Macromolecules 1981, *14*, 1698–1700

Observation of the Side-Chain Motion of Polypeptides in Solution by Measurement of Picosecond Dielectric Relaxation[1]

Haruki Nakamura*

Department of Applied Physics, Faculty of Engineering, University of Tokyo, Hongo, Tokyo, Japan

Satoru Mashimo

Department of Physics, Faculty of Science, Tokai University, Hiratsuka-shi, Kanagawa, Japan

Akiyoshi Wada

Department of Physics, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.

Received June 3, 1981

ABSTRACT: A new dielectric relaxation for poly(γ-benzyl L-glutamate) in dioxane solution with a critical frequency near 1 GHz was observed by measurement of picosecond dielectric relaxation using the method of time domain reflectometry (TDR). Since (1) the main chain of the polypeptide keeps its α-helical structure and (2) this relaxation does not depend upon the molecular weight, it is thought to be a rotational relaxation of the side-chain dipole. The observed residual dipole moment is around 1.4 D, and the activation energy is 2–3 kcal/mol, which are values consistent with those obtained in other studies.

Introduction

Dielectric relaxation studies have been used for observing electric dipole moments and the dynamics of biological macromolecules such as polypeptides[2] and proteins.[3] The usual approach has been to observe the overall rotation of the electric dipoles of the macromolecules by measurements of dielectric spectra below 10 MHz using a transformer bridge. In contrast to these frequency domain measurements, time domain measurements have been developed, with the advantage of fast measurement covering a wide frequency range.[4–6] In particular, the recent development of the method of time domain reflectometry (TDR) has made it possible to observe picosecond dielectric relaxation in macromolecular solutions.[6]

Recent studies have revealed that the side chains of proteins do not maintain their rigid structures[7–10] and that their internal motions, on a picosecond time scale,[7] play important roles in the catalytic reactions of proteins.[11]

In the present report, we describe the measurement of picosecond dielectric relaxation of helical poly(γ-benzyl L-glutamate) ((Bzl-Glu)$_n$) solutions. The observed relaxation is thought to be due to the internal motion of the ester groups of the side chains. This direct observation of the local motion of small dipoles of side chains will give useful information on the structural fluctuation of protein molecules.

Experimental Section

The molecular weights of the three (Bzl-Glu)$_n$ samples were determined by gel permeation chromatography[12] (mol wt 7400 and 11 500) and viscosity (mol wt 95 000). Dioxane was distilled before use.

The TDR measuring system employed for the difference method was almost the same as that described by Cole et al.[6] A step pulse of 270-mV pulse height with a rise time of less than 50 ps was generated by a pulse generator (Hewlett-Packard HP1105 and 1106b). A sampling time base (Hewlett-Packard HP1811A) and a transient recorder (Data Lab DL905) sampled and digitized the signal reflected at the interface between the sample cell and the semiflexible coaxial line (Junkosha DGM010, DC-18.5 GHz). The sample cell, with a N-type connector, consists of a Kel-F insulating washer and an inner and an outer conductor made of stainless steel. A very short inner conductor 1.6 mm long was used in order to cover the range up to 5 GHz. The temperature inside the cell was controlled within ±0.1 °C by circulating water at a constant temperature. The whole system was controlled and the results were analyzed by a minicomputer (Yokogawa Hewlett-Packard YHP2108). The incident pulse (V_0), the reflection from the solvent (R_s), and the reflection from the solution (R_x) were successively measured and the $V_0 - R_s$ and $R_s - R_x$ differences were Fourier transformed. Finally, a complex dielectric spectrum $\epsilon^*(\omega)$ was obtained by a recursion calculation. The timing jitter errors in V_0, R_s, and R_x were reduced to less than 0.2 ps with the help of a timing marker signal simultaneously measured with each reflection.

Table I
Picosecond Dielectric Relaxation of Several (Bzl-Glu)$_n$ Samples in Dioxane Solutions at 20 °C

M_w	concn, % w/w	τ, ps	$\Delta\epsilon/c$, mL/g	μ_{res}, D
7 400	2.9	140	1.2_5	1.5_6
	4.8	150	1.0_6	1.4_3
	6.7	120	1.0_4	1.4_2
11 500	4.9	140	0.9_0	1.3_0
95 000	4.8	130	0.9_3	1.3_2

