^{13}C and ^{1}H n.m.r. dynamic study of poly(γ-benzyl-L-glutamate) poly(β-benzyl-L-aspartate) and poly(L-alanine) in solution

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^{1}H T_1 relaxation times were measured in solutions of poly (γ-benzyl-L- glutamate), PBLG, and poly(β-benzyl-L-aspartate), PBLA, and ^{13}C T_1 relaxation times, *NOE* factors and line widths in solutions of the two polypeptides and of poly(L-alanine), PLA, over a broad range of solvent composition ($CDCl_3$–trifluoroacetic acid) including the helix–coil transition. PBLG was also studied through temperature-induced inverted helix–coil transition. Most relaxation parameters change smoothly over the studied range, and the observed changes correspond to increase of mobility with increasing content of trifluoroacetic acid and with increasing temperature except for PBLG which undergoes an inverted helix–coil transition with temperature. Analysis of experimental data by means of the isotropic model yielded, for the backbone carbons of all three polypeptides, $\tau_{eff} \sim 2 \times 10^{-8}$ sec in the helix and $\tau_{eff} \sim 1 \times 10^{-9}$ sec in the coil form. For the side-chain carbons, τ_{eff} decreases in the range 10^{-9}–10^{-10} sec with increasing distance from the backbone, with τ_{eff} values consistently lower in the coil form. Results of analysis by means of a model with two correlation times are discussed.

INTRODUCTION

The conformational transition of polypeptides in solution from the ordered helix to the disordered random coil state presents a complicated problem in studies of polypeptide chain dynamics[1–4]. The numerous nuclear magnetic resonance (n.m.r.) studies of the conformations of polypeptides are mostly based on measurements of chemical shifts, coupling constants or band shapes in n.m.r. spectra[1–3]. With the development of pulsed n.m.r. methods dynamic studies of polypeptides have become possible, based on the measurements of both relaxation times and *NOE* factors of individual nuclei in the backbone and in the side chains. So far this method has been applied to only a few polypeptides. Most detailed data are available for solutions of poly(γ-benzyl-L-glutamate) from ^{13}C[5,6] and ^{1}H[7–9] n.m.r. relaxation measurements. Based on ^{13}C T_1 relaxation times, line widths and *NOE* factors of poly(γ-benzyl-L-glutamate) in the helix and random coil forms, Allerhand *et al.*[6] have found that the extreme narrowing condition[10] applies for the effective correlation times τ_{eff} of all carbon nuclei in the random coil form, and for the aromatic carbons in the helix form. Due to rapid internal rotations, for the side chain carbons τ_{eff} gradually decreases with increasing distance from the backbone both in the helix and in the coil forms. Analysis of ^{13}C T_1 relaxation time measurements leads to the conclusion that τ_{eff} of the backbone carbons in the helix form corresponds to overall molecular reorientation, whereas in the random coil form, rapid segmental motions of the chains determine the relaxation process. The behaviour of the side chain in the range of the helix–coil transition has been reported in a number of papers[7–9,11,12] mainly by means of ^{1}H and ^{13}C chemical shifts. From these studies it follows that in the helix form of poly(γ-benzyl-L-glutamate) the side chain is extended into the solution, and stacking of the phenyl rings is indicated[11,12]. For poly(β-benzyl-L-aspartate), evidence of ring stacking has not been found[12,13]. As more abrupt changes of chemical shifts are observed at higher concentrations of trifluoroacetic acid (TFA) for side chain atoms than for the backbone atoms, it is assumed that the backbone helix formation is preceded by a conformational change of the side chain[14]. ^{1}H T_1 relaxation measurements of poly(γ-benzyl-L-glutamate) have been applied only in a study of the dynamics of aromatic protons in the range of the helix–coil transition[7–8]. This study showed that the ^{1}H relaxation process of aromatic protons is determined primarily by the side chain motions and overall molecular reorientation is of minor importance. In spite of this, the dependence of relaxation rate on TFA contents, exhibits a change of slope in the range of the helix–coil transition.

