

NMR Studies on Poly(γ -methyl-L-glutamate) Solutions

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ABSTRACT: Proton spin-lattice relaxation time, T_1 , and chemical shift of solvent protons (dichloroacetic acid—chloroform or dichloroethane) were measured on poly(γ -methyl-L-glutamate) (PMLG) solutions. It was found that T_1 and chemical shift were considerably affected by the conformational transition of PMLG, and peculiar behavior did exist. These were discussed in relation to the results on the solvent mixtures. The order of the magnitudes of the interactions between PMLG and the solvent molecules was found to be as follows: carboxyl proton of dichloroacetic acid (DAC) acting as a random-coil solvent \gg methine group of DCA $>$ helix-forming solvent.

It was proposed in connection with the results on amide solutions that the interaction between PMLG and DCA should be a weak hydrogen bonding rather than protonated form interactions. We also measured the dependence of T_1 on PMLG concentration, and discussed the results in terms of PMLG—solvents and PMLG—PMLG intermolecular interactions.

KEY WORDS Spin-Lattice Relaxation / Chemical Shift / Poly(γ -methyl-L-glutamate) / Conformational Transition / Solvent /

Conformational transitions in polypeptides have been extensively studied, and various qualitative and quantitative interpretations of their phenomena have been proposed based on experimental results, such as optical rotatory dispersion, circular dichroism, infrared spectra, nuclear magnetic resonance, and viscosity measurements.¹ However, in most cases, discussions did not, partly due to the lack of experimental data, take the effect of solvent—polypeptide interaction into consideration as the essential point. We consider that information on the solvent—polypeptide interaction should assist in understanding in detail the mechanism or molecular picture of conformational transitions of polypeptides. It is well recognized that nuclear magnetic relaxation studies provide valuable information on local molecular motions and intermolecular interactions in liquids, and that they are capable of distinguishing between different types of molecular motions or interactions.²

The possibility of studying the relaxation phenomena of chemically shifted nuclei within a complex molecular system and sensitivity of nuclear magnetic relaxation to intermolecular interactions or molecular associations whose life

time is very short (10^{-10} — 10^{-12} sec) may be advantages of this technique not found in other relaxation techniques.

Recently, Glasel³ studied deuteron magnetic spin-lattice relaxation experiments on D_2O solutions of various macromolecules, and discussed the interactions of water with various conformations of macromolecules. Liu and Lignowski⁴ reported on chemical shift studies of trifluoroacetic acid (TFA) in poly(γ -benzyl-L-glutamate) (PBLG) solutions, and suggested that the changes in chemical shift of the acid proton due to the presence of polypeptide would provide valuable information about helix-random coil transitions of polypeptides.

In this paper some experiments on proton spin-lattice relaxation time, T_1 , and chemical shift of solvent molecules (both of helix and random coil solvents) are carried out on poly(γ -methyl-L-glutamate) (PMLG) solutions. Based on the results obtained, we discuss the interaction between solvent and PMLG and compare it with the results of our previous report⁵ on T_1 and the chemical shift of *N*-methylacetamide (NMA) and *N,N*-dimethylacetamide (DMA). The peculiar behavior of T_1 and the chemical shift observed at the helix—random

coil transition region of PMLG are also reported.

For the helix—random coil solvent mixtures we chose chloroform—dichloroacetic acid(DCA) and 1,2-dichloroethane(DCE)—DCA systems, and experiments were carried out in the temperature and composition range which cover the helix—random coil transition of PMLG.

In those systems we have studied, the carboxyl proton should experience the proton-exchange reaction. Therefore, to have a fully quantitative discussion about the mechanisms of the behavior of the spin relaxation and chemical shift in those systems, it will be necessary to carry out measurements of spin-spin relaxation times and the line shapes of the spectrum. However we would like to discuss in this report the roles of solvent molecules through the difference of T_1 's and chemical shifts with and without PMLG in the solutions, though there may inevitably be some quantitative discussion about experimental results.

