# Effect of Side-Chain Substituents on the Thermodynamics and Dynamics of Helical Polypeptides. I. Vapor Sorption Studies

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ABSTRACT: Sorption of dimethylformamide onto poly(benzyl L-asparate) ((BzlAsp)$_n$), poly(methyl L-glutamate) ((MeGlu)$_n$), poly(carbobenzoxy-L-lysine) ((CbzLys)$_n$), poly(carbobenzoxy-L-ornithine) ((CbzOrn)$_n$), poly(L-phenylalanine ((Phe)$_n$), and poly(L-alanine) ((Ala)$_n$) has been studied. The data clearly showed strong binding sites exist in (Ala)$_n$, (Phe)$_n$, (MeGlu)$_n$, and (BzlAsp)$_n$. The data were analyzed according to two polymer–solvent mixing models, umpenetrable rod and Flory–Leonard side-chain mixing, to two solid–gas sorption models, Langmuir and finite layer BET, and to various combinations of these models. Three dimethylformamide soluble polymers, (CbzLys)$_n$, (CbzOrn)$_n$, and poly(benzyl L-glutamate), clearly show the sorption thermodynamics to be controlled by solvent–polymer side-chain mixing. The sorption thermodynamics of the non-dimethylformamide-soluble polymers (BzlAsp)$_n$, (MeGlu)$_n$, (Phe)$_n$, and (Ala)$_n$ is clearly different from the sorption thermodynamics of the soluble polymers. The isotherms suggest that (MeGlu)$_n$ and (BzlAsp)$_n$ have ordered side chains. The temperature dependence of the (MeGlu)$_n$ and (Phe)$_n$ isotherms yields estimates for the heat of condensation onto the strong binding sites of 3–4 kcal in excess of the heat of condensation of dimethylformamide. For the nonsoluble polymers the isotherms alone do not provide a unique choice among the sorption model considered.

Vapor sorption onto polypeptides of rodlike, $\alpha$-helical structure has been reported.[1,2] The studies indicate that the free energy associated with the initial dissolution of the macromolecules is primarily controlled by polymer side chain–solvent mixing. The nature and the size of the side chains appear to be the controlling factors which determine the solubility of the helical polypeptides. The present papers report work on the dissolution properties of a number of polypeptides, each with different side chains, in dimethylformamide. In this paper, equilibrium vapor pressures of dimethylformamide over polymer solutions measured in the composition range 60–100 vol % polymer by the vapor sorption method are reported. In the following paper, the results of proton magnetic resonance studies of the interaction between dimethylformamide and the polymers, the mobility of the dimethylformamide molecules adsorbed by the solid polymers, and the effect of the dimethylformamide molecules on the mobility of polymer side chains will be presented.

## Experimental Section

Poly($\epsilon$-carbobenzoxy-L-lysine) ((CbzLys)$_n$), poly($\delta$-carbobenzoxy-L-ornithine) ((CbzOrn)$_n$), poly($\gamma$-benzyl L-glutamate) ((BzlGlu)$_n$), poly($\gamma$-methyl L-glutamate) ((MeGlu)$_n$), poly($\beta$-benzyl L-aspartate) ((BzlAsp)$_n$), poly(L-phenylalanine) ((Phe)$_n$), and poly(L-alanine) ((Ala)$_n$) were obtained from New England Nuclear. Molecular weights of the polymers are 690,000, 460,000, 310,000, 275,000, and 180,000 for (CbzLys)$_n$, (CbzOrn)$_n$, (BzlGlu)$_n$, (MeGlu)$_n$, and (BzlAsp)$_n$, respectively. The molecular weights of (Phe)$_n$ and (Ala)$_n$ are not known. The polymers were vacuum dried for at least 24 hr at 60° before use. Dimethylformamide (Fisher Scientific Co., reagent grade) was vacuum distilled and dried before use.

The vapor sorption apparatus and the method of obtaining equilibrium solvent vapor pressures has been described previously.[2] To compute the volume concentration, the polymer and solvent specific volumes were assumed to be concentration independent. The specific volumes of (BzlGlu)$_n$, (MeGlu)$_n$, (BzlAsp)$_n$, (Phe)$_n$, and (Ala)$_n$ were taken[1,3-5] as 0.787, 0.76, 0.765, 0.805, and 0.83 cm$^3$ g$^{-1}$, respectively. The specific volume of (CbzOrn)$_n$ was assumed to be the same as (CbzLys)$_n$ and was taken[6] as 0.847 cm$^3$ g$^{-1}$. The specific volume of dimethylformamide at 25 and 15° was taken as 1.058 and 1.048 cm$^3$ g$^{-1}$, respectively.