In this paper we have attempted to determine the relation between the dynamics of the backbone and of the side chain in solutions of three synthetic polypeptides, poly(γ-benzyl-L-glutamate), poly(β-benzyl-L-aspartate) and poly(L-alanine), by measuring the ^{1}H and ^{13}C T_1 relaxation times, ^{13}C line widths and *NOE* factors over a broad range of compositions

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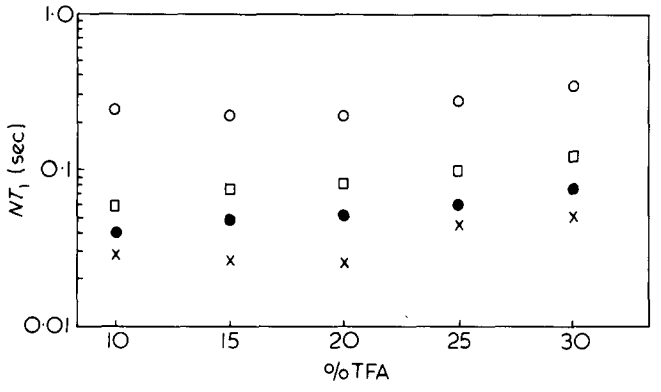

Figure 1 ^{13}C T_1 relaxation times of poly(γ-benzyl-L-glutamate) 15% (w/v) in $CDCl_3$–TFA solvent mixture; x, α-C; ●, β-C; □, γ-C; ○, benzyl C

of $CDCl_3$–TFA mixtures and of temperature which include the helix–coil transition.

EXPERIMENTAL

The samples of the polypeptides [poly(γ-benzyl-L-glutamate) S416 (PBLG), poly(β-benzyl-L-aspartate) 440B (PBLA) and poly(L-alanine) 371A (PLA)] were obtained from the Biophysics Laboratory, Portsmouth. The sample PBLG S416 has a relatively narrow molecular weight distribution with M_w = 24 000 and degree of polymerization (DP) 110; the sample PBLA 440B has a DP 315 and the sample PLA 371A has a DP 100. Additional characteristics of these samples are given by Bradbury and collaborators[15,16].

For the measurement of n.m.r. spectra, solutions of the polypeptides in mixtures of $CDCl_3$ and TFA were prepared at a concentration of 2% w/v for ^1H and 15% w/v (PBLG and PLA) or 17% w/v (PBLA) for ^{13}C measurements. The solutions were not degassed, as it is known[17] that in solutions of polypeptides the effect of oxygen can be neglected. The ^1H T_1 relaxation times were measured, at 270 MHz on the Brucker WH-270 spectrometer, by means of the inversion-recovery method with the pulse sequence 180°–T–90° with a 90° pulse length 14.8 μsec, repetition time 15 sec and 15 scans.

The ^{13}C T_1 relaxation times were measured at 15 MHz on the FX-60 Jeol spectrometer, by the same method, with proton noise decoupling, 90° pulse length 19 μsec, repetition time 5 sec and 1200 scans. T_1 values were calculated from peak heights, with an accuracy of ± 10%. The T_2 relaxation times were determined from measured line widths $\Delta\upsilon$ by means of the relation $T_2 = 1/\pi\Delta\upsilon$, with an accuracy of ±15%. *NOE* factors were measured by the gated decoupling technique with a pulse repetition time 10 sec, and evaluated from integrated line intensities. The accuracy of *NOE* factors is estimated to be ±10 to 20%.

RESULTS

Helix–coil transitions

From experimental and theoretical studies it is known[1–2,18] that the character of the helix–coil transition (its abrupt or gradual occurrence, ease of its induction) depends not only on the structure of the polypeptide, but also on its molecular weight and on the concentration of the sample. All these properties affect the external conditions (solvent composition, temperature) required to induce the transition. As our ^1H and ^{13}C spectra were measured with different polypeptide concentrations, the helix–coil transition requires different solvent compositions and temperature ranges. Therefore the conformational states of the polypeptide samples under investigation were identified by means of the known chemical shift and line width dependences in ^1H and ^{13}C n.m.r. spectra[11–16,19–20].