EXPERIMENTAL

Materials

The high-molecular weight PMLG sample with a viscosity average molecular weight of about 7.8×10^4 was supplied from Kyowa Hakko Co. Ltd. DCA, DCE, and chloroform were reagent grade purchased from Tokyo Kasei Co. Ltd. Chloroform was purified by the usual procedure immediately before use. DCA and DCE were used without further purification because impurities were not observed in the NMR spectrum. After the oxygen dissolved in solutions was removed by cyclic repetition of freeze-pump-thaw degassing in the NMR sample tube, the sample tube was sealed off under vacuum (10^{-5} — 10^{-6} mmHg) and subjected to NMR experiments immediately.

NMR Measurements

The NMR spectrometer used was the JNM-C-60H model operated at 60MHz and equipped with a temperature variable apparatus by the Japan Electron Optics Laboratory. Measurements of T_1 were made by the adiabatic rapid passage and saturation recovery methods. Experimental errors were smaller than $\pm 3\%$ in the case of T_1 values longer than about 1 sec,

while in the case of T_1 shorter than 1 sec, errors approached $\pm 5\%$.

The chemical shifts were measured by the usual side-band-technique using tetramethylsilane(TMS) as the external reference. Bulk susceptibility corrections were not carried out.

RESULTS AND DISCUSSION

PMLG—DCA—Chloroform solution

In this solvent mixture, it has been well established by ORD and viscosity measurements⁶ that PMLG undergoes conformational transition from random coil (in the low temperature region) to helix (in the high temperature region) at the temperature 30—40°C in the volume fraction of DCA about 65%. The temperature dependence of T_1 of solvent protons in the solvent mixture and in PMLG solution ($c=4.6$ g/100-ml solvent) are shown in Figures 1 and 2, respectively. In the solvent mixture, the linear dependence of $\ln T_1$ and $1/T(^{\circ}\text{K}^{-1})$ is observed for all protons over the experimental temperature range. This means that in the solvent mixture the microscopic molecular motions can be expressed well by the Arrhenius-type equation, $T_1 = T_{10} \exp(-E_a/RT)$, and may obey the thermally activated process characterized by the single activation energy, E_a . However, in PMLG solution the linear dependence does not hold

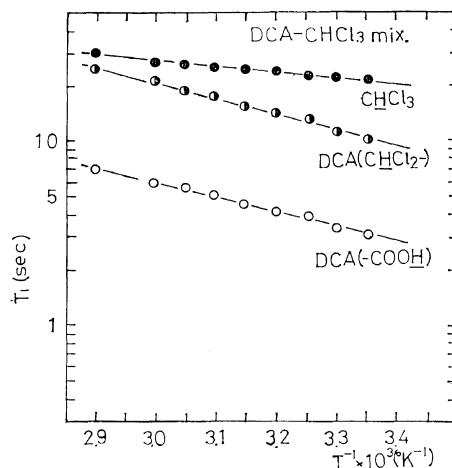


Figure 1. Temperature dependence of T_1 of solvent protons in DCA(65 vol %)—chloroform(35 vol %) mixture.

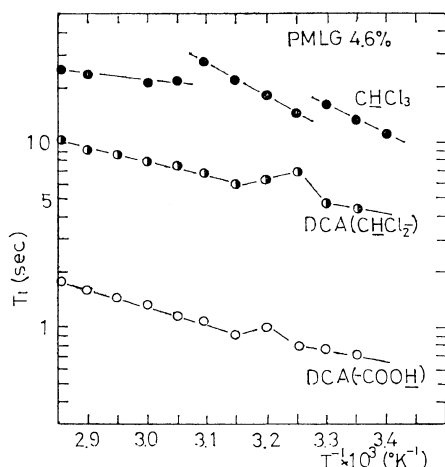


Figure 2. Temperature dependence of T_1 of solvent protons in PMLG solution: solvent, DCA(65 vol %) + chloroform(35 vol %); PMLG concentration, 4.6 g/100-ml solvent.