## Results and Discussion

Vapor pressure ratios $P_1/P_1^0$ for systems (CbzLys)$_n$–dimethylformamide, (CbzOrn)$_n$–dimethylformamide, (MeGlu)$_n$–dimethylformamide, (BzlAsp)$_n$–dimethylformamide, (Phe)$_n$–dimethylformamide, and (Ala)$_n$–dimethylformamide are plotted as a function of solvent volume fraction in Figures 1–6. All samples were in powder form, except for (BzlAsp)$_n$ which was in fibrous form. For each system, two or three samples of different weights ranging from 50 to 100 mg were used. The data were taken randomly by increasing or decreasing solvent vapor pressures. For (CbzLys)$_n$–dimethylformamide, (BzlAsp)$_n$–dimethylformamide, and (Ala)$_n$–dimethylformamide mixtures, the data were taken at 25° only. For (MeGlu)$_n$–dimethylformamide mixtures, data were taken at 45, 25, and 15°. For both (CbzOrn)$_n$–dimethylformamide and (Phe)$_n$–dimethylformamide mixtures, data were taken at 25 and 15°. Solvent activities for (BzlGlu)$_n$–dimethylformamide solutions at 25, 20, and 10° in the concentration range 70–100 vol % polymer have been reported previously.[2]

(CbzLys)$_n$ is known to dissolve in dimethylformamide with the $\alpha$-helical conformation.[6,7] Both the (CbzLys)$_n$ and the (CbzOrn)$_n$ polymers used in the present work have been found soluble (>10 wt %) in dimethylformamide in the $\alpha$-helical conformation. (BzlAsp)$_n$ is reported to be insoluble in dimethylformamide.[8] (MeGlu)$_n$, (BzlAsp)$_n$, (Phe)$_n$, and (Ala)$_n$ have been found insoluble (<0.1 wt %) at room temperature. The difference in polymer solubility is clearly reflected in the $P_1/P_1^0$ *vs.* $v_1$ plots shown in Figures 1–6. While the concentration dependence of $P_1/P_1^0$ is virtually indistinguishable between the (CbzLys)$_n$–dimethylformamide and (CbzOrn)$_n$–dimethylformamide systems, and between the (MeGlu)$_n$–dimethylformamide and (BzlAsp)$_n$–dimethylformamide systems, the shape of the curves for soluble polymers and nonsoluble polymers is clearly different.

Theories of solutions of rodlike particles have been limited either to very dilute athermal solutions[9,10] or to the

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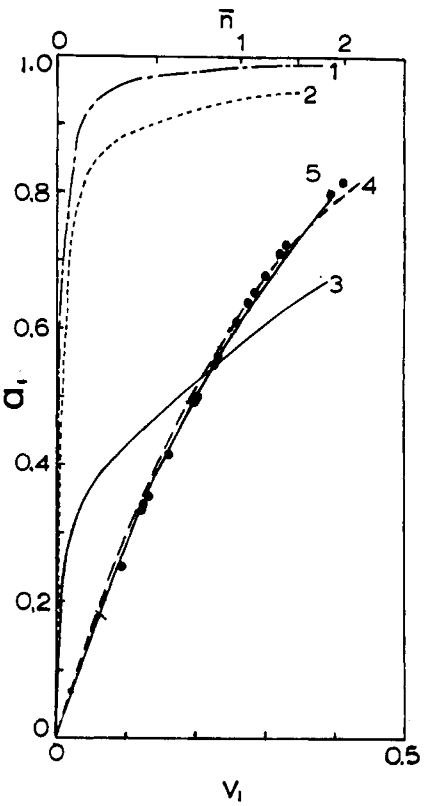

**Figure 1.** Vapor sorption of dimethylformamide on (CbzLys)$_n$ at 25°: (●) ascending, (x) descending; impenetrable rod (eq 1-3) isotherms with $\chi$ = 0(1), −0.1(2) or −1.0(3); Flory-Leonard side-chain mixing model (eq 4-5) with $\chi$ = 0(4); BET model (eq 8) with parameters given in Table I (5).

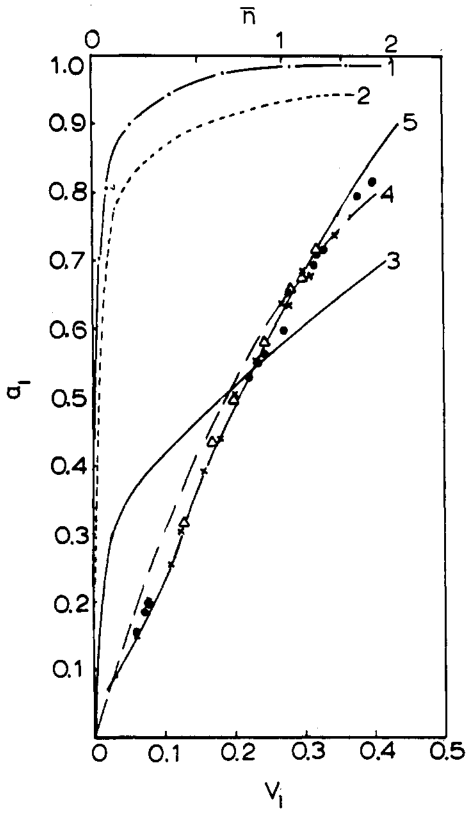

**Figure 2.** Vapor sorption of dimethylformamide on (CbzOrn)$_n$ at 25 and 15°: (△) sample 1, 25°; (●) sample 2, 25°; (x) sample 2, 15°; theoretical isotherms as in Figure 1.