For PBLG, the helix–coil transition can be induced both by solvent composition, and by temperature. At room temperature, a 15% solution of PBLG in $CDCl_3$ with 10% TFA is found to be in the helical form, whereas in 30% TFA it is in the coil form. This random coil PBLG undergoes an inverted helix–coil transition with increase of temperature. The sample in 30% TFA can thus be reverted to the helical form by heating; at 55°C the helix predominates, but the transition is not quite complete. In 2% solutions PBLG is in the coil form by 7% TFA at room temperature; with 7% TFA however, the transition to the helix is complete at 60°C.

For 2% solutions of PBLA in $CDCl_3$–TFA mixtures at room temperature, the helix–coil transition takes place in the range 0.5–5% TFA. In 17% solutions of PBLA, 20% of TFA are needed for complete transition to the coil form; the limit for complete transition to the helical form is not easy to determine at this PBLA concentration, because at low TFA contents, a very large broadening of the PBLA lines is observed in the n.m.r. spectra, probably due to an aggregation of the polypeptide molecules. The lowest acid content used in our ^{13}C n.m.r. measurements of PBLA was 5% TFA. Some of the relaxation parameters described below indicate that in 17% solutions of PBLA in $CDCl_3$ with 5% TFA the transition to the helix is not complete.

For PLA at room temperature, the helix–coil transition extends over a broad range of solvent composition[19,20]. Our ^{13}C n.m.r. spectra were measured at two solvent compositions 35 and 75% TFA/$CDCl_3$ respectively. According to the cited references, the helix form should predominate at the former, and the coil form at the latter TFA/$CDCl_3$ composition.

Poly(γ-benzyl-L-glutamate). The values of ^{13}C T_1 relaxation times, *NOE* factors and line widths for PBLG in solvent compositions corresponding to the helix and coil forms both at room temperature and at an elevated temperature are summarized in *Table 1*. The overall dependence of ^{13}C T_1 for values for α-C and that for the non-aromatic carbons of the side chain on solvent composition, and the dependence for a solvent with 30% TFA on temperature, are shown in *Figures 1* and *2*, respectively. All these data show that

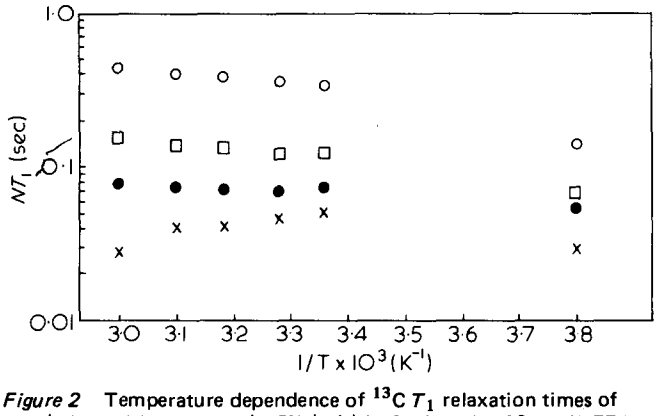

Figure 2 Temperature dependence of ^{13}C T_1 relaxation times of poly(γ-benzyl-L-glutamate) 15% (w/v) in $CDCl_3$ with 30 vol % TFA. x, α-C; ●, β-C; □, γ-C; ○, benzyl C

the changes of T_1 relaxation times through the helix–coil transition range are relatively small: the relaxation times of the helix (T_{1H}) and coil (T_{1C}) forms differ at most by a factor of 2. From *Figures 1* and *2* it is evident that the different carbon atoms have different behaviours. In the case of solvent-induced helix-coil transition, the T_1 values of α-C pass through a minimum, and also the behaviour of the T_1 values of the benzyl carbon is not linear. For all carbons, $T_{1H} < T_{1C}$. However, in the case of the temperature induced inverted helix–coil transition, this inequality holds only for α-C. The behaviour of T_1 values for this carbon in *Figure 2* may be considered analogous to the behaviour shown in *Figure 1*, with the minimum absent because, at the highest temperature measured (55°C), the transition to the helix state is not yet complete. For the β-C, ^{13}C T_1 values show little change with temperature; for the more distant side-chain carbons the T_1 values are clearly increasing with increasing temperature, so that for all these carbons, $T_{1H} > T_{1C}$. A similar trend is observed for ^1H T_1 values of all protons in the temperature-induced helix-coil transition (*Figure 3*), i.e. even the T_1 of the proton on α-C increases with increasing temperature.

