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Microwave Absorption in a Helical Polypeptide Molecule

By Mansel Davies, P. Maurel [*] and A. H. Price

Edward Davies Chemical Laboratories, University College of Wales, Aberystwyth

Microwave absorption measurements (3, 8.5, 36 and 72 GHz) are reported on three different molecular weight samples of poly(γ-benzyl-L-glutamate), PBLG, in two solvents. In each case a distinct absorption, often quite narrow (the Fuoss-Kirkwood parameter $\beta \simeq 1.0$), which is almost temperature independent is centred between 2 and 15 GHz. Its frequency, intensity and contour are not inconsistent with a helical-spring mode suggested to account for the abnormal increase in $(\alpha_{\parallel} - \alpha_{\perp})$ between the visible and 6 MHz. Other possible origins are considered.

In inert solvents such as dioxan and trans-1,2-dichloroethylene (t-dce) the typical synthetic polypeptide poly(γ-benzyl-L-glutamate) (PBLG) is well known to have a regular helical form of diameter *ca* 1.8 nm, a pitch of 0.54 nm, each turn of the helix incorporating 3.6 aminoacid residues.[1] Dielectric polarization and relaxation studies[2] confirm these details, giving an effective dipole moment and reorientational relaxation time (τ) (e.g., $\mu = 4100 \times 10^{-30}$ C m; $\tau = 32\,\mu s$ for $\overline{M}_w = 190\,000$ in dioxan) which are measures of the length of the helix. With electric fields *ca.* 50 kV cm^{-1}, it is possible to closely approach dielectric saturation in solution,[3] i.e. essentially complete alignment of the helices in the electric field; and from permittivity changes as a function of the applied field at a frequency (*ca.* 6 MHz) too high for the permanent dipoles to contribute greatly, the anisotropy of the polarizability of the helix ($\alpha_{\parallel} - \alpha_{\perp}$) at this frequency has been evaluated.[4] This Kerr constant determination shows the microwave molecular anisotropy to be at least five times that of the visible (n_D) value. This abnormally high ratio of the total distortion polarizability ($\alpha_{atomic} + \alpha_{electronic}$) to the electronic component ($\alpha_{electronic}$) which alone contributes in the visible region (n_D), is emphasized when one recalls that for most organic molecules α_{atomic} is of the order of ten per cent of $\alpha_{electronic}$. The abnormally high α_{atomic} for the helical PBLG has been ascribed[4] to the non-rigidity of the helix, the successive turns of which are held in place by hydrogen bonds and other van der Waals forces.

Irrespective of the foregoing evidence for pronounced flexibility of the helix along its cylindrical axis, the established structure and character of this conformation in the polypeptides necessarily require the presence of vibrational modes of the helix arising from quasi-harmonic displacements as of a spiral spring along its length.

This paper reports and discusses the presence of absorptions near 14 GHz in PBLG solutions.

EXPERIMENTAL

Dielectric loss (ε'') measurements on solutions of PBLG in dioxan and t-dce were made at 3, 8.5, 36 and 72 GHz. At 3 and 8.5 GHz the Dakin and Works[5] variation of the Roberts and von Hippel method was used and at 36 and 72 GHz a substitution method as described by Kramer.[6] The solute dielectric loss factors were analyzed using the Fuoss-Kirkwood equation

$$\cosh^{-1} \frac{\varepsilon''_m}{\varepsilon''} = \beta \ln \frac{f_m}{f} \tag{1}$$

[*] on leave from Laboratoire de Chimie Structurale, Faculté des Sciences, Montpellier, France.

where f is the frequency, the subscript m refers to the parameters at the absorption maximum and β is an empirical distribution parameter $(0<\beta\leqslant1 : \beta = 1$ corresponds to the Debye conditions).

