrn)_n$, (△) $(BzlGlu)_n$, (x) $(MeGlu)_n$, (⊙) $(BzlAsp)_n$.

The line shape of the nmr spectra of each of the solid polymers can be treated as if it were a single, broad absorption. The spectra obtained from the mixtures of polymers and dimethylformamide-d_7 can be resolved approximately into two components, as shown in Figure 1b. By numerical integration, the relative number of protons in each component may be determined. The ratio, R, of the number of protons in the narrow to the broad component is plotted as a function of composition in Figure 3. Inasmuch as even in the absence of solvent the spectra cannot be fitted to a simple mathematical form, such as a Gaussian line shape, the resolution of the spectra for samples containing deuterated solvent was not done with the aid of analytical line shapes. Although there is a certain amount of arbitrariness, the general form of the composition variation of the R values is considered to be meaningful.

As a result of the slow motion of the polymer the direct nuclear dipole interactions are not averaged to zero and the line width of the proton resonance of the polymer is very broad even in the presence of considerable solvent, as can be seen from Figures 1 and 2. The resonance is so broad that in the high resolution Varian A-60 spectrometer it appears as a part of the base line. Consequently, by sorption of nondeuterated dimethylformamide onto the polymer the dimethylformamide protons could be studied. The solvent proton resonance was much narrower than that of the polymer on which it was sorbed, and was the order of 100–500 Hz. The line width was a function of the composition of the system as well as the nature of the

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Table I

Polymer	System Composition (mol of DMF[a]/mol of Monomer)	DMF Sharp Component Line Width (Hz)	Line Width at 0.25 mol of DMF/mol of Monomer (Hz)	Estimate of Bound DMF (mol of DMF[a]/mol of Monomer)
(Ala)$_n$	0.35-0.15	80-160	125	0.05
(MeGlu)$_n$	0.3-0.17	200-300	225	0.12
(BzlGlu)$_n$	0.75-0.2	150-300	275	0.16
(Phe)$_n$	0.65-0.2	150-400	375	0.16
(ClzOrn)$_n$	0.6-0.4	250-400	>500	0.33
(BzlAsp)$_n$	0.55-0.25	300-500	>500	0.36

[a] DMF = dimethylformamide.

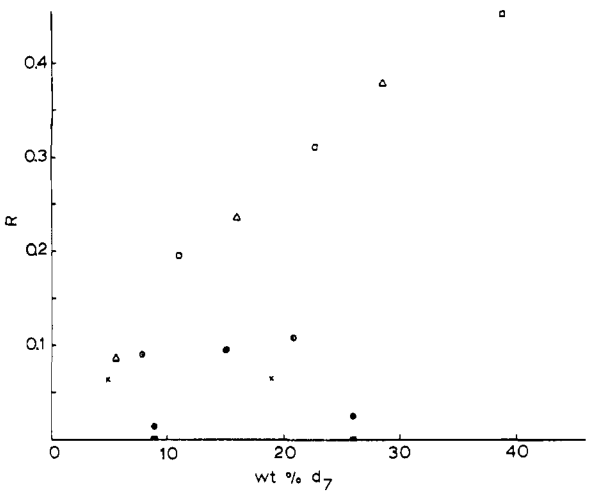

Figure 3. *R*, the ratio of the number of protons in the narrow to the broad component, as a function of dimethylformamide-d_7 content: (□) (CbzOrn)$_n$, (△) (BzlGlu)$_n$, (x) (MeGlu)$_n$, (○) (BzlAsp)$_n$, (●) (Phe)$_n$, (■) (Phe)$_n$ after exchange.

polymer to which it was sorbed. It was noted also that the signal intensity at a fixed solvent composition depended on the nature of the polymer on which the dimethylformamide was sorbed, suggesting that some dimethylformamide is bound for sufficiently long times to be dipolar broadened into the apparent base line. This was put on a semiquantitative basis by the use of Wilmad nmr microtubes with cylindrical cavity, so that the entire sample was monitored by the detector. As a reference the intensity of known quantities of glycerol was measured under the same instrumental conditions. In this manner an estimate could be made of the number of solvent molecules bound per monomer. The results are summarized in Table I, ranked in order of increasing line width. Solvent dissolved on (Ala)$_n$ exhibits the narrowest line width, and the spectrum is composed of a well-defined methylformyl proton doublet over the entire composition range that was studied. By contrast no above base-line solvent signal was detected when 0.15 mol of dimethylformamide/mol of monomer was sorbed onto (BzlAsp)$_n$. Even in the highest solvent composition studied the nmr signal was broad, and the methyl and formyl protons were not resolved. When compared at a fixed solvent composition, such as 0.25 mol of dimethylformamide/mol of monomer (Table I), the wide variation in line width is evident and is a reflection of the difference in polymer-solvent interaction.