The values of ^{13}C *NOE* factors are shown in *Table 1*; in no case do they reach the maximum value of 3 required for groups with isotropic motion under the extreme narrowing condition

$$4\pi^2(\upsilon_H + \upsilon_C)^2 \tau_{eff}^2 \ll 1$$

The dependence of *NOE* factors on solvent composition (*Figure 4*) is typical of anisotropic relaxation of the polymer chains[21]. The increase in mobility along the side chain is demonstrated by *NOE* factors increasing with increasing distance from the chain backbone. For α-C and β-C, the plot of *NOE* factors exhibits a break in the region of the helix–coil transition. With the exception of this region for these two carbons, and in the whole measured range for all other carbons, the *NOE* factors increase with increasing content of TFA.

Changes of ^{13}C line widths with solvent composition and temperature are summarized in the last columns of *Table 1*. It is seen that the line widths decrease with increasing distance from the backbone, and that the lines are generally broader in the helix than in the coil form; the difference is largest for the backbone carbons and smaller for the side chain carbons. This is true also of the helix form which has been induced by an increase of temperature.

Poly(β-benzyl-L-aspartate). The results of measurements of ^{13}C n.m.r. spectra of PBLA are summarized in *Table 2*. The ester and amide carbon data are not included because these two peaks are not resolved in the spectra. Although the measurements of PBLA were less detailed than of PBLG and the occurrence of the T_1 minimum for α-C was not verified, the general trend of the ^{13}C T_1 values for the solvent-induced helix–coil transition is similar to that for PBLG. The same is true for the *NOE* factors which increase with increasing concentration of TFA, and for a given concentration of TFA,

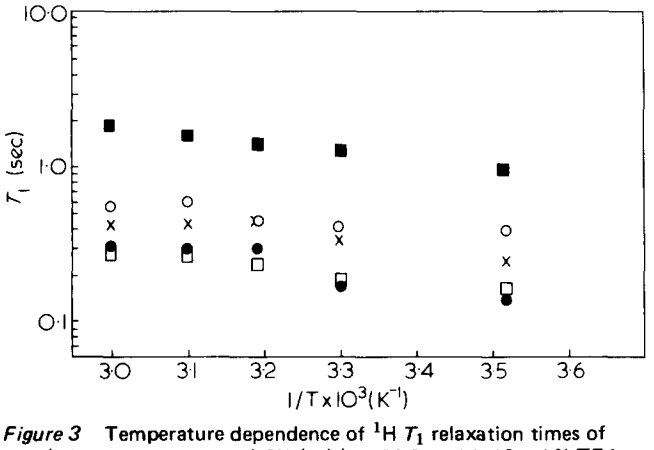

Figure 3 Temperature dependence of ^1H T_1 relaxation times of poly(γ-benzyl-L-glutamate) 2% (w/v) in $CDCl_3$ with 10 vol % TFA. x, α-CH; ●, β-CH_2; □, γ-CH_2; ○, benzyl CH_2; ■, phenyl proton

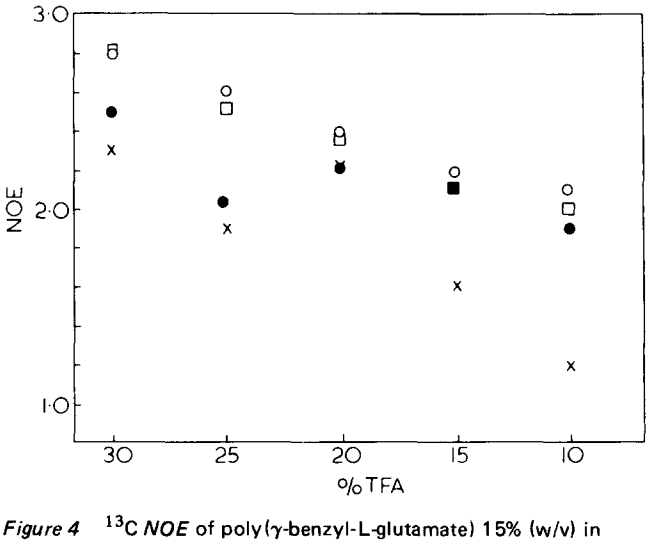

Figure 4 ^{13}C *NOE* of poly(γ-benzyl-L-glutamate) 15% (w/v) in $CDCl_3$ solvent mixture; x, α-C, ●, β-C, □, γ-C, ○, benzyl C