over the whole experimental temperature range, and at about 30–40°C abnormal behavior is observed in all protons. This temperature range corresponds to the random coil–helix transition temperature observed by ORD and viscosity measurements. Hence we consider that this peculiar temperature dependence of the solvent protons may reflect the random coil–helix transition of PMLG, and this conformational transition of PMLG does influence the molecular motions of solvent molecules in so far as the spin-lattice relaxation rates are changed. Similar characteristics of T_1 of solvent D_2O at the conformational transition region of macromolecules were reported by Glasel.³ At the temperature ranges either higher, where the helical conformation of PMLG is assumed dominant, or lower, where the random–coil conformation is preferred, than this transition temperature, the linear dependence of $\ln T_1$ is again observed. In particular, at the higher region the slopes of $\ln T_1$ against $1/T$ are almost equal to those observed in the solvent mixture. This means that solvent molecules in PMLG solution recover their characteristics inherent in the solvent mixture, since the helical conformation of PMLG becomes stable and interactions between solvents and PMLG may be so weakened as to not influence the molecular activated process. This

is not observed in the case of the lower temperature region, where in PMLG the random–coil conformation assumed to interact with solvents in a stronger way than the helical conformation. It should be noted that chloroform acting as a helix-forming solvent exhibits the same peculiar dependence to that of DCA acting as a random–coil solvent. It is found quantitatively by means of spin-lattice relaxation measurements that chloroform scarcely interacts with PMLG even at the random-coil conformation region.

Hence, it is assumed that these peculiar dependences of T_1 may not be caused by the change in the direct interaction of solvent molecules with PMLG, such as the chemical bonding or molecular association. We suggest that this behavior may be due to the rapid fluctuation of random coil \rightleftharpoons helix interconversion, and through this rapid fluctuation the temperature of the lattice may increase sufficiently to reduce the spin-lattice relaxation rates, $1/T_1$, of solvent protons. However, there is a lack of reliable evidence of such a rise in lattice temperature, and further studies will be necessary to examine the mechanism in detail. In plots of $\ln T_1$ vs. $1/T$ for PMLG solutions at different PMLG concentrations ($c=2.4$ and 8.0 g/100-ml solvent), the same peculiar temperature dependence of T_1 can also be observed. The dependence of T_1 of the solvent protons on

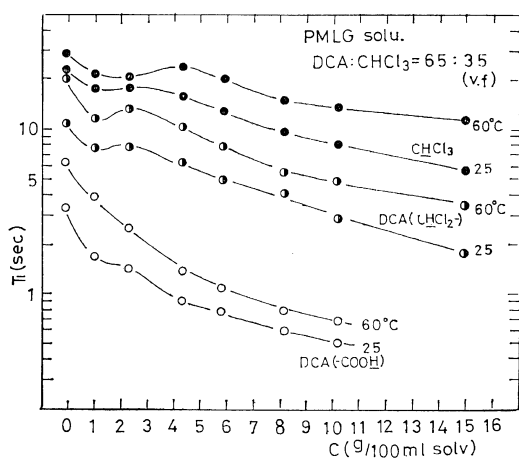


Figure 3. Concentration dependence of T_1 of solvent protons in PMLG solutions; solvent, DCA(65 vol %) + chloroform(35 vol %).

PMLG concentration is shown in Figure 3 at 60°C (helical conformation) and 25°C (random-coil conformation) in PMLG—DCA(65 vol %)—chloroform (35 vol %) solutions. The concentration dependence is similar between 60 and 25°C, and T_1 's of chloroform and methine(DCA) protons exhibit the maximum at a certain concentration region. We consider this concentration dependence may reflect the effect of PMLG—PMLG and PMLG—solvent intermolecular interactions on solvent molecules. Although the details of the mechanism are not clear, it may be speculated that at the low concentration region PMLG—solvent interaction predominates over PMLG—PMLG interaction, but at the moderate concentration region the PMLG—PMLG interaction becomes appreciable, and solvent molecules are released from PMLG—solvent interaction to some extent. This mechanism seems to explain why the relaxation rates, $1/T_1$, of the solvent protons, except for the carboxyl proton of DCA, decrease at the moderate concentration region. The carboxyl group of DCA does interact most strongly with PMLG and is not loosened from PMLG even in moderate concentration regions. It is interesting that concentrations dependences are almost identical for both the case of the helical conformation and the random-coil conformation of PMLG.