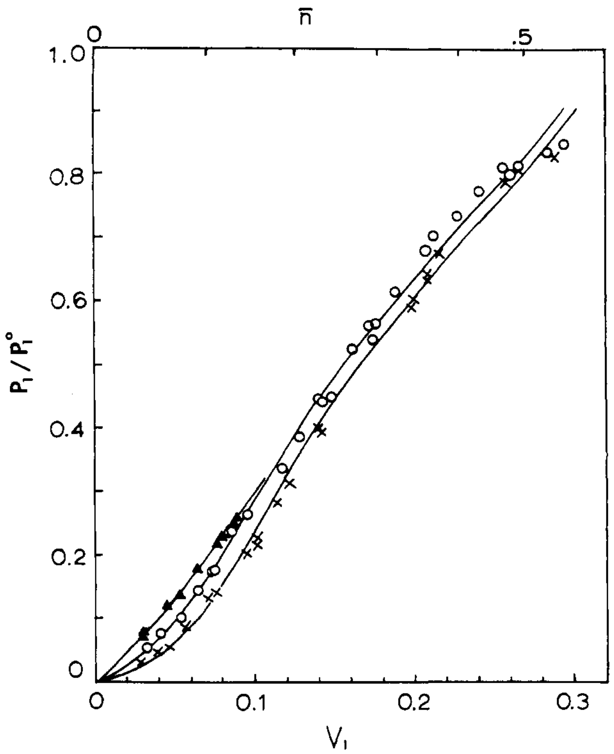

**Figure 3.** Vapor sorption of dimethylformamide on (MeGlu)$_n$ at 45, 25, and 15°: (▲) 45°; (●) 25°; (x) 15°; theoretical lines are BET isotherms with parameters given in Table I.

approximate approach of lattice theory.[11-13] In Flory's theory of solutions of impenetrable, rodlike particles, the activity $a_1$ of solvent in the anisotropic phase is given by

$$a_1 = v_1 \exp\{[(y - 1)/x]v_2 + \frac{2}{y} + \chi v_2^2\} \qquad (1)$$

where $v_1$ and $v_2$ are, respectively, the volume fractions of solvent and solute, $x$ is the polymer axial ratio, $\chi$ is the thermodynamic interaction parameter, and $y$ is a disorientation parameter. At high polymer concentrations, where the rods are perfectly ordered, $y$ is equal to unity. At lower concentrations, it is given by the solution of

$$v_2 = \left[\frac{\chi}{(x - y)}\right]\left[1 - \exp\left(-\frac{2}{y}\right)\right] \qquad (2)$$

At high concentrations, where $y = 1$ and perfect alignment of long rods prevails at equilibrium, $a_1$ is given by the expression for a solution with ideal entropy of mixing

$$a_1 = x_1 \exp(\chi v_2^2) \qquad (3)$$

where $x_1$ is the mole fraction of solvent. Flory's theory has been found in substantial agreement with experimental results in dilute solutions;[14] however, the theory was unable to explain the vapor sorption data obtained at high polymer concentrations.[1,2] To account for this discrepancy, Flory and Leonard[1] considered the mixing of solvent with flexible, disoriented polymer side chains. Assuming that mixing of solvent with randomly oriented side chains dominates the concentrated solution thermodynamic behavior, the following equation was derived to correlate their data

$$a_1 = v_1' \exp[v_2' + \chi(v_2')^2] \qquad (4)$$

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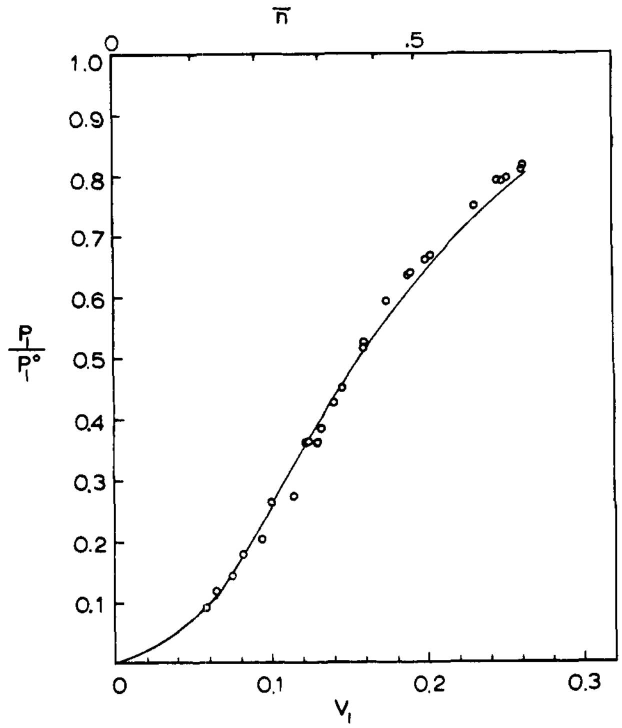