Table 1 ^{13}C n.m.r. parameters of PBLG

	T_1 (msec)				*NOE*			$(\pi\Delta\upsilon)^{-1}$ (msec)			
% TFA	10	10	30	30	10	30	30	10	10	30	30
Temperature (°C)	25	57	25	59	25	25	55	25	59	25	55
CO Amide	440	370	570	>1000	1.63	–	–	28	–	50	–
CO Ester	960	480	>1000		2.60	(2.9)	–	46	–	47	–
C_1 Aromatic	>1000	>1000	>1000	>1000	2.25	–	–	50	–	50	–
C_{2-6} Aromatic	580	1250	840	1070	2.35	(2.8)	–	44	–	50	–
C Benzyl	118	169	170	220	2.10	2.80	2.60	31	32	45	28
γ-C	30	69	60	78	2.00	2.80	2.60	19	25	27	17
β-C	19	30	38	39	1.90	2.50	2.40	12	13	19	13
α-C	30	20	50	28	1.20	2.30	1.40	10	11	19	6

Table 2 ^{13}C n.m.r. parameters of PBLA

	T_1 (msec)				*NOE*			$(\pi\Delta\upsilon)^{-1}$ (msec)			
% TFA	5	7	20	20	5	20	20	5	7	20	20
Temperature (°C)	25	25	25	57	25	25	58	25	25	25	55
C_1 Aromatic	>1000	>1000	>1000	>1000	1.25	1.75	2.00	27	51	53	61
C_{2-6} Aromatic	500	580	600	1080	2.30	2.35	2.90	43	44	50	51
C Benzyl	86	80	100	240	1.95	2.35	2.50	7	22	28	43
β-C	26	25	29	30	1.60	2.10	2.20	4	9	14	18
α-C	33	40	50	90	1.47	1.90	2.50	4	11	16	33

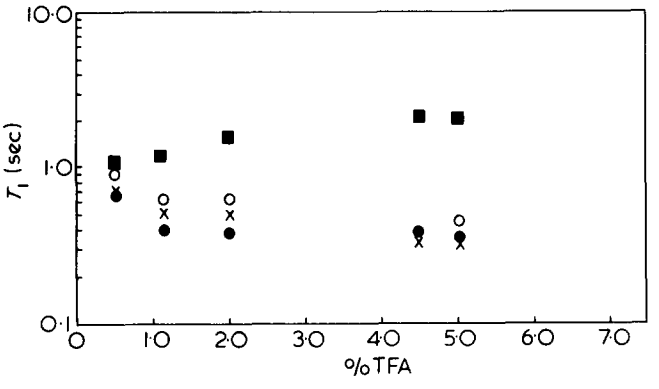

Figure 5 ^1H T_1 relaxation times of poly(β-benzyl-L-aspartate) 2% (w/v) in $CDCl_3$-TFA solvent mixture, x, α-CH, ●, β-CH_2, ○, benzyl CH_2, ■, phenyl proton

increase with the distance of the carbon atom from the backbone. Similarly as found for PBLG, they do not reach the value 3 for any carbon. Contrary to PBLG, the line widths of the side chain carbons increase upon transition into the helix state as do the line widths of the α-C. In this polypeptide the formation of the helix is not induced by an increase of temperature of the coil form in TFA/$CDCl_3$, i.e. there is no inverted helix–coil transition. Heating the sample leads to reduction of line width and to growth of the *NOE* factors and ^{13}C T_1 values of all carbon atoms measured. The plot of ^1H T_1 relaxation times vs. solvent composition is shown in *Figure 5*. Pronounced changes are observed only for aromatic protons ($T_{1H} < T_{1C}$) and α-CH groups ($T_{1H} > T_{1C}$), whereas for the other proton groups little change is observed. An increase of temperature leads to a small increase of ^1H T_1 relaxation times.