In Table I, relaxation rates of solvent protons in the solvent mixture and in PMLG solution ($c=4.6$ g/100-m³ solvent) denoted by $1/T_{1s}$ and $1/T_{1p}$, respectively, are shown. We estimate, as shown in the last column of the table, the difference in the relaxation rates between the solution and the solvent mixture, $(1/T_{1p}-1/T_{1s})$, which is considered to reflect the magnitudes of PMLG—solvent interactions. From Table I,

it is found that these values are larger in the case of the random-coil conformation of PMLG (25°C) than in the case of the helix conformation(60°C). This may be attributed to the fact that the random-coil conformation interacts with solvents in a stronger way than the helical conformation does.

The effect of PMLG—solvent interaction on spin-lattice relaxation rates is most pronounced for the carboxyl group of DCA. At both temperatures(25 and 60°C), the order of these magnitudes is as follows: DCA(-COOH) > DCA(CHCl₂-) > CHCl₃. It is recognized clearly that chloroform hardly interacts with PMLG. The order of the magnitudes of interactions estimated on PMLG solution corresponds closely to the result on NMA and DMA solutions obtained previously.⁵ We summarize several conclusive remarks as follows

- (1) There exists a peculiar temperature dependence of T_1 of solvent protons in the temperature range where PMLG should undergo conformational transition from random coil to helix. It is considered this may be due to the rapid fluctuation of the interconversion between random coil \rightleftharpoons helix conformation of PMLG.
- (2) In the region where the helical conformation of PMLG is considered to be stable, solvent molecules recover to some extent their characteristics in their solvent mixture.
- (3) The carboxyl group of DCA interacts most strongly with PMLG, but the interaction between chloroform and PMLG is scarcely appreciable.
- (4) There exists a maximum in the dependence of T_1 on PMLG concentration except for the carboxyl group of DCA.
- (5) Chloroform scarcely interacts with PMLG. However, in the dependence of T_1 on temper-

Table I. Spin-lattice relaxation rates of solvent protons in DCA—chloroform solvent mixture ($1/T_{1s}$) and in PMLG solution ($1/T_{1p}$)^a

Temp, °C	DCA(-COOH), sec ⁻¹			DCA(CHCl ₂ -), sec ⁻¹			CHCl ₃ , sec ⁻¹		
	T_{1s}^{-1}	T_{1p}^{-1}	$T_{1p}^{-1}-T_{1s}^{-1}$	T_{1s}^{-1}	T_{1p}^{-1}	$T_{1p}^{-1}-T_{1s}^{-1}$	T_{1s}^{-1}	T_{1p}^{-1}	$T_{1p}^{-1}-T_{1s}^{-1}$
25	0.30	1.42	1.12	0.10	0.23	0.13	0.04	0.08	0.04
60	0.21	0.81	0.60	0.05	0.17	0.12	0.03	0.05	0.02

^a $(1/T_{1p}-1/T_{1s})$ means the difference in relaxation rates. PMLG concentration, 4.6 g/100-m³ solvent.

ature and PMLG concentration, peculiar behavior could be found. These facts suggest

that chloroform may play some role in the conformational transition of PMLG.

PMLG—DCA—DCE Solution

From ORD measurements,⁷ PMLG is known to undergo helix—random-coil transition in this solvent mixture in which the volume fraction of DCA varies from 70–80% at 25°C. That is, at a smaller fraction of DCA (<70%) the helical conformation is stable and at a larger fraction (>80%) the random-coil conformation becomes stable. In Figures 4 and 5, respectively, the dependence of T_1 of solvent protons on volume fraction of DCA in the solvent mixture and PMLG solution ($c=4.1$ g/100-ml solvent) are shown at several temperatures between 25 and 50°C.