Results

Parts a and b of Figure 1 show the time domain signals of $V_0 - R_s$ and $R_s - R_x$ for 4.8% w/w (Bzl-Glu)$_n$ (mol wt 7400) in dioxane at 20 °C. The Fourier-transformed dielectric spectra observed at 20 and 54 °C are indicated in Figure 2, and the Cole–Cole plots are shown in Figure 3. Table I summarizes the results of the dielectric relaxation time (τ) and the relaxation increment ($\Delta\epsilon$), together with the calculated residual dipole moment (μ_{res}) for several samples at 20 °C. An uncertainty of ±10% for both τ and $\Delta\epsilon$ is unavoidable. Each residual dipole moment was estimated by the usual equation for a spherical molecule in a nonpolar solvent using Onsager's formula,[13] with $\Delta\epsilon$ given by an extrapolation of the Cole–Cole plot. Here, the residual dipoles were assumed to be uncorrelated. It is clearly seen in Table I that the residual dipole moment is 1.4 ± 0.1 D, that the relaxation time is around 140 ps, and that neither depends on concentration or molecular weight. Figure 4 shows the Arrhenius plot of the dielectric relaxation time for the sample of mol wt 7400, and the activation energy was found to be 2–3 kcal/mol.

Discussion

Since the main chain of a polypeptide maintains its α-helical conformation in dioxane solution, the relaxation

0024-9297/81/2214-1698$01.25/0

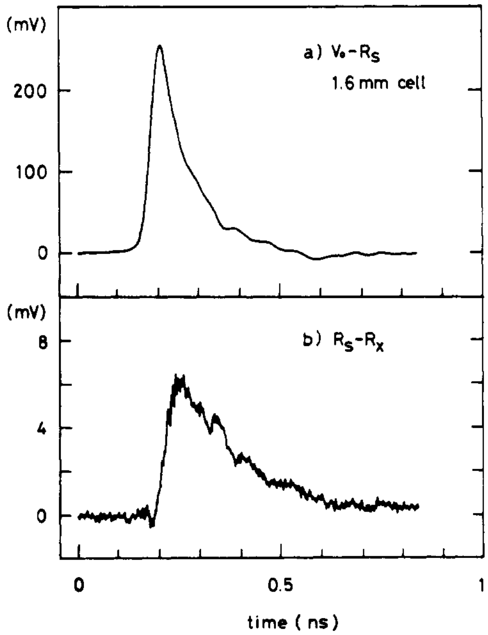


Figure 1. Time domain signals of (a) $V_0 - R_s$ and (b) $R_s - R_x$ for 4.8% w/w (Bzl-Glu)$_n$ (mol wt 7400) in dioxane at 20 °C, using a 1.6-mm inner conductor. The 1024 digital points are obtained after averaging each record of V_0, R_s, and R_x 400 times.

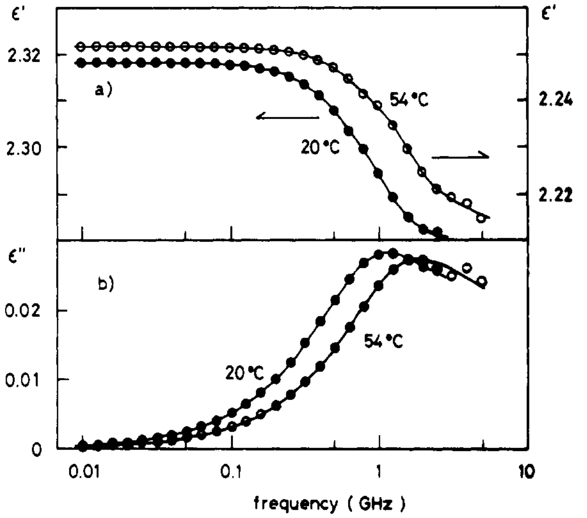


Figure 2. Dielectric spectra of 4.8% w/w (Bzl-Glu)$_n$ (mol wt 7400) in dioxane at 20 (●) and 54 (○) °C: (a) dispersion curves ($\epsilon'(\omega)$); (b) loss curves ($\epsilon''(\omega)$).

time due to its overall rotation should be longer than 10 ns at 20 °C, even for the sample with the smallest molecular weight, 7400.[2,14] A possible origin of the fast dielectric relaxation observed might be an accordion-like vibration of the α helix, which has been studied by far-infrared spectroscopy.[15] However, both the overall rotation and the accordion-like vibration of the α helix should depend strongly on the molecular weight of (Bzl-Glu)$_n$. A proton-jumping mechanism[16] at the hydrogen bond between the main chain of the α helix is another candidate for the dielectric relaxation. Though there are no data for either the relaxation time or the activation energy which clearly establish proton jumping, there is no reason at present to ignore this possibility.