Poly(L-alanine). For PLA only ^{13}C n.m.r. spectra have been measured and these results are summarized in *Table 3*. The changes of T_1 values and *NOE* factors of α-C over the helix–coil transition range correspond to the changes observed for the two preceding polypeptides. Even for the methyl group, *NOE* factors do not reach the value 3, and they differ relatively little for the helix and coil forms. With increasing concentration of TFA, the widths of the α-C and CH_3 lines decrease; this decrease is more pronounced for the CH_3 band than for α-C. The width of the line of the amide carbon does not change.

DISCUSSION

It is well known that in the range of the helix–coil transition, experimental data can be affected by the properties of the sample such as its molecular weight and polydispersity[1,2,15,16,22]. Most affected are the widths of some lines, which exhibit a maximum value in the centre of the helix–coil transition; for this reason, in the region of the helix–coil transition, line widths cannot be used for characterization of internal motion. Also the values of the T_1 relaxation times and *NOE* factors can be affected by sample polydispersity in this region, and may differ according to the method of intensity measurement (peak or integrated line intensities). Therefore our experimental data were evaluated quantitatively only for conditions sufficiently removed from the centre of the transition.

Table 3 ^{13}C n.m.r. parameters of PLA (25°C)

	T_1 (msec)		*NOE*		$(\pi\Delta\upsilon)^{-1}$ (msec)	
% TFA	35	75	35	75	35	75
CO amide	530	790	1.60	1.90	37	36
CH_3	130	200	1.90	2.20	12	38
α-C	30	56	1.54	1.90	6	1

As is customary, our ^{13}C relaxation data were first analysed by means of the isotropic motional model with an effective correlation time τ_{eff}[10,23]. For this calculation it is of importance that by detailed measurement of the T_1 dependence on solvent composition for PBLG (*Figure 1*) it has been proved that the α-C correlation times calculated from T_1 for the helix and coil forms lie on different sides of a T_1 minimum. The experimental minimum T_1 value 0.025 sec in this case is near to the theoretical value 0.023 sec. The results of analysis by means of the isotropic approximation are summarized in *Table 4*. Satisfactory agreement of correlation times calculated from T_1 *NOE* and line widths is obtained for α-C in the helix form of PBLG, for which isotropic reorientation of the helix backbone may be characterized by a τ_{eff} 1.5×10^{-8} sec. In all other cases, τ_{eff} values calculated from the three relaxation parameters differ considerably, indicating the inadequacy of the isotropic approximation. Even so, some characteristics of the molecular dynamics may be obtained from this comparison.

τ_{eff} values calculated from T_1 for the α-C of the other two polypeptides are very close to the corresponding value for PBLG, those calculated from *NOE* are lower, those calculated from line widths are higher by about a factor of 2. This indicates anisotropy (or distribution of correlation times) of helix reorientation, with the more rapid motional modes approximating the isotropic reorientation of the PBLG helix. Slower motional modes are evident and very similar in PBLA and PLA. This anisotropy of helix reorientation in PBLA and PLA might be due to helix aggregation but the possibility that the high τ_{eff} values calculated from

Table 4 Effective correlation times

	τ_{eff} (nsec)[a]				
	α-C	β-C	γ-C	Benzyl C	Aromatic C_{2-6}
PBLG					
Helix	18.00	1.60	0.85	0.20	0.09
10% TFA	11.00	3.40	3.00	2.70	2.10
	17.00	3.20	1.55	0.80	0.53
Coil	1.10	0.65	0.39	0.14	0.06
30% TFA	2.20	1.75	1.00	1.00	1.00
	6.00	1.55	0.92	0.52	0.46
PBLA					
Predominantly	22.00	1.0		0.28	0.09
helix, 5% TFA	6.54	5.20		3.20	2.20
	50.00	23.00		11.00	0.56
Coil	1.10	0.90		0.23	0.08
20% TFA	1.70	2.70		2.10	2.10
	8.00	2.40		0.85	0.44
PLA					
Predominantly	18.00	0.11			
helix 35% TFA	5.88	3.50			
	40.00	0.49			
Predominantly	0.95	0.07			
coil 75% TFA	3.40	2.40			
	18.00	0.40			

