Side chain motions and electrical polarizability of helical polypeptides

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In a study of the dielectric properties of helical polypeptides the contribution of side chain motions in the electric polarizability has been considered. A model of hindered rotating dipolar groups is presented, the results of which, compared with the experimental results, strongly suggest the co-operative character of the motions in poly(γ-benzyl-L-glutamate) and poly(γ-methyl-L-glutamate) molecules.

INTRODUCTION

Synthetic polypeptides of the formula $-(\text{CO–CHR–NH})_n-$ are known to form helical structures in many solvents[1]. The fairly rigid helical backbone is stabilized by almost unidirectional dipolar bonds CO · · · HN. As a result a huge molecular dipole moment comes into existence, which is proportional to the degree of polymerization[2]. Moreover, the side chain groups can be polar and their orientation and motion may influence the total molecular dipole moment of the components:

$$M_i = \mu_i + \alpha_{ij}E_j + \beta_{ijk}E_jE_k + \gamma_{ijkl}E_jE_kE_l + \dots \qquad (1)$$

where μ is the permanent moment and where the moment induced in an electric field, E, is determined by the electric polarizabilities $\alpha, \beta, \gamma, \dots$ of the first and the higher orders, respectively. Up to the present time only the permanent moment of polypeptides has been widely discussed from the conformational point of view[3–6]. This is surprising if the availability of the experimental data on the polarizability, α, is taken into account. Data for α are reported below and compared with those obtained from the simple model of side chain polarizability.

The polarizability of a solute, either α^0 in an optical field or α^e in an electric field, can be estimated from the increments $\Delta n = n_{12} - n_1$ or $\Delta\epsilon = \epsilon_{12} - \epsilon_1$ with subscripts 1, 2 and 12 denoting solvent, solute and solution, respectively. In order to obtain the data relating only to the induced moment, the electric polarizability should be determined under conditions preventing the orientation of whole helices in the measuring field; in this way the contribution:

$$\alpha_{0r} = \mu^2/3kT(1 + \omega^2\tau^2)$$

from the helix dipole orientation could be neglected. Therefore the dielectric increment $\Delta\epsilon$ should be measured at sufficiently high frequencies, ω, or at fixed frequency for solutes of sufficiently high relaxation times, τ. Under these conditions, and neglecting also the anisotropy of the internal field acting on molecules, F, we obtain the following results for dilute solutions with negligible density increments:

$$\Delta\epsilon = 4\pi N_2\left(\alpha_2^e\frac{F_2}{E} - \alpha_1^e\frac{F_1}{E}\frac{M_2}{M_1}\right) \qquad (2a)$$

$$\Delta n = \frac{2\pi}{n_1}N_2\left(\alpha_2^0\frac{F_2}{E} - \alpha_1^0\frac{F_1}{E}\frac{M_2}{M_1}\right) \qquad (2b)$$

where N_2 is the number of macromolecular entities of molecular weight M_2 in unit volume of solution. It must be noticed that the association processes occurring in polypeptide solutions[7–10] influence the concentration and the polarizability of macromolecules when estimated from equation (2) but it does not change the polarizability per side group. This latter feature is of further interest.

EXPERIMENTAL

Two polypeptides were examined to determine their polarizabilities: poly(γ-methyl-L-glutamate) (PMLG) of molecular weight 300 000 and poly(γ-benzyl-L-glutamate) (PBLG) of molecular weight 125 000, both the samples from Schwarz–Mann (Lots 7763 and Y3633). Additionally five other PBLG samples: 120 000, 100 000, 75 000, 60 000 and 46 000 were measured to discuss the helix dipole contribution to dielectric increment. The mean molecular weights were estimated by the manufacturer, Miles–Yeda Ltd, from the intrinsic viscosity in DMF. Solutes as well as solvent (1,4-dioxane (BDH) for spectroscopy) were dried and used without further purification.

The dielectric increment was measured at 25.0°C for solutions concentrated, whenever possible, in the range from 0.5×10^{-3} to 20×10^{-3} g/g. The DM01 WTW dipolmeter working at 2 MHz equipped with MLF 1D cell was applied. The optical data were calculated using $dn/dc = 0.124$ cm^3/g for PBLG[9] or assuming the additivity of the group refractions for PMLG.