**Figure 4.** Vapor sorption and BET isotherms of dimethylformamide on $(\mathrm{BzlAsp})_n$ at 25°.

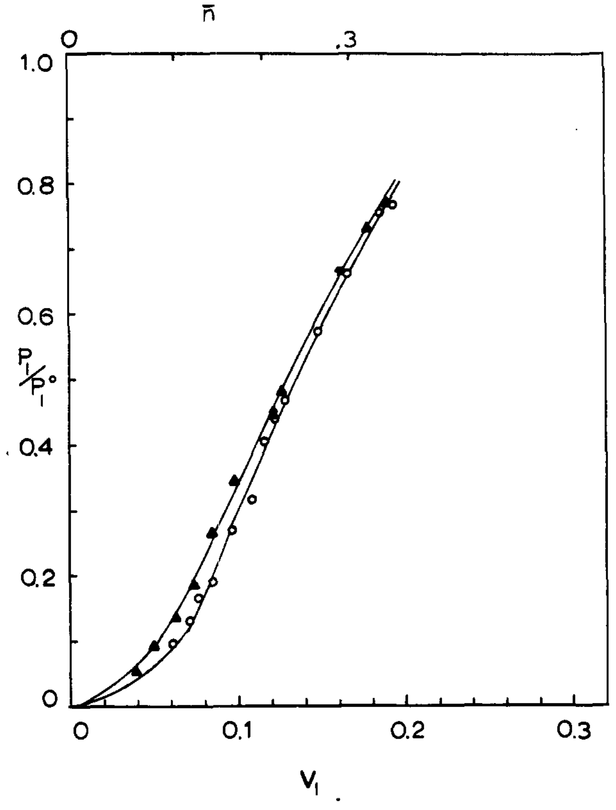

**Figure 5.** Vapor sorption and BET isotherms of dimethylformamide on $(\mathrm{Phe})_n$ at 25 and 15°: (▲) 25°; (●) 15°.

here $v_1'$ is the volume fraction of solvent calculated on the basis of mixing being confined to the side chains. The relationship of $v_1'$ and $v_2'$ to $v_1$ and $v_2$ is given by

$$\frac{v_2'}{v_1'} = \left(\frac{M_s}{M_u}\right)\left(\frac{v_2}{v_1}\right) \qquad (5)$$

where $M_s$ and $M_u$ are, respectively, the molecular weights of the side chain and of the entire peptide unit. If $\chi$ values of 0.35, 0.2, 0, and 0.6 were assumed for the systems $(\mathrm{BzlGlu})_n$-pyridine, $(\mathrm{BzlGlu})_n$-dichloroethane, $(\mathrm{BzlAsp})_n$-chloroform, and $(\mathrm{BzlGlu})_n$-dimethylformamide, respectively, the experimental data were found to be in good agreement with the results calculated from eq 4.[1,2]