[a] τ_{eff} determined from T_1 (first row), from *NOE* (second row), from $(\pi\Delta\nu)^{-1}$ (third row)

line widths are only apparent and due to incompleteness of transition to helix in the measured range cannot be excluded.

τ_{eff} values for α-C in the coil forms of the three studied polypeptides are lower by an order of magnitude than those for the helix. They are remarkably similar for the three substances and indicate a similar type of motional anisotropy (or distribution).

Some information on the dynamics of the side chain carbons may likewise be obtained from the τ_{eff} values in *Table 4*. Even here, τ_{eff} values calculated from T_1 are consistently lower than those calculated from line widths. This difference is particularly pronounced in the helix form of PBLA where aggregation evidently dominates the line width values. τ_{eff} values calculated from T_1 are in all cases slightly lower in the coil than in the helix, they decrease systematically with increasing distance from the backbone, and in all cases correlate well for PBLG and PBLA. From *Table 4* it is also evident that the τ_{eff} (T_1) values of α-C in the coil form are only slightly higher than for the corresponding τ_{eff} values of the side chain carbons with which they fall in line whereas in the helix forms, τ_{eff} values for α-C and β-C differ by an order of magnitude. From this it may be concluded that the T_1 relaxation process of backbone carbons in the coil form is dominated by segmental motions of a similar type to the segmental motions of the side chain, whereas in the helix it is dominated by over-all helix reorientation which is slower by at least an order of magnitude.

We have also attempted to correlate the analysis of side chain dynamics based on ^{13}C relaxation data with the results of proton T_1 measurements. For the relaxation time T_1 expressed as

$$T_1^{-1} = T_1^{-1}{}_{intra} + T_1^{-1}{}_{inter}$$

with the second term corresponding to all inter-group and inter-molecular interactions, in the extreme narrowing limit for the methylene protons[24] we obtain the relation

$$T_1^{-1}{}_{intra} = 3/2\frac{\gamma^4 h^2}{r^6} \cdot \tau_{eff}$$

By neglecting all but intra-group interactions, this relation may be used to calculate the τ_{eff} values for side chain methylene protons in the coil forms of the studied polypeptides from ^1H T_1 relaxation data (*Figures 3* and *5*). The calculated correlation times for the benzyl CH_2 group of PBLA (0.83×10^{-10} sec) and for the benzyl and γ-CH_2 groups of PBLG (1.00×10^{-10} sec, 2.34×10^{-10} sec) are in reasonable agreement with τ_{eff} values calculated for the respective carbons from ^{13}C relaxation data (*Table 4*).

Although some interesting information on the dynamics of the polypeptides could be extracted from ^{13}C relaxation data by means of the isotropic model, from the above analysis and discussion it is quite clear that the description of the dynamics of these systems by means of a single correlation time is only a very crude approximation. Therefore we have also tried to describe the dynamics of the side chain by means of a model considering the internal rotations of the side chain in addition to the reorientation of the backbone. We have used the relations for ^{13}C T_1, T_2 and *NOE*, derived by Doddrell *et al.*[25] for the simplest model of this type, considering an isotropic reorientation of the polymer backbone characterized by τ_R, and only one side chain rotation characterized by τ_G. Of the peptides under investigation, this model should be suitable for describing the dynamics of the β-C in the helix form of PBLG, and to a lesser extent for the analysis of relaxation data of the β-C in the coil form of PBLG and for both the helix and coil forms of the other two polypeptides. By means of the relations of Doddrell *et al.*[25], the values of T_1, T_2 and *NOE* were tabulated for a physically reasonable range of τ_R and τ_G values, and a match with experimental values of the relaxation parameters was sought. In some cases, a match for all three parameters could not be found. The results of this analysis are summarized in *Table 5*.