Discussion

Polymer Line Width. In previous papers[1,2] we have shown that there is a qualitative difference in the sorption isotherms of the highly dimethylformamide soluble polymers (BzlGlu)$_n$, (CbzLys)$_n$, and (CbzOrn)$_n$ and the dimethylformamide-insoluble polymers (MeGlu)$_n$, (BzlAsp)$_n$, (Phe)$_n$, and (Ala)$_n$. In the former the dissolution thermodynamics were controlled by the entropy of solvent-polymer side-chain mixing whereas in the latter there was little evidence that this entropy source was a contributing factor. The polymer nmr line widths as a function of solvent composition likewise show a qualitative difference between the dimethylformamide soluble and insoluble polymers, as can be seen in Figure 2. With the soluble polymers continuing addition of solvent gives greater flexibility to the side chains. As the dipolar interaction becomes averaged out the side-chain proton spectrum collapses into a fairly narrow line. In dilute solution the width of the polymer spectrum is controlled by the protons more or less rigidly held in or near the helix backbone.

Addition of solvent to the nonsoluble polymer (BzlAsp)$_n$ results in only a slight drop in the nmr second moment. Calculations based on the Van Vleck equations indicate that at room temperature in the solid polymers rotation is occurring in the phenyl and perhaps in the attached methylene group.[7] As viewed from the solvent composition-polymer line width measurements the addition of solvent allows the side chain to have flexibility not much greater than in the solid state. This suggests that the sidechains are ordered in the presence of dimethylformamide, consistent with the sorption isotherms.[2] The approximate resolution of the spectrum into a narrow and a broad component, as shown in Figure 3, is consistent with the notion of rather inflexible side chains.

The recent nmr studies by Bradbury *et al.*[8] show that there is a preferred side-chain orientation in (BzlAsp)$_n$-$CDCl_3$-CF_3COOH solutions. Theoretical calculations carried out by Yan *et al.*[9] indicate that for helical polyaspartate esters *in vacuo,* there are four possible preferred side-chain conformations, two longitudinal and two transverse to the helical axis. In transverse conformation, side chains wrap tangentially about the backbone at right angle to the helix axis; while in longitudinal conformation, side chains lie more nearly parallel to the helix axis. Recent nmr studies of Silverman *et al.*[10] exclude the possibility that a transverse conformation is preferred for left-handed α-helical (BzlAsp)$_n$ in chloroform solution. Although the exact nature of the side chain conformation in (BzlAsp)$_n$ is still not known, it seems highly likely that the side chains are ordered.

The (Phe)$_n$ data are similar to those for (BzlAsp)$_n$. Nuclear magnetic resonance data on solid (Phe)$_n$ have previously[4] indicated that the phenyl group has some mo-

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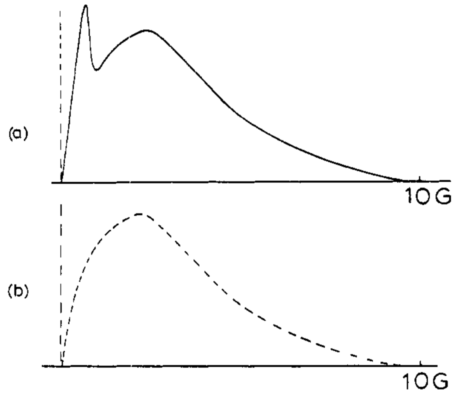

Figure 4. Nuclear magnetic resonance spectra for $(\mathrm{Phe})_n$ and 8.9 wt % dimethylformamide-d_7 mixture (a) before exchanged dimethylformamide-d_7 was desorbed, (b) after exchanged dimethylformamide-d_7 was desorbed and new dimethylformamide-d_7 was added.

tional freedom at room temperature. Addition of solvent increases the freedom, though it does not appear to produce the complete motional freedom observed at high temperature for $(\mathrm{Phe})_n$.[4] Thus, the restricted rotation at room temperature may be a result of local steric conflict which is not altered by addition of solvent. Again the nmr data are consistent with the sorption isotherms.[2]

Small addition of dimethylformamide to $(\mathrm{MeGlu})_n$ results in a substantial drop in line width, which is relatively unaffected by further addition of solvent. Comparison with calculated line widths indicates that the side chain has considerable, but not complete, motional freedom. The ratio of the narrow to wide components (Figure 3) suggests that there is considerable side-chain restriction. In concurrence with this the sorption isotherm[2] was not describable by the solvent-side-chain mixing model.