RESULTS

Model of side chain polarizability

Consider the simplified geometry of a polypeptide mole-

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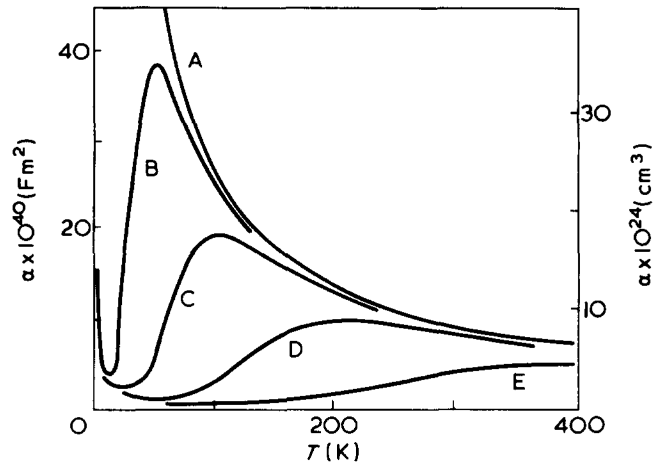

Figure 1 Mean polarizability α of side group vs. temperature, T. Calculations for $\mu_s = 1$ D (3.3×10^{-30} cm) and $\Delta\theta = \pi/16$. A, 0.0kJ/mol; B, 1.25 kJ/mol; C, 2.5 kJ/mol; D, 5.0 kJ/mol; E, 10.0 kJ/mol

cule with stiff side groups, where the dipole moment, μ_s, of a group rotates around an axis perpendicular to the axis of the helix[5]. The calculation of side chain polarizability can be outlined as follows.

The contribution of a dipolar group to the polarizability is:

$$\alpha_{ii} = \lim_{E_i \to 0} \partial\langle\overline{\vec{\mu}_s \cdot \vec{i}}\rangle/\partial E_i \tag{3}$$

The momentary orientation of a side group is characterized by the angle θ between the dipole and the molecular axis. The averaging $\langle \ldots \rangle$ is carried out through all the possible orientations of potential energy $V(\theta)$:

$$V(\theta) = U(\theta) - \vec{\mu}_s \cdot \vec{E} \tag{4}$$

where $U(\theta)$ is potential energy for dipole rotation, assumed to be independent of the orientation of neighbouring dipoles. The averaging $\overline{\ldots}$ is carried out through the orientation of axes of side group rotation, all of equal probabilities with respect to molecular symmetry. After averaging we obtain, for low fields:

$$\alpha_{11} = \frac{\mu_s^2}{kT}(\langle\cos^2\theta\rangle - \langle\cos\theta\rangle^2) \tag{5a}$$

$$\alpha_{22} = \alpha_{33} = \frac{\mu_s^2}{2kT}(\langle\sin^2\theta\rangle - \langle\sin\theta\rangle^2) \tag{5b}$$

which results in the mean polarizability, α, of a side group:

$$\alpha = \frac{\mu_s^2}{3kT}(1 - \langle\cos\theta\rangle^2 - \langle\sin\theta\rangle^2) \tag{5c}$$

where

$$\langle f(\theta)\rangle = \int_{-\pi}^{+\pi} f(\theta) e^{\beta U(\theta)} d\theta \Big/ \int_{-\pi}^{+\pi} e^{\beta U(\theta)} d\theta \tag{6}$$

and β has here its usual significance: $\beta = -1/(kT)$.