It is seen for the β-C in the helix form of PBLG that all three relaxation parameters could be matched to physically reasonable values of τ_R and τ_G. The τ_R thus obtained agrees well with the τ_{eff} value obtained by isotropic analysis of α-C relaxation data. This strongly supports the assumption about the isotropic reorientation of the PBLG helix. The correlation time for reorientation about the α-C–β-C bond appears to be shorter by almost two orders of magnitude than the helix reorientation. For the helix form of PBLA a physically reasonable match to τ_R and τ_G could be found only for T_1 and *NOE* data. In spite of that, the τ_R and τ_G

Table 5 Correlation times τ_R and τ_G (nsec) for β-C

Polypeptide	% TFA	τ_R	τ_G	Matching ^{13}C parameters
PBLG				
Helix	10	10.0	0.20	T_1, T_2, *NOE*
Coil	30	2.0	0.10	T_1, *NOE*
PBLA				
Helix	5	10.0	0.10	T_1, *NOE*
Coil	20	5.0	0.09	T_1, *NOE*
PLA				
Helix	35	60.0	0.04	T_1, T_2, *NOE*

values obtained for the β-C for the PBLA helix are very similar to the corresponding values for the PBLG helix. This indicates that the lack of agreement with T_2 values need not be caused by any inadequacy in the applied model, but possibly to specific line-width effects due, e.g. to aggregation.

Also for the β-C in the coil forms of PBLG and PBLA, agreement with physically reasonable τ_R and τ_G values could only be obtained for T_1 and *NOE* data. In this case the reason probably lies in the anisotropic character of the backbone chain reorientation. The τ_R values resulting from this analysis are slightly shorter than in the helix forms, while the τ_G values are almost equal to those found in the corresponding helices. Therefore it seems that the dynamics of the side chains, at least the more rapid motional modes characteristic of it, are little affected by the conformational state of the polypeptide backbone.

The β-C in the PLA helix is the only case where a good match was obtained for all three relaxation parameters in a case with clearly anisotropic reorientation of the backbone. The obtained value of $\tau_G = 4 \times 10^{-11}$ sec agrees well with methyl group reorientation times found in similar polymer systems[10,25]. The obtained τ_R shows a larger difference from the corresponding τ_{eff} value than was the case with the other two polypeptides. It is difficult to decide to what extent this result is real or fortuitous. For the β-C in the PLA coil, agreement between experimental relaxation parameters and physically reasonable τ_R and τ_G values could not be obtained.

It is evident that even the model with the two correlation times is not adequate for the full treatment of the studied systems.

In conclusion, the main results following from the measurement of the relaxation parameters of the three polypeptides examined in a broad range of composition of $CDCl_3$–TFA mixtures, and in a broad temperature range can be summarized as follows:

Backbone mobility in both the helix and coil forms of the polypeptides depends but little on the type of polypeptide, and must therefore be relatively independent of the structure and dynamics of the side chain. For all three polypeptides, relaxation of the backbone carbons in the helix form is dominated by helix reorientation with an effective correlation time of the order of 10^{-8} sec, while in the coil form the relaxation mechanism is dominated by segmental motions, with and effective correlation time of 10^{-9} sec shorter by an order of magnitude.

Even though the effective correlation times of backbone carbons in the helix and coil forms differ by an order of magnitude, the corresponding difference in T_1 values is extremely small. This is caused by the fortuitous fact that the T_1 values of the two states lie on different branches of the T_1 vs. τ_c curve.

Over the whole range studied, including the helix–coil transition, the change of T_1 relaxation times is continuous both for the backbone and for the side chain carbons. A sharp change is only observed in the behaviour of *NOE* factors of α- and β-C, and in the line-widths, where it has been interpreted theroretically already in a number of preceding papers. The change of relaxation parameters generally corresponds to growth of mobility with increasing content of the TFA and increasing temperature; the only exception to this rule is observed for α- and β-C in those cases where helix formation is induced by an increase of temperature. For the dynamics of the more distant side-chain carbons, solvent composition and temperature are more important determining factors than the conformation of the polypeptide chain.

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