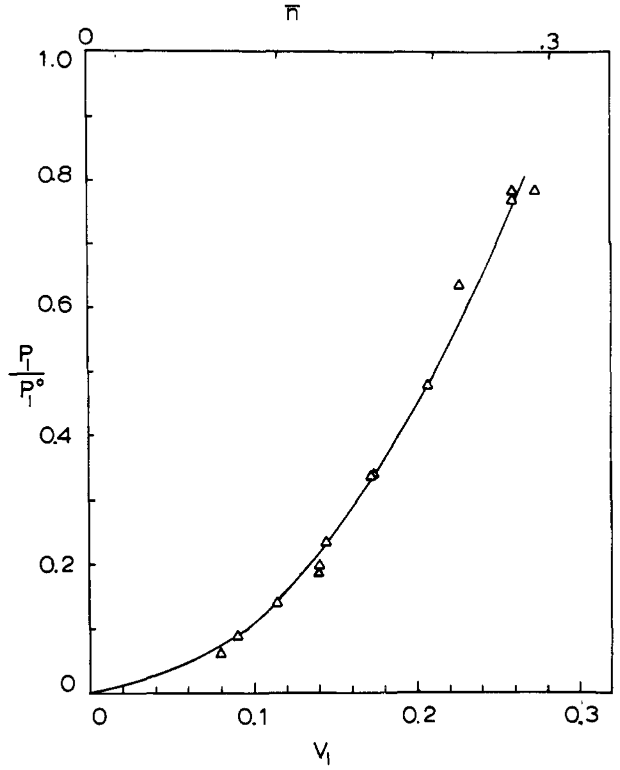

**Figure 6.** Vapor sorption and BET isotherm on dimethylformamide on $(\mathrm{Ala})_n$ at 25°.

In Figures 1 and 2, $a_1$ *vs.* $v_1$ calculated from eq 1-3 is plotted for $\chi$ values of 0, −0.1, and −1.0. Also shown in both figures is the $a_1$ *vs.* $v_1$ plot calculated from eq 4 for a $\chi$ value of 0. The results again indicate the inapplicability of the impenetrable rod model at high polymer concentrations and the explicability of the data in terms of the solvent-side-chain mixing model.

Perhaps it is not surprising that the concentration dependence of $a_1$ for the $(\mathrm{CbzLys})_n$-dimethylformamide system is indistinguishable from that for the $(\mathrm{CbzOrn})_n$-dimethylformamide system. Both $(\mathrm{CbzLys})_n$ and $(\mathrm{CbzOrn})_n$ dissolve in dimethylformamide as the $\alpha$-helical conformation, and a difference of one $CH_2$ group in the side chain is not expected to cause a significant difference in the entropy of mixing.

As shown in Figure 2, temperature variation of the solvent activity in $(\mathrm{CbzOrn})_n$-dimethylformamide solution at fixed solution composition was not detectable. Similar results were obtained for $(\mathrm{BzlGlu})_n$-dimethylformamide solutions.[2] Using calorimetric data[15] for $(\mathrm{BzlGlu})_n$-dimethylformamide system, the calculated heat of mixing is ~300 cal/mol, a value very close to the one calculated from a 4% difference in solvent activity (the estimated maximum temperature dependence[2]). At the same $v_1$, the solvent activity in the $(\mathrm{CbzLys})_n$-dimethylformamide system is observed to be lower than in the $(\mathrm{BzlGlu})_n$-dimethylformamide system.[2] The enthalpy of mixing for $(\mathrm{CbzLys})_n$-dimethylformamide and $(\mathrm{CbzOrn})_n$-dimethylformamide is, as shown above, very small. Since the side

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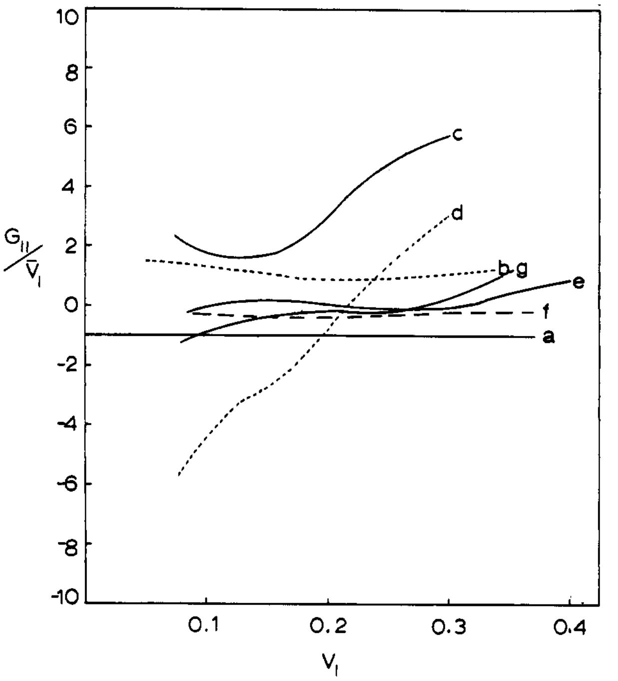

**Figure 7.** Cluster function ($G_{11}/\bar{v}_1$) as a function of $v_1$: (a) ideal solution; (b) benzene-rubber; (c) dimethylformamide-(BzlGlu)$_n$[2], 25°; (d) dimethylformamide-(BzlAsp)$_n$, 25°; (e) dimethylformamide-(CbzLys)$_n$, 25°; (f) dimethylformamide-(CbzOrn)$_n$, 25°; (g) dimethylformamide-(CbzOrn)$_n$, 15°.