For the sake of simplicity in analytical calculations the square well potential for $U(\theta)$ was chosen, that is:

$$U(\theta) = 0 \quad \text{for } \theta_0 - \Delta\theta < \theta < \theta_0 + \Delta\theta$$

and

$$U(\theta) = \Delta U \text{ for } \theta < \theta_0 - \Delta\theta \text{ and } \theta_0 + \Delta\theta < \theta \tag{7}$$

where $\Delta\theta$ is the amplitude of angular motions of the side group dipole around its mean orientation θ_0. In this case the mean polarizability is given by:

$$\alpha = \frac{\mu_s^2}{3kT}\left\{1 - \left(\frac{\sin\Delta\theta}{\Delta\theta + \pi/A}\right)^2\right\} \tag{8}$$

where $A = \exp(\Delta U/kT) - 1$ may be considered as a measure of the rotational barrier height. A typical set of polarizability curves is shown in *Figure 1* for different barrier heights in the case of small librations. The curves are similar in shape to those obtained by White in his model of hindered rotating dipoles[11]. A substantial difference exists only in the lowest temperature region where, for the model discussed, the polarizability increases independently of the form of an assumed potential.

The effect of hindering the rotational motions on the induced dipole moment can be presented in a more clear form by introducing the correction factors R_α and R_γ for dipolar side group polarizabilities, defined as:

$$R_\alpha = \alpha/\alpha_s \qquad R_\gamma = \gamma/\gamma_s \tag{9}$$

where

$$\alpha_s = \mu_s^2/(3kT)$$

and

$$\gamma_s = -\mu_s^4/(45k^3T^3) \tag{10}$$

are referred to free rotating dipoles, μ_s. The factor R_β is not considered here because $\beta_s = 0$ for the dipole orientation. From *Figure 2* it is seen that the destruction of the side chain ordering revealed here by the R_α values, occurs in a rather broad region of temperature and practically no sharp transition is predicted for substantially hindered motions.

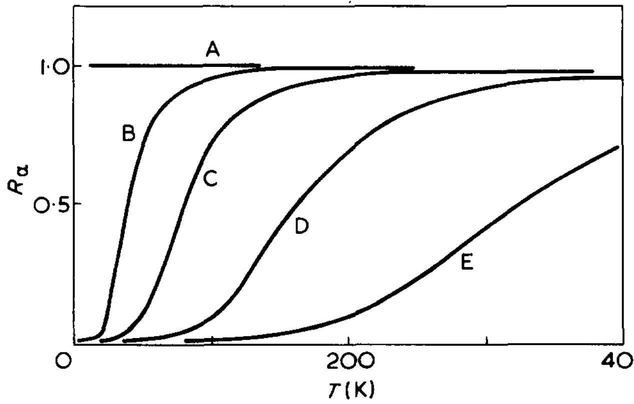

Figure 2 Correction factor R_α for polarizability vs. temperature, T. Calculations for the same case as in the *Figure 1*. A, 0.00 kJ/mol; B, 1.25 kJ/mol; C, 2.5 kJ/mol; D, 5.0 kJ/mol; E, 10.0 kJ/mol

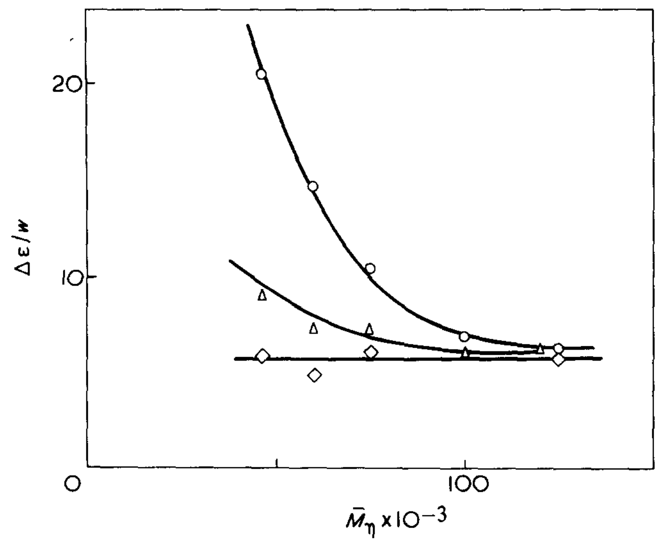

Figure 3 Specific dielectric increment $\Delta\varepsilon/w$ vs. mean molecular weight, $\bar{M}_\eta$, of PBLG samples. Dioxane solutions: ○, 0.5×10^{-3} g/g; △, 1.0×10^{-3} g/g; ◇, 1.5×10^{-3} g/g

Estimation of molecular polarizability

The computations presented above as well as the experimental estimation of side chain polarizability are both based on neglecting the contribution from helix dipole orientation. The validity of this assumption for the examined samples should first be discussed in detail.