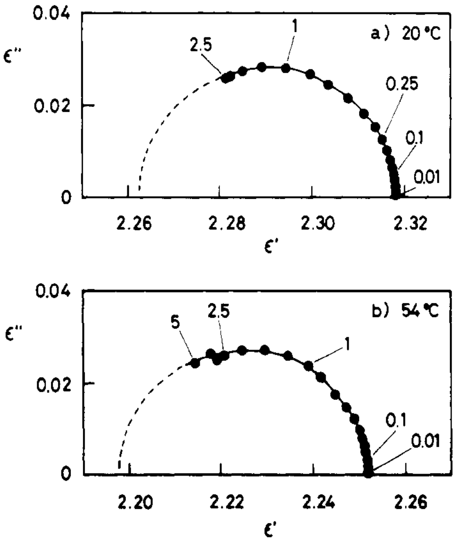


Figure 3. Cole–Cole plots at (a) 20 and (b) 54 °C. The numbers in the figures indicate frequencies (GHz).

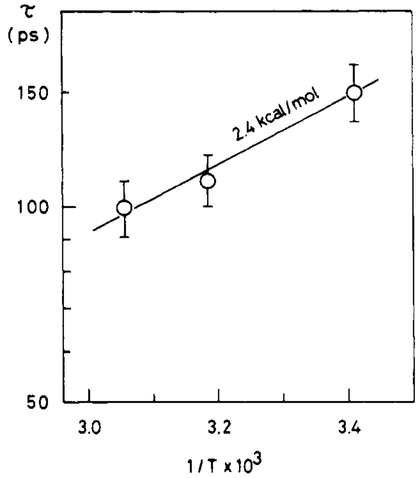


Figure 4. Arrhenius plot of the dielectric relaxation time for (Bzl-Glu)$_n$ (mol wt 7400) in dioxane solution (4.8% w/w). Error bars are indicated, and the solid line gives an activation energy of 2.4 kcal/mol.

The last and the most probable origin of the relaxation is a local rotation of a small electric dipole due to the ester group of the side chain extending into the solvent. The reasons are as follows: (1) The observed relaxation time depends very little upon the molecular weight and is the same order as 3 times the rotational correlation time of Bzl-CH$_2$ observed by ^{13}C NMR[17] and ^{1}H NMR.[18] (2) The Cole–Cole plot in Figure 2 indicates that it is almost a single relaxation with little dispersion of the relaxation time, suggesting a simple rotation of local dipoles. (3) The value of the residual dipole moment calculated as a side-chain moment, 1.4 D, is a little smaller than those of free ester molecules, such as methyl acetate (1.74 D) and ethyl acetate (1.86 D). The moment of the ester group, calculated by taking different angles of the internal rotation into account, is also between 1.5 D (in the trans position) and 3.5 D (in the cis position).[2] There may be a weak restriction on the side-chain motion, such as in the "wobbling-in-a-cone" model.[19] (4) The activation energy, 2–3 kcal/mol, is almost the same as that estimated from the spin–lattice relaxation time, T_1, of β-CH$_2$ and Bzl-CH$_2$ by ^{1}H NMR measurement.[18]

In previous studies, the side-chain moments have been found to orient, in a long-time average, antiparallel to the main-chain moment.[2] In the present study, it has been demonstrated that the side chain still has a considerable amount of internal freedom of motion, the characteristic frequency of which is in the gigahertz region.

Acknowledgment. We thank Dr. K. Nagayama for helpful discussions. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Japan.

References and Notes

(1) This work was performed at the Department of Physics, Faculty of Science, University of Tokyo, Tokyo, Japan.
(2) Wada, A. *Adv. Biophys.* **1976**, *9*, 1–63.
(3) Grant, E. H.; Sheppard, R. J.; South, G. P. "Dielectric Behaviour of Biological Molecules in Solution"; Clarendon Press: Oxford, 1978.
(4) Husimi, Y.; Wada, A. *Rev. Sci. Instrum.* **1976**, *47*, 213.
(5) Nakamura, H.; Husimi, Y.; Wada, A. *J. Appl. Phys.* **1981**, *52*, 3053.
(6) Cole, R. H.; Mashimo, S.; Winsor, P., IV. *J. Phys. Chem.* **1980**, *84*, 786.
(7) McCammon, J. A.; Gelin, B. R.; Karplus, M. *Nature (London)* **1977**, *267*, 585.
(8) Wagner, G.; Wüthrich, K. *Nature (London)* **1978**, *275*, 247.
(9) Frauenfelder, H.; Petsko, G. A.; Tsernoglou, D. *Nature (London)* **1979**, *280*, 558.
(10) Artymiuk, P. J.; Blake, C. C. F.; Grace, D. E. P.; Oatley, S. J.; Phillips, D. C.; Sternberg, M. J. E. *Nature (London)* **1979**, *280*, 563.
(11) Patel, D. J.; Canuel, L. L. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1398.
(12) Nagayama, K.; Okada, S. *Polym. J.* **1973**, *5*, 97.
(13) Fröhlich, H. "Theory of Dielectrics"; Clarendon Press: Oxford, 1958.
(14) Nakamura, H.; Husimi, Y.; Parry-Jones, G.; Wada, A. *J. Chem. Soc., Faraday Trans. 2* **1977**, *73*, 1178.
(15) Itoh, K.; Shimanouchi, T. *Biopolymers* **1970**, *9*, 383.
(16) Davies, M. *J. Chem. Educ.* **1969**, *46*, 17.
(17) Allerhand, A.; Oldfield, E. *Biochemistry* **1973**, *12*, 3428.
(18) Nagayama, K. *Biophysics* (in Japanese) **1976**, *16*, 68.
(19) Kinoshita, K., Jr.; Kawato, S.; Ikegami, A. *Biophys. J.* **1977**, *20*, 289.

