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Fluorescence and FTIR Studies on the pH-Dependent Conformational Change of Poly(methacrylic acid) in Aqueous Solutions

Kenichi Nakashima,* Yoshinori Fujimoto, Takayuki Anzai, Jian Dong,† Hidetoshi Sato,† and Yukihiro Ozaki†

Department of Chemistry, Faculty of Science and Engineering, Saga University, 1 Honjo-machi, Saga 840-8502

†Department of Chemistry, School of Science, Kwansei-Gakuin University, Uegahara, Nishinomiya 662-8501

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Fluorescence and FTIR spectroscopy have been used to investigate the pH-dependent conformational change of poly(methacrylic acid) (PMA) in aqueous solutions. Fluorescence intensity and lifetime, and other fluorescence parameters of the 1-pyrenemethanol probe were measured as a function of pH in PMA aqueous solutions. The fluorescence data obtained show that PMA undergoes a sharp coiling ⟷ uncoiling change around pH 5. FTIR spectra of PMA were successfully observed by an attenuated total reflection technique. The FTIR data show that the carboxyl groups of PMA are ionized progressively over the wide pH range from 3 to 8, and that PMA takes an expanded conformation under basic conditions. These results make it clear that although the ionization of the carboxyl groups takes place progressively, the conformational change of PMA is brought about abruptly.

It is known that some polyacids containing carboxyl groups undergo a pH-dependent conformational change in dilute aqueous solutions.[1—31] When the pH of the solutions is low enough to suppress the ionization of the carboxyl groups, the polyacids take a contracted form due to hydrogen-bonding between the carboxyl groups and/or due to other interactions such as hydrophobic interaction. As the pH is increased and the degree of ionization (α) of the carboxyl group reaches a certain value (α = 0.2—0.3 in many cases), a conformational change from a contracted to expanded form is induced as a result of the repulsion between carboxylate anion groups.

Poly(methacrylic acid) (PMA) is one of such polyacids. Many studies have been done on PMA in aqueous solutions, because this polymer has a peculiar titration curve unobserved for other polyvinylic compounds.[4—8,12] Various techniques have been used for the investigations: potentiometry,[4—6] viscometry,[7,8] light scattering,[9,10] small angle X-ray scattering (SAXS),[11] NMR,[12,13] and spectroscopy such as IR,[14,15] Raman,[2,3] UV,[14,16] and fluorescence.[1,17—27]

In spite of numerous studies, there still remain issues about three aspects: (1) whether the conformational change is cooperative or progressive; (2) what kind of interaction is a dominant factor for inducing the conformational change; (3) what is the nature of the polymer structure. As to the first problem, Liquori et al. demonstrated from investigation by potentiometry that the transformation of the chain structure is cooperative.[16] Fluorescence studies by Anufrieva et al. also supported the predominance of cooperative change.[17] On the other hand, Koenig et al. argued from laser Raman data that the conformational change of PMA is dependent on the tacticity of the polymer.[2,3] They concluded that the conformational changes of the syndiotactic and atactic PMA occur progressively, while that of the isotactic PMA has a cooperative nature. However, their Raman data reflect the local conformational change of PMA rather than the overall conformational change.[2,3] Therefore, there remains ambiguity on the overall conformational change. As to the second problem, hydrogen-bonding between carboxyl groups, van der Waals interaction, and hydrophobic interaction have been considered as the possible factors. Although the majority of studies stress the role of hydrophobic interaction, similar to the case for proteins,[6—8,16,17] some studies contradict the importance of hydrophobic interaction.[14,28,29] As to the third problem, we refer the interested readers to a recent short review by Morawetz.[30] He claimed in the review that the contracted forms of PMA in aqueous solutions are far from compact spheres from which the solvent is almost excluded. Alternatively, he proposed that the PMA chain behaves as a random coil in a theta solvent.

As so far stated, there is a contradiction between conclusions which were drawn by different groups by different techniques.[2,3,16,17] Therefore, we are convinced that it is necessary to apply two or more techniques to the same sample for delineating a correct picture of the conformational state of PMA. By virtue of the rapid progress of modern spectroscopy, it seems possible to obtain more precise and detailed information than ever before about the conformational change of PMA in aqueous solutions. In this study, we have reexamined these long-standing problems by a combination of FTIR and fluorescence spectroscopy, especially focusing on the first problem: i.e. whether the conformational change is cooperative or progressive. Fluorescence spectroscopy

is a useful technique to obtain information on a conformational change and/or association of polymer chains.[1,17—27] On the other hand, IR spectroscopy is a powerful tool for detecting the ionization and hydrogen-bonding of carboxyl groups.[31—38] Despite this merit, IR spectroscopy has not been a conventional technique for aqueous solutions because the strong absorption of IR light by water heavily interferes with measurements. Using an attenuated total reflection (ATR) technique, however, we have successfully observed the IR spectra of PMA in aqueous solutions.

Experimental

A stock solution of sodium salt of PMA (average molecular weight: 15000; concentration: 25 wt%) was obtained from Polysciences Inc., and was used as received. The fluorescence probe, 1-pyrenemethanol (PyM), was purchased from Molecular Probes Inc.

A stock solution of 100 μM (1 M = 1 mol dm^{-3}) PyM was prepared with methanol. Portions of the stock solution of the fluorescence probe were transferred into 10-mL volumetric flasks, and the solvent was evaporated by gentle heating under a nitrogen-gas stream. Then, the volumetric flasks were filled with an aqueous solution of PMA (1.0 g dm^{-3} or 10 g dm^{-3}), followed by ultrasonic agitation for 10 min. The pH of each solution was adjusted by titration with 0.1 M HCl or 0.1 M NaOH aqueous solution. The samples were stored in the dark for one night before fluorescence measurements.

The solutions for IR measurements were prepared by the same procedure as that for fluorescence measurements except that the step of adding the fluorescence probe was skipped. To estimate the tacticity of PMA, the solution samples were cast onto CaF_2 plates and carefully dried by vacuum evaporation. By comparison with IR data of isotactic poly(sodium methacrylate),[3] we can safely rule out the possibility that the PMA sample contains a high content of isotactic regularity. However, it requires further characterization to clarify whether the sample contains appreciable syndiotactic stereoregularity since atactic and syndiotactic PMA have close IR spectra.

IR spectra were recorded at a resolution of 4 cm^{-1} on a Nicolet Magna 550 FTIR spectrometer or a JASCO 7300 FTIR spectrometer. The sample holder was an ATR unit from JASCO or Nicolet with fixed angle of 45° and multiple reflection path (5 times). A sample was put on a horizontally mounted crystal of ZnSe. The sample chambers were purged with dried air or pure nitrogen gas to remove moisture of water, which introduces noise-like rotational-vibrational absorption bands on the ATR spectra. The ATR spectra are expressed as difference spectra, which are obtained by subtracting the background absorption of water from the sample absorption.

Fluorescence spectra were observed with a Hitachi F-4000 spectrofluorometer. The spectra were corrected by the use of a standard tungsten lamp with a known color temperature. Fluorescence lifetime (τ) measurements were done with a Horiba NAES 1100 time-resolved spectrofluorometer that uses a time-correlated single photon counting technique.[39]

Results and Discussion

Fluorescence Studies on the Conformational Change. Non-covalently bound fluorescent probes so far used for studying PMA solutions are pyrene,[1] auramine,[17,21] Acridine Orange,[17] 9-methylanthracene,[24,25] and 9,10-dimethylanthracene.[25] In this study, we used PyM because this probe has several advantages over the former ones: PyM affords various fluorescence parameters