Solvent Line Widths. One would not expect the polar dimethylformamide molecule to strongly interact with the nonpolar $(\mathrm{Ala})_n$ side chain, and the disappearance of only 5% (Table I) of the solvent could easily be explained by tight binding to the ends of the presumably helical $(\mathrm{Ala})_n$ molecules in this rather low molecular weight polymer.[2] It is thus not surprising that the solvent exhibits the sharpest spectrum when sorbed on $(\mathrm{Ala})_n$. Although a similar argument could be made for $(\mathrm{Phe})_n$, the line width is considerably larger. This may be a result of interaction of the phenyl ring with the solvent. Of considerable interest is the fact that a significant amount of dimethylformamide appears to be tightly bound, which is consistent with the sorption isotherms.[2] As with $(\mathrm{Ala})_n$ the molecular weight was low suggesting a possibility of bonding to the ends of the chains such as the non-hydrogen-bonded NH groups. If so, isotopic exchange might occur between dimethylformamide-d_7 and the NH protons. In fact, the ratio of narrow- to wide-lined polymer intensities (Figure 3) is consistent with the sharp line arising from loosely held, H-D-exchanged dimethylformamide. This view was further strengthened when a $(\mathrm{Phe})_n$ sample was equilibriated with an excess of dimethylformamide-d_7 for 24 hr, the solvent removed, and fresh dimethylformamide-d_7 added. The spectrum did not now show a sharp line superimposed on a much wider line (Figure 4). Further exchange studies suggest that the exchangeable protons do not constitute the entire NH pool, but only a static fraction of them. Thus it is presumably dimethylformamide molecules hydrogen bonded to end units or to other exposed NH units, such as a D-isomer placement or disordered ends, which have a broad spectrum. As a result of this exchange, the polymer second moments shown in Figure 2 are probably smaller than the true values, as any protonated dimethylformamide resulting from exchange will undoubtedly contribute negligibly to the line width.

The remaining polymers are all of high molecular weight and hence there is an insignificant contribution from potential bonding at the end of the helix. With $(\mathrm{CbzOrn})_n$ the dimethylformamide may tightly bond to the side-chain NH and account for the significant fraction of bound solvent molecules. The situation with $(\mathrm{BzlAsp})_n$ must, however, be different. The fact that the side chains are ordered in $(\mathrm{BzlAsp})_n$ while not in $(\mathrm{BzlGlu})_n$ may be a result of stereochemistry. The dimethylformamide may strongly interact with the polar ester groups in such a manner as to contribute to the side-chain ordering in $(\mathrm{BzlAsp})_n$. Their strong interaction results in a rather wide solvent line width and in an altered isotherm when compared with $(\mathrm{BzlGlu})_n$. This strong interaction is definitely not with NH groups as no isotopic exchange occurred with dimethylformamide-d_7 and $(\mathrm{BzlAsp})_n$ or $(\mathrm{MeGlu})_n$. Although both isotherms and polymer nmr line widths were qualitatively different between $(\mathrm{BzlGlu})_n$ and $(\mathrm{MeGlu})_n$, the solvent nmr line widths are nearly identical.

Summary. Using nmr, isotopic exchange and sorption isotherms[2] the following conclusions can be drawn concerning the dissolution of various helical polymers in dimethylformamide. (1) It is necessary that the helical polymer has a flexible side chain in order to be soluble, as has been suggested.[11] (2) The length of the side chain is not the determining factor as geometrical factors can introduce specific interactions which can dominate the entropy of solvent-side-chain mixing. (3) In the absence of flexible side chains the sorption isotherms are best described by the BET equation. (4) Polymer line-width measurements as a function of solvent composition can be a useful tool in helping to understand the sorption and solution properties of polymers.

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