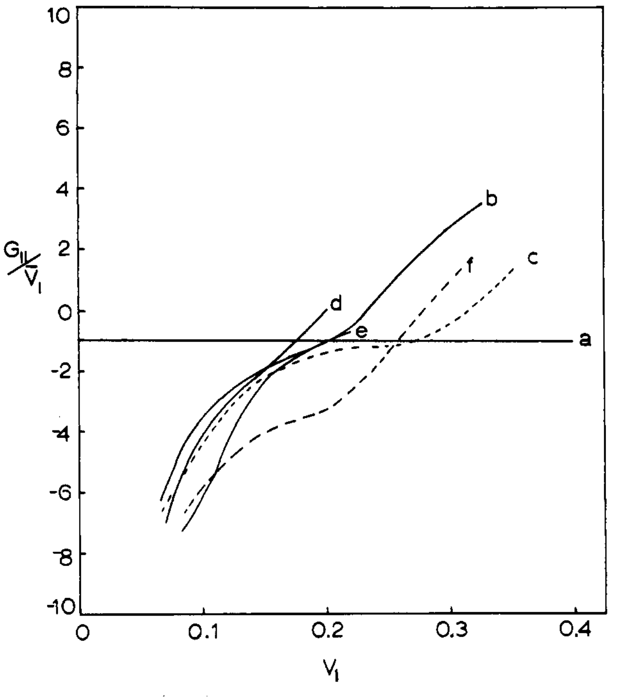

**Figure 8.** Cluster function as a function of $v_1$: (1) ideal solution; (b) dimethylformamide-(MeGlu)$_n$, 25°; (c) dimethylformamide-(MeGlu)$_n$, 15°; (d) dimethylformamide-(Phe)$_n$, 25°; (e) dimethylformamide-(Phe)$_n$, 15°; (f) dimethylformamide-(Ala)$_n$, 25°.

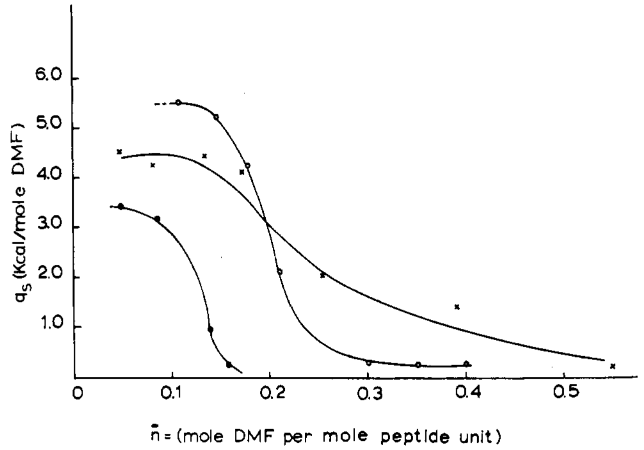

**Figure 9.** Isosteric heats of sorption as a function of $\bar{n}$ (moles of dimethylformamide sorbed per mole of monomer): (●) dimethylformamide-(MeGlu)$_n$, 25-45°; (x) dimethylformamide-(MeGlu)$_n$, 15-25°; (○) dimethylformamide-(Phe)$_n$, 15-25°.

chain of (CbzLys)$_n$($(CH_2)_4NHCOOCH_2C_6H_5$) is longer than the side chain of (BzlGlu)$_n$($(CH_2)_2COOCH_2C_6H_5$), the entropy of solvent-side-chain mixing is expected to be considerably larger in the (CbzLys)$_n$-dimethylformamide system than in the (BzlGlu)$_n$-dimethylformamide system. The increase in entropy of mixing, therefore, would reduce the solvent activities.

Inspection of Figures 1 and 2 shows that $a_1$ increases smoothly with $v_1$ and there is no region where solvent activity is constant. Also observed is the absence of sigmoidal shape in the $a_1$ *vs.* $v_1$ curve near $v_1 = 0$. Such behavior was observed also in the systems studied previously,[1,2] indicating the lack of biphasic regions in the concentration and temperature range studied, and the lack of any ordering in the side chains of solid (CbzLys)$_n$, (CbzOrn)$_n$, and (BzlGlu)$_n$.

In contrast to the similarity in sorption properties of (BzlGlu)$_n$, (CbzLys)$_n$, and (CbzOrn)$_n$, the four nonsoluble polymers, (Phe)$_n$, (Ala)$_n$, (BzlAsp)$_n$, and (MeGlu)$_n$, are strikingly different. While $a_1$ increases smoothly with $v_1$ in the (BzlGlu)$_n$-dimethylformamide system, a point of inflection is observed near $v_1 = 0.1$ in each of the four systems. At constant composition, $P_1/P_1^0$ also shows a temperature dependence in both the (MeGlu)$_n$-dimethylformamide and (Phe)$_n$-dimethylformamide systems, in contrast to the (BzlGlu)$_n$-dimethylformamide system.[2]