PBLG samples were obtained from Miles-Yeda Ltd., Israel. Solvents were dried with molecular sieves and distilled before use. Solutions were allowed to stand for 2 to 3 days at about 320 K before measurement. A series of measurements at 36 GHz showed a solute dielectric loss proportional to the polypeptide concentration.
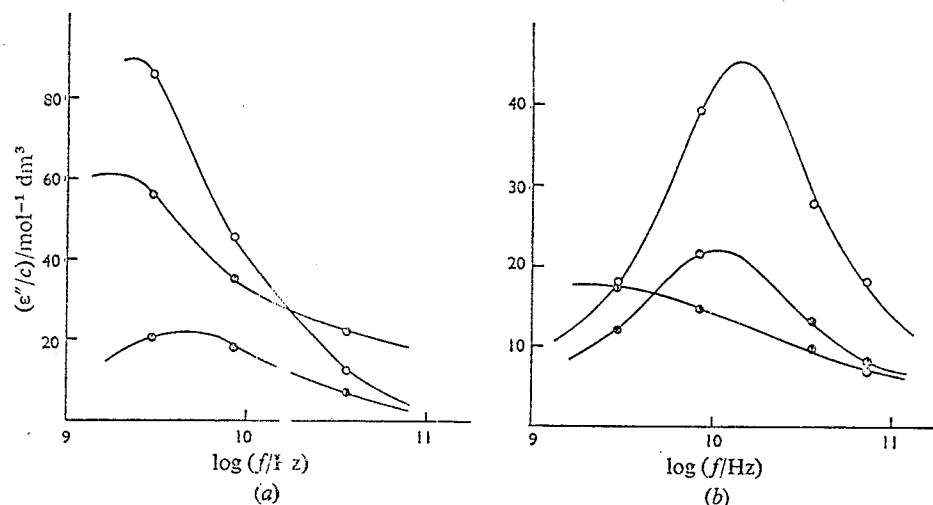

FIG. 1.—Dielectric absorption (ε''/c) in PBLG solutions as a function of frequency (a) trans-1,2-dichloroethylene solutions, (b) dioxan solutions : ⟨, $\bar{M}_w$ PBLG = 46 000 ; O, $\bar{M}_w$ PBLG = 76 000 ; ⊙, $\bar{M}_w$ PBLG = 110 000 (in dioxan multiply ordinate by 10).

Fig. 1 shows the dielectric absorption for the polypeptide solutions investigated. The derived dielectric parameters are listed in table 1. The effective dipole moments were calculated from ε''_m values using the Onsager equation (with $(\varepsilon_{12}-\varepsilon_0) = 2\,\varepsilon''_m/\beta$)

$$\mu^2 = \frac{9kT\varepsilon}{Nc}\frac{(2\varepsilon_{12}+n_2^2)^2(2\varepsilon''_m/\beta)}{\varepsilon_{12}(2\varepsilon_{12}+\varepsilon_1)(n_2^2+2)^2} \tag{2}$$

where ε is the permittivity of vacuum, n the refractive index, N Avogadro's constant, the subscripts 12, 1 and 2 refer to the solution, solvent and solute respectively, c is the polypeptide concentration : n_2 (= 1.71) was estimated from molar refraction data. The estimated uncertainty is ±10 % in the relaxation times and ±8 % in the dipole moments.

TABLE 1.—MICROWAVE DIELECTRIC PARAMETERS OF PBLG SOLUTIONS

solvent	$\bar{M}_w$	PBLG concentration $10^4\,c/$ mol dm^{-3}	$10^3\,\varepsilon''_m$	β	$\left(\frac{\varepsilon''_m}{c\beta}\right)/$ mol^{-1} dm^3	$f_m/$ GHz	$10^{30}\mu/$ C m	$\left(\frac{10^{30}\mu}{z^{\frac{1}{2}}}\right)*/$ C m
trans-1,2-	46 000	2.35	5.3	0.88	25.6	4.5	65	4.5
dichloroethylene	75 000	1.20	10.8	0.95	94.8	2.3	122	6.6
	110 000	0.68	4.0	0.70	85.1	1.6	100	4.5
dioxan	46 000	5.33	9.5	0.40	44.5	2.0	85	5.5
	75 000	1.73	7.9	1.0	45.7	14.5	87	4.7
	110 000	0.91	19	0.85	246	11.4	197	8.8

* Z is the degree of polymerization.

DISCUSSION

The interpretation of these results is complicated by association. Powers and Peticolas [7] suggest that PBLG molecules in solvents of low permittivity are associated in an antiparallel manner. Dielectric studies [2] confirm the reasonableness of this suggestion; the permittivity of PBLG solutions in dioxan tends to a constant value over the concentration range we have studied.

Despite this complication two molecular mechanisms deserve consideration. These are (a) the longitudinal vibration of the helix (c.f. a free spiral spring) and (b) the rotation of polar side groups in the helix which would be expected at a frequency between 1 MHz and 1 THz depending on the barrier restricting rotation. Both mechanisms would contribute to the polarization parallel to the helical axis.