In the solvent mixture, the maximum of T_1 at about 80% DCA is observed for carboxyl and methine protons of DCA. We believe that this may reflect the variation in the acid—acid association interaction depending upon the solvent compositions. Recently, in this laboratory, studies concerned with the effects of acid—acid association and acid—solvent interaction on spin-lattice relaxation of acid were examined.⁸ It was found that these effects are pronounced and similar maxima or minima of T_1 were observed on DCA—chloroform, acetic acid—carbon tetrachloride, deuteriochloroform, and hexadeuteriobenzene solutions. In DCA—DCE mixture, in addition to this behavior of T_1 ,

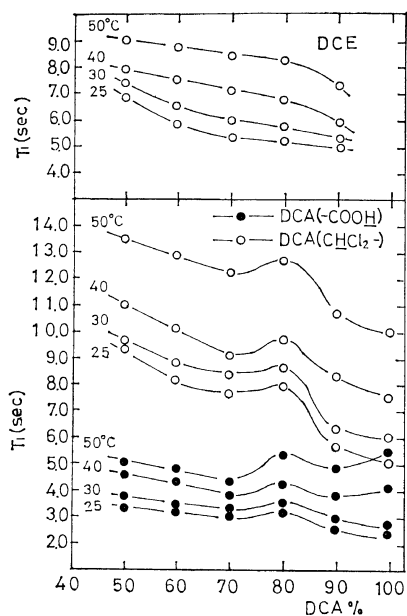


Figure 4. T_1 of solvent protons vs. volume fraction of DCA in DCA—DCE solvent mixture.

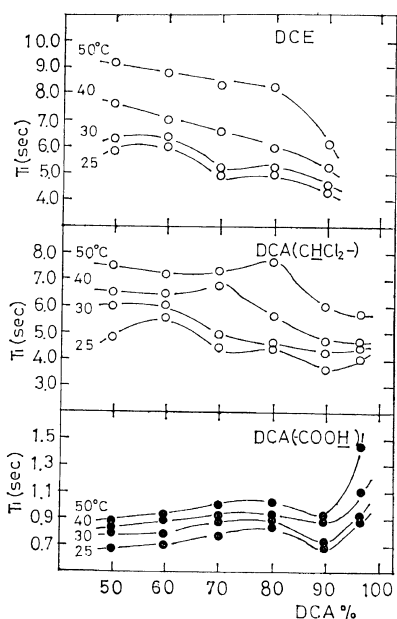


Figure 5. T_1 of solvent protons vs. volume fraction of DCA in PMLG solution ($c=4.1$ g/100-ml solvent).

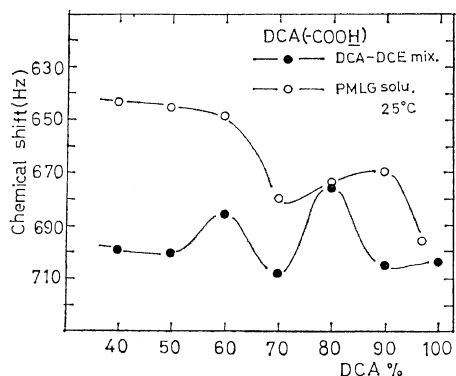


Figure 6. Chemical shift of DCA(-COOH) vs. volume fraction of DCA in DCA—DCE solvent mixture (●) and in PMLG solution (○); PMLG concentration, 4.1 g/100-ml solvent. Chemical shifts were measured from external TMS.

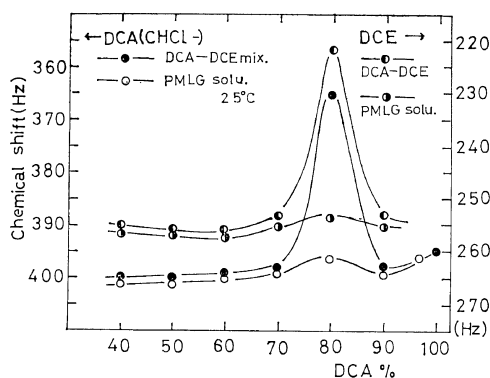


Figure 7. Dependence of chemical shift of DCA(CHCl_2^-) and DCE on volume fraction of DCA in DCA-DCE solvent mixture (\bullet , \circ) and in PMLG solution (\bigcirc , \bigcirc); PMLG concentration, 4.1 g/100-ml solvent. Chemical shifts were measured from external TMS.