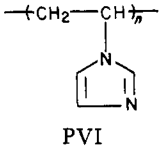
Chain Characteristics and Counterion Binding of Poly(*N*-vinylimidazole) and Its Protonated and Quaternized Salts

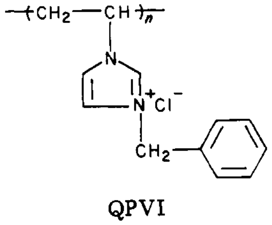
J. S. Tan* and A. R. Sochor

Research Laboratories, Eastman Kodak Company, Rochester, New York 14650.
Received March 5, 1981

ABSTRACT: Intrinsic viscosity, light scattering, and turbidimetric titration were used to characterize the solution behavior of poly(*N*-vinylimidazole) and its charged polycations, including the protonated and the quaternized forms. The unperturbed dimensions of these chain molecules are not sensitive to the solvents or the side groups at the quaternary ammonium site but are sensitive to the counterions. The characteristic ratio C_∞ is 15–18 for polymers with the stronger binding chloride ion as the counterion but increases to 28 when the counterion is replaced by the weaker binding acetate or propionate ion. The order of counterion binding is similar to the order for the Hofmeister series. The persistence lengths estimated according to the scheme of Yamakawa and Fujii based on the wormlike-chain model are 22–23 Å for neutral poly(*N*-vinylimidazole) and 20 Å for the quaternized salt.

Poly(*N*-vinylimidazole) (PVI) and its quaternary ammonium salt poly(3-benzyl-1-vinylimidazolium chloride) (QPVI) can be considered as model polymers for investigating the interactions of similar polymers with neutral salts, complexing metal ions, and dye molecules. Substantial work has been reported concerning viscometric, potentiometric, and conductometric behavior of PVI and its quaternary salts in aqueous media,[1-3] the catalytic effect of imidazole-containing polymers,[4] and complex formation of PVI with metal ions.[5] Little effort has been made to investigate chain flexibility of these polymers as affected by the nature of the solvents, the type of neutral electrolytes present, or the structure of the side group at the quaternized site.


PVI


QPVI

We report here the solubility behavior of PVI, QPVI, and the protonated polymer HPVI. The unperturbed dimensions of these polymers were determined by light scattering and intrinsic viscosity. These results are related to counterion binding data obtained by turbidimetric titration. The effect of counterion binding on dye binding will also be discussed. The conformational change of PVI upon protonation, as studied by potentiometry, viscometry, and ^{1}H NMR was reported elsewhere.[6]

Experimental Section

Materials. PVI was synthesized by polymerization of *N*-vinylimidazole (BASF Chemicals) with azobis(isobutyronitrile) as the catalyst. Distilled monomer (150 g) was dissolved in 980 mL of benzene and polymerized at 60 °C under a nitrogen atmosphere for 48 h with 1.3 g of catalyst. The polymer was precipitated as a white powder. The solid was collected by filtration and was oven-dried at 35 °C. Two batches of the whole polymer were used for further fractionation in this study. Previous NMR studies suggested that this polymer is atactic.[6]

PVI was fractionated by stepwise precipitation with two solvent–nonsolvent pairs: *n*-butyl alcohol + *n*-hexane (for polymer batch I) and methanol + toluene (for polymer batch II). The coacervate liquids were freeze-dried, and the solids were heated at 40 °C under vacuum in the presence of P_2O_5 for several weeks before use. The weight-average molecular weights, $\bar{M}_w$, of the fractions, as determined by light scattering in methanol, ranged from 1.3×10^6 to 5.5×10^4.

Portions of several of the PVI fractions in batch I were then converted into the quaternized form. An excess of benzyl chloride was added to each PVI fraction dissolved in dimethylformamide. The mixture was heated at 85 °C on a steam bath for 3 h to allow complete quaternization. The quaternized polymer was precipitated by addition of acetone. The solids were puified by redissolving them in methanol and precipitating them with acetone. These steps were repeated once again before the polymer was dried

0024-9297/81/2214-1700$01.25/0