The characteristics of the longitudinal vibration may be briefly summarized:

(i) Its frequency: the adjacent turns of the helix are principally held by the ($>$N—H---O) H-bonds of which there are 3.6 per turn. A recent summary of H-bond force-constants [8] shows that in somewhat similar structures, i.e., in the phenol-amine systems, this factor is near 8 N m^{-1}. On this basis the longitudinal modulus per turn of the PBLG helix is expected to be 29 N m^{-1}. Using this value and the expression for the principal vibrational frequency of a helical spring [9]

$$f = \frac{a}{2L}\left(\frac{k}{M}\right)^{\frac{1}{2}} \qquad (3)$$

where a is the pitch of the helix (0.54 nm), L the length of the helix, k the force constant per turn and M the mass per turn, a value of $f = 16.8$ GHz is estimated for the PBLG molecule ($\overline{M}_w = 110\,000$). The observed critical frequencies (table 1) for this polypeptide are in dioxan 11.4 GHz and in t-dce 1.7 GHz. The agreement is good in dioxan solution, but the results in t-dce imply a force constant of only 0.8 N m^{-1} per H-bond. It is, of course, only a first approximation to assume the bonding force is restricted to the H-bonds: other inter-chain interactions (attractive and repulsive) will contribute to the resultant force between the turns. Solvation or molecular association would presumably increase the effective mass of the vibrating unit and reduce the vibrational frequency, whilst kinks or other irregularities in the helix would produce other deviations. Unfortunately the microwave absorption is too small for adequate measurement at concentrations which eliminate association.

Reference to table 1 shows that the critical frequencies of the absorptions in t-dce and for the 75 000 and 110 000 PBLG in dioxan are inversely proportional to $\overline{M}_w$ as expected from eqn (3).

(ii) Its intensity: the molecular polarizability term $\alpha_{\parallel} - \alpha_{\perp}$ measured at 6 MHz can be converted into an equivalent dipole moment ($\alpha \equiv \mu^2/3kT$) and hence the intensity of the absorption estimated. For the ($\alpha_{\parallel} - \alpha_{\perp}$) value reported [4] for PBLG of $\overline{M}_w = 220\,000$ in dioxan, the length of the helix is about twenty times its diameter and it is an acceptable approximation to neglect $\alpha_{\perp}$ in comparison with $\alpha_{\parallel}$. The present observations do not relate to the same molecular weight but we assume $\alpha_{\parallel}$ is proportional to the length of the helix. On that basis the intensities ($\varepsilon''_m/\beta c$) calculated for the PBLG samples studied are $14 \text{ mol}^{-1} \text{ dm}^3$ for $\overline{M}_w = 46\,000$; $22.5 \text{ mol}^{-1} \text{ dm}^3$ for $\overline{M}_w = 75\,000$ and $33.2 \text{ mol}^{-1} \text{ dm}^3$ for $\overline{M}_w = 110\,000$. Comparison with the observed intensities ($\varepsilon''_m/\beta c$) listed in table 1 shows that there is no regular trend in these values which are two to three times larger than those anticipated from the one ($\alpha_{\parallel} - \alpha_{\perp}$) published value. The uncertainty in the evaluation [10] of ($\alpha_{\parallel} - \alpha_{\perp}$) at 6 MHz is sufficient to accommodate a variation of this magnitude.

(iii) The absorption contour: an intra-molecular mode, whether of helical-spring or side-group rotational origin, could approach a simple Debye-type contour ($\beta = 1$). The helix would *inter alia* show a distribution ($\beta < 1.0$) from the distribution of molecular weights but unless all polar side groups behaved similarly they would each have characteristic frequencies appearing as separate Debye-type absorptions. The occurrence of all the anticipated intensity in a single absorption which (table 1) often approaches $\beta = 1$, supports the suggestion of its helical-spring origin.

(iv) The temperature dependence: the "helical spring" vibrational mode would be a resonant absorption essentially independent of temperature. In solution the half-width of such a resonance-vibrational absorption would be increased to the limit of $\beta = 1$ (even for a single molecular species) by collisional and other molecular interactions. Significantly, measurements at 36 GHz show no variation in the dielectric loss factor over a temperature range of 40 K

The alternative origin i.e., side group rotation, should produce a critical frequency which is independent of $\overline{M}_w$, but solvent dependent if any solvation or association differs between dioxan and t-dce. The critical frequency in t-dce is much lower than expected for the free rotation of the side groups: c.f. f_m for benzyl acetate (a molecule of comparable volume to that of the side group) in benzene solution is 12 GHz. The intensity of the absorption (for free rotation of the side groups) should correspond to a dipole moment proportional to the square root of the degree of polymerization (Z in table 1). Despite their scatter, the last column entries in table 1 do not rule out this condition. The previously mentioned temperature studies show that the activation energy involved in the process is less than 4 kJ mol^{-1}. This would be an exceptionally low barrier for the side-group rotation. Judged by this criterion the absorption does not arise from side-group mobility but is more nearly of a quasi-resonant character.

Neither of the mechanisms considered is in complete agreement with the experimental findings. The limitations imposed by association and solubility restrict the prospects of measurements on a simpler solute state but it may prove possible to follow changes in the absorption with the transformation to the random coil configuration.

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