chemical shifts of all kinds of solvent protons also exhibit high field shift at 80-% DCA (see Figures 6 and 7). In PMLG solution at 25°C, the behavior of T_1 is different to that in the solvent mixture, especially for methine(DCA) and DCE protons. However, at the higher temperatures where PMLG is considered not to undergo definite helix to coil transition at 80-% DCA the behavior of T_1 gradually approached that in the solvent mixture. These differences of T_1 at 25°C which are observed in PMLG solution and in the solvent mixture may arise from the conformational transition of PMLG. However, variation in the contributions from PMLG-solvent, DCA-DCE, and DCA-DCA interactions according to the change of DCA

content makes the problem very complicated.

In Table II, relaxation rates at 25°C of solvent protons in the solvent mixture and in PMLG solution denoted by $1/T_{1s}$ and $1/T_{1p}$, respectively, are shown together with $(1/T_{1p}-1/T_{1s})$ values. The relaxation rates, $1/T_{1s}$, $1/T_{1p}$, and $(1/T_{1p}-1/T_{1s})$, of carboxyl and methine protons of DCA are roughly equal to those values in PMLG-DCA-chloroform solution at 25°C, as seen from Tables I and II. As $(1/T_{1p}-1/T_{1s})$ values of the acid proton are the largest and those of DCE protons the smallest, the carboxyl group of DCA would interact most strongly with PMLG, whereas the interaction between DCE and PMLG is not appreciable. The order of the magnitudes of the interactions between PMLG and solvents parallels that in PMLG-DCA-chloroform solution.

In Figure 6 and 7, respectively, the dependence of chemical shifts of solvent protons on the solvent composition at 25°C are shown with and without PMLG. In the solvent mixture, at 80-% DCA, the chemical shifts of all protons exhibit high field shifts, which correspond to the maximum of T_1 at 80-% DCA. Similar tendencies were also observed in DCA-chloroform mixture.⁵ It may be speculated that this behavior of T_1 's and chemical shifts may be due to the change in the liquid structure of the solvent mixture. This abnormal behavior is interesting in view of the fact that PMLG undergoes conformational transition near this solvent composition. And this suggests the importance of the role of the solvent or solvent structure at conformational transitions of bio-

Table II. Spin-lattice relaxation rates of solvent protons in DCA-DCE solvent mixtures ($1/T_{1s}$) and in PMLG solutions ($1/T_{1p}$), at different fractions of DCA^a

DCA : DCE	DCA(-COOH), sec ⁻¹			DCA(CHCl_2^-), sec ⁻¹			DCE, sec ⁻¹		
	T_{1s}^{-1}	T_{1p}^{-1}	$T_{1p}^{-1}-T_{1s}^{-1}$	T_{1s}^{-1}	T_{1p}^{-1}	$T_{1p}^{-1}-T_{1s}^{-1}$	T_{1s}^{-1}	T_{1p}^{-1}	$T_{1p}^{-1}-T_{1s}^{-1}$
50:50	0.29	1.43	1.14	0.11	0.20	0.09	0.14	0.17	0.03
60:40	0.31	1.40	1.09	0.12	0.17	0.05	0.17	0.17	0.00
70:30	0.30	1.25	0.95	0.13	0.23	0.10	0.20	0.20	0.00
80:20	0.30	1.11	0.81	0.13	0.23	0.10	0.20	0.20	0.00
90:10	0.42	1.43	1.01	0.18	0.29	0.11	0.22	0.23	0.01
100:0	0.43	1.11	0.68	0.20	0.24	0.04	—	—	—

^a $(1/T_{1p}-1/T_{1s})$ means the difference in relaxation rates.
PMLG concentration, 4.1 g/100-ml solvent.