An alternate and perhaps a more informative way to see the difference in sorption properties between nonsoluble polymers ((Phe)$_n$, (Ala)$_n$, (BzlAsp)$_n$, and (MeGlu)$_n$) and soluble polymers ((BzlGlu)$_n$, (CbzLys)$_n$, and (CbzOrn)$_n$) is through the use of the Zimm-Lundberg clustering function $G_{11}/\bar{v}_1$,[16,17] which is

$$\frac{G_{11}}{\bar{v}_1} = -v_2[\partial(a_1/v_1)/\partial a_1]_{P,T} - 1 \qquad (6)$$

where $G_{11}$ is the cluster function and $\bar{v}_1$ is the partial molar volume of component 1. An additional term belonging to eq 6 involving the isothermal compressibility has been dropped, as it is small in condensed systems. The clustering function is defined such that $v_1(G_{11}/\bar{v}_1)$ is the mean number of type 1 molecules in the neighborhood of a given type 1 molecule. For an athermal binary solution of equal size molecules, the clustering function is independent of concentration and equal to −1.

The $a_1$ *vs.* $v_1$ values of the data presented here were fitted to a power series in activity by a least-squares fit. The clustering function was determined according to eq 6 and is shown in Figure 7 and Figure 8. Also shown in Figure 7 are (BzlGlu)$_n$-dimethylformamide values determined previously[2] and the values of nearly athermal solutions of a flexible polymer, *i.e.*, benzene-rubber solutions.[17] The clustering function for (MeGlu)$_n$-dimethylformamide at 45° is not shown as the data cover only a narrow concen-

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Table I
Sorption Models and Least-Squares Parameters

| Polymer | $(BzlGlu)_n$[a] | $(CbzLys)_n$ | $(CbzOrn)_n$ | $(MeGlu)_n$ | | | $(BzlAsp)_n$ | $(Phe)_n$ | | $(Ala)_n$ |
|---|---|---|---|---|---|---|---|---|---|---|
| $T$ (°C) | 10–25 | 25 | 15–25 | 15 | 25 | 45 | 25 | 15 | 25 | 25 |
| DP | 1415 | 2635 | 1850 | | 1925 | | 880 | | 130[b] | 60[c] |
| Impenetrable Rod (IR)[d] | | | | | | | | | | |
| | —[e] | — | — | — | — | — | — | — | — | — |
| Solvent-Side-Chain Mixing (FL)[f] | | | | | | | | | | |
| $\chi$ | 0.69 ± 0.001 | 0 ± 0.01 | 0 ± 0.01 | — | — | — | — | — | — | — |
| Langmuir (L)[g] | | | | | | | | | | |
| $n_s$ | — | — | — | — | — | 0.17 ± 0.01 | — | 0.8 ± 0.2 | 1.1 ± 0.3 | 0.46 ± 0.05 |
| $b$ | — | — | — | — | — | 7.0 ± 0.9 | — | 1.0 ± 0.3 | 0.6 ± 0.2 | 1.7 ± 0.3 |
| BET[h] | | | | | | | | | | |
| $n_s$ | 0.25 ± 0.01 | 0.54 ± 0.01 | 0.48 ± 0.01 | 0.15 ± 0.002 | 0.15 ± 0.002 | 0.17 ± 0.008 | 0.21 ± 0.002 | 0.13 ± 0.001 | 0.13 ± 0.001 | 0.13 ± 0.002 |
| $c$ | 0.64 ± 0.08 | 1.9 ± 0.2 | 2.6 ± 0.3 | 11.6 ± 1.2 | 7.0 ± 0.9 | 4.1 ± 0.4 | 12.3 ± 1.4 | 22.0 ± 2.7 | 13.1 ± 1.0 | 12.6 ± 1.6 |
| $m$ | 9 | 11 | 10 | 8 | 8 | 8 | 8 | 6 | 6 | 4 |
| Langmuir + Impenetrable Rod (L + IR) | | | | | | | | | | |
| $n_s$ | — | — | — | 0.48 ± 0.07 | 0.5 ± 0.1 | 0.38 ± 0.08 | 0.7 ± 0.1 | 0.30 ± 0.04 | 0.35 ± 0.06 | 0.26 ± 0.02 |
| $b$ | — | — | — | 3 ± 1 | 2.3 ± 0.8 | 1.9 ± 0.6 | 2.5 ± 0.7 | 5.5 ± 1.9 | 3.5 ± 1.4 | 5 ± 1 |
| $\chi$ | — | — | — | −0.3 ± 0.2 | −0.2 ± 0.2 | −1.3 ± 0.5 | −0.3 ± 0.2 | −0.3 ± 0.2 | −0.3 ± 0.4 | −0.3 ± 0.2 |
| L + FL | | | | | | | | | | |
| $n_s$ | — | — | — | 0.8 ± 0.3 | | | 0.8 ± 0.3 | | | 0.18 ± 0.01 |
| $b$ | — | — | — | 0.6 ± 0.3 | | | 0.7 ± 0.4 | | | 8 ± 2 |
| $\chi$ | — | — | — | 1.0 ± 0.00 | | | 1.0 ± 0.00 | | | 0 ± 0.00 |
| Model of best statistical fit | FL | FL | FL | BET | BET | BET | BET | BET | BET | BET |