The mean relaxation time for the polypeptides used is of the order of 10^{-4} sec for PBLG 125 000, when estimated from the Kerr constant dispersion at a concentration of 0.3×10^{-3} g/g[12], and of the order of 10^{-3} sec for PMLG 300 000 when calculated from molecular dimensions. These high values alone cannot be any argument for the assumption discussed because of a sample polydispersity. Fractions of low molecular weight, characterized by a much lower τ may well contribute to helix orientation at 2 MHz and then polydispersity may influence the dielectric increment measured. The theoretical analysis of this effect is not possible because of unknown molecular weight distributions, especially in the strongly associated dioxane solutions used. Thus the polydispersity effect should be excepted, based on experimental facts. It now appears that the measurement of the specific dielectric increment, $\Delta\varepsilon/w$, as a function of molecular weight for different PBLG concentrations was justified.

From the results presented in *Figure 3* one can see that for sufficiently high molecular weights, influenced by concentration but always below examined molecular weight 125 000, the increment does not depend on mean molecular weights, mean relaxation times or their distribution in the sample. Therefore, at a molecular weight of 125 000, the contribution from helix orientation must be excluded in solutions concentrated above 0.5×10^{-3} g/g, most likely as a result of strong association. The above is also in agreement with the frequency measurements performed precisely by Gupta *et al.*[13]. For non-fractionated PBLG of molecular weight 59 000 above 1 MHz there is practically no residual contribution from helix orientation as clearly seen in *Figures 1* and *6* of ref 13.

The molecular parameters (mean polarizabilities and correction factors of polypeptides under examination) are listed in *Table 1*. They were obtained from equation (2) and from equations (9) and (10), respectively. The polarizabilities of solvent molecules were obtained by applying Lorentz–Lorenz and Clausius–Mossotti relations. The difference between external and internal fields acting on macromolecules was neglected. To calculate the correction factors the dipole moments of ester side groups were assumed to be 1.8 debyes (6.0×10^{-30} cm). It was also assumed that the polarizability α, due to reorientation of side groups, is merely the difference between electric polarizability, α^{ε}, and optical polarizability, α^0, which amounts to including the classical atomic polarizability into α. However, the error introduced by this procedure is generally smaller than the optical polarizability, and data from *Table 1* show that it practically does not influence the values of correction factors.

DISCUSSION

It is of great interest that in all the cases the experimental R_α values clearly exceed unity. Such behaviour cannot be expected for any chosen form of the hindering potential (see *Figure 2*). To explain this fact we must assume that the side group movements are not independent of one another, but that they move cooperatively. In such a case a strong enhancement of polarizability occurs, e.g. when n dipoles move in phase the side chain polarizability as well as the correction factor increase n times as compared with the same dipoles rotating independently.

Cooperative processes usually lead to sharply marked temperature transitions, suggested also for the side chain order–disorder conformational melting[5]. This suggestion seems to be supported by changes in the dielectric permittivity observed in PBLG–PBDG film mixtures and is probably due to the melting of the side chain stacking ordering[14,15].

Another available correction factor R_γ for the third order polarizability also requires the assumption of cooperative motions. Recalculation of the recent data on PBLG hyperpolarizability[16] gives $\gamma = 0.6 \times 10^{-32}$ e.s.u. (7.4×10^{-58} SI units) per side group which corresponds to $R_\gamma = -1.8$ in contradiction to the positive value expected for independent dipoles. The positive hyperpolarizability, γ, accounting for the negative R_γ due to neighbouring dipole interactions and correlations within the molecule was also observed by means of the non-linear dielectric effect in systems composed of small molecules[17,18].

Assumption of the model of polarizability for systems with collectively moving dipoles is strongly required to draw any quantitative conclusions on the character of side group motion and orientation in helical polypeptides. For this reason we will not try to discuss the side chain conformation in detail on the basis of the dipole moments, permanent or induced, but here we only pick out the cooperative nature as the important, although commonly ignored, aspect.

Table 1 Mean polarizabilities and correction factors of helical polypeptides in 1,4-dioxane*

	Mean polarizability of side group		
Polypeptide	$\alpha^0 \times 10^{24}$ (cm^3) at 5.5×10^{14} Hz	$\alpha^{\varepsilon} \times 10^{24}$ (cm^3)† at 2.0×10^6 Hz	Correction factor R_α
PBLG	38.8	207 ± 10	6.3 ± 0.3
PMLG	13.1	From 194 ± 10	6.9 ± 0.3
		to 267 ± 20	9.7 ± 0.7
PMLG‡	13.1	129 ± 10	4.4 ± 0.4

* Solutions concentrated below 10^{-3} g/g; † in SI units: 1 cm^3 = 1.1126×10^{-16} Fm^2; ‡ gel phase

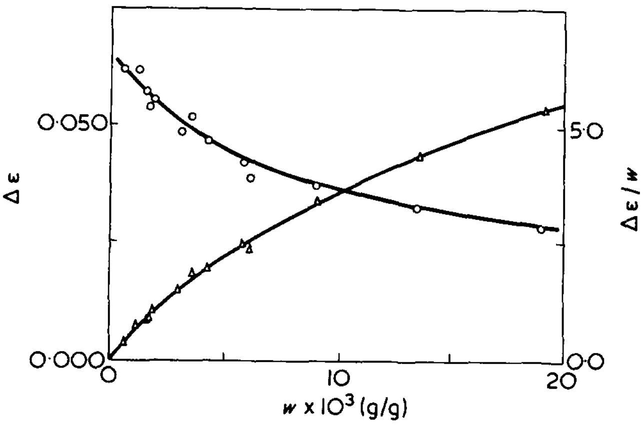

Figure 4 Dielectric increment Δε (△) and specific dielectric increment Δε/w vs. concentration (○) of PBLG 125000 in dioxane

Final remarks should be made on the influence of intermolecular interactions. In our experiment the polarizability of PMLG markedly decreases when the solutions, even when very dilute, transform to the gel phase (see *Table 1*). This phase also occurs in PBLG dissolved in benzene or toluene but not in dioxane[7]. In all these systems the gel phase can be induced by the action of high intensity electric field[12]; however, the electric properties of gel deposits, concentrated in this way, were not examined. We have also observed a significant decrease of about 60% in the specific increment related to polarizability, Δε/w, when PBLG was concentrated in dioxane from 0.5×10^{-3} to 20×10^{-3} g/g (*Figure 4*). Similarly Adachi *et al.*[19] observed that the high frequency dielectric permittivity of PBLG in 1,2-dichloromethane decreased with the concentration and was followed by the displacement of the melting range to higher temperatures, as seen in *Figure 3* of ref 19. These effects are due to side-by-side interactions of helical macromolecules prevailing in associated solutions[8,20,21], which effectively restrict the motion of side chain dipoles in the interface between neighbouring helices. In the terms of this work, it should be said that the entanglements and interactions of side chains in this region raise the energy barrier for collective motions and decrease the range of side chain orientation allowed and/or the degree of the cooperative effect. The first of the above conclusions has been experimentally proved by Adachi *et al.*[19].

Further investigations of the side chain structure of synthetic polypeptides are now in progress, including the study of the permanent dipole moment as well as the anisotropy of electric polarizability.

CONCLUSIONS

Measurements of the electric properties, in this case the mean electric polarizability, of helical polypeptides could reveal aspects of helical secondary structure. With the limitation to the assumed model of raising the permanent and induced dipole moments it seems to be possible to obtain information on the cooperative motion and orientation of polar groups relative to the macromolecular axis. This is a problem of great interest with regard to the study of the internal arrangement of polypeptides, subtle in its nature and easily influenced by temperature, solvent and association.

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