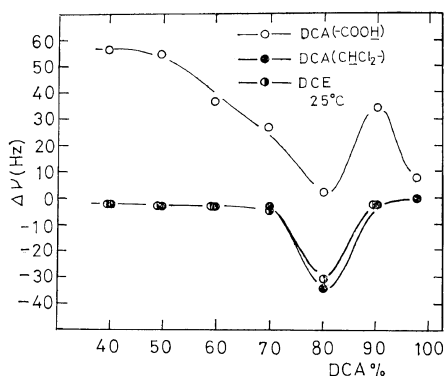


Figure 8. Chemical shift difference $\Delta\nu$ vs. volume fraction of DCA.

polymers. However, these anomalous shifts decrease in the presence of PMLG, and a similar disappearance of the high field shifts was observed in the presence of NMA or DMA in the case of the DCA—chloroform mixture. This means that solvent mixtures should lose their characteristics or liquid structures in the presence of PMLG or amides.

In Figure 8 the dependence of chemical shift difference, $\Delta\nu$, on DCA content is shown (25°C). We define $\Delta\nu$ as follows

$\Delta\nu = \delta$ in the solvent mixture $-\delta$ in PMLG solution. Thus the positive $\Delta\nu$ indicates that chemical shifts of solvent protons show high field shift due to the presence of PMLG or PMLG—solvent interaction. Very interesting results are obtained for all protons, especially at about 80% DCA where PMLG undergoes helix-random coil transition. It is considered that this strange behavior of $\Delta\nu$ may be due to the effect of the conformational transition of PMLG on the solvent molecules. In respect of $\Delta\nu$ of methine proton of DCA and DCE, except for at 80% DCA, $\Delta\nu \approx 0$. This means that the effects of PMLG or PMLG—solvent interaction at these regions do not influence chemical shifts of these protons. However, at 80% DCA these effects are appreciable and correspond to the fact that spin-lattice relaxations are affected by the presence of PMLG and the conformational transition of PMLG at 25°C.

In the plot of $\Delta\nu$ vs. DCA content, the maximum and minimum for the carboxyl proton of DCA occur near the helix—coil transition

region. Similar results were reported for the TFA proton in PBLG—TFA—benzene and PBLG—TFA—chloroform solutions by Liu and Lignowski.⁴ Another interesting point is that $\Delta\nu$ is positive over the experimental range of DCA content. In NMA or DMA—DCA—chloroform solution,⁵ $\Delta\nu$'s of carboxyl proton of DCA are large negative values, that is $\Delta\nu$'s experience opposite signs in PMLG and in amide solutions.

In amide solutions, from the results of chemical shift and spin-lattice relaxation, we propose for amide—DCA interactions the following:

- (a) protonated amide—dissociated acid direct interaction,
- (b) protonated amide—dissociated acid indirect interaction,
- (c) protonated amide—acid hydrogen bonding, and
- (d) amide—acid hydrogen bonding and so on.

However, in PMLG solution strong interactions such as (a), (b), and (c) mentioned above, which may induce large down field shift of the carboxyl proton, may not exist, and an interaction such as a weak hydrogen bonding may be considered predominant. These suggestions are in accord with the conclusions of Stewart, *et al.*,⁹ Ferretti, *et al.*,¹⁰ and Liu and Lignowski.⁴

In summary

- (1) In PMLG—DCA—DCE solution, spin-lattice relaxations of solvent protons are affected by the conformational transition of PMLG. This is in accordance with the results on PMLG—DCA—chloroform solution.
- (2) In this solution, the interaction between PMLG and carboxyl group of DCA is most remarkable and DCE scarcely interacts with PMLG.
- (3) In the plot of $\Delta\nu$ vs. DCA content, strange behavior believed to be due to the conformational transition of PMLG exists.
- (4) The sign of $\Delta\nu$ of carboxyl proton of DCA is opposite on PMLG solution and on amide solutions. This leads to the conclusion that the interaction between PMLG and DCA may be a weak one such as a weak hydrogen bonding rather than protonated-form interactions considered predominant in amide—acid interactions.

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