[a] Data taken from ref 2. [b] Estimated from intrinsic viscosity in trifluoroacetic acid assuming $\langle r^2\rangle/nl^2 = 15$. [c] Estimated from intrinsic viscosity in dichloroacetic acid assuming $\langle r^2\rangle/nl^2 = 15$. [d] Calculated according to eq 1-3. [e] — indicates a poor fit. [f] Calculated according to eq 4-5. [g] Calculated according to eq 7. [h] Calculated according to eq 8.

tration range. Both the $(CbzLys)_n$-dimethylformamide values and $(CbzOrn)_n$-dimethylformamide values are sensibly independent of concentration, and their values lie between the value for an ideal solution of equal size molecules and the value for athermal, flexible chain polymers $[z/(z-2)]$, if $z$, the effective coordination number, is taken as 4. The $(BzlGlu)_n$-dimethylformamide curve is similar to those for $(CbzOrn)_n$-dimethylformamide and $(CbzLys)_n$-dimethylformamide but lies above them. At the lowest solvent activities there is considerable uncertainty in the $G_{11}/\bar{v}_1$ values determined from the experimental data. When $G_{11}/\bar{v}_1 < -1$ there is a tendency for like molecules to segregate from one another, either because of the difference in molecular volumes or because of the net attractive forces between unlike molecules.[17] The $(MeGlu)_n$-dimethylformamide and $(BzlAsp)_n$-dimethylformamide values increase rapidly with $v_1$ from a value of *ca.* −7 at $v_1 = 0.05$ to 4 at $v_1 = 0.35$. Similar behavior is also observed with $(Phe)_n$-dimethylformamide and $(Ala)_n$-dimethylformamide.

The shape of the adsorption isotherms as well as the numerical values of the cluster function suggest the existence of strong binding sites in $(BzlAsp)_n$, $(MeGlu)_n$, $(Ala)_n$, and $(Phe)_n$, and that the initial sorption process is not dominated by the entropy of polymer–solvent mixing as given by eq 1, 3, and 4. Looked upon as an adsorption one must postulate that in addition to strong binding sites "multilayer" adsorption exists in order to get the observed curvature at high solvent activities. These arguments are further strengthened when the isosteric heats of sorption ($q_s$) are determined as a function of composition, as shown in Figure 9. At low coverage the heats of adsorption amount to several kilocalories, but decrease rapidly as the amount bound increases, suggestive of BET type adsorption. This type of sorption seems particularly surprising for $(Phe)_n$ and $(Ala)_n$, where the impenetrable rod model as given by eq 3 was expected to be the model of choice. It is surprising also to find such a large difference between $(BzlGlu)_n$ and $(BzlAsp)_n$, in contrast to the small difference between $(CbzLys)_n$ and $(CbzOrn)_n$.

In discussing models for isotherms the solvent (sorbate) activity is plotted typically as a function of the amount absorbed (number of moles of sorbate bound per mole of monomer, $\bar{n}$). The top abscissa scale in Figures 1–6 is $\bar{n}$. When viewed as $\bar{n}$ *vs.* $P_1/P_1^0$ the sorption isotherms of the nonsoluble polymers look very much like BET isotherms. In order to get a better understanding of the meaning of the isotherms the experimental data for each polymer was fitted by a nonlinear least-squares procedure to six phenomenological isothermal equations: the impenetrable rod model, eq 1–3; the solvent-side-chain mixing model, eq 4–5; the Langmuir isotherm as given by

$$\bar{n} = n_s bx/(1 + bx) \tag{7}$$

where $n_s$ is the number of binding sites per monomer and $x$ is $P_1/P_1^0$; the BET isotherm as given by

$$\bar{n} = n_s cx[1 - (m + 1)x^m + mx^{m+1}]/\\(1 - x)(1 - x + cx - cx^{m+1}) \tag{8}$$

where $n_s$ is the number of strong binding sites per monomer and $m$ is the maximum "multilayer thickness;" a combination of Langmuir and impenetrable rod sorption (L + IR); a combination of Langmuir and solvent-side-chain mixing sorption (L + FL). The parameters so determined 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.

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# 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($\epsilon$-carbobenzoxy-L-lysine) ((CbzLys)$_n$), poly($\delta$-carbobenzoxy-L-ornithine) ((CbzOrn)$_n$), poly($\gamma$-benzyl L-glutamate) ((BzlGlu)$_n$), poly($\gamma$-methyl L-glutamate) ((MeGlu)$_n$), poly($\beta$-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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(1) J. H. Rai and W. G. Miller, *Macromolecules*, 5, 45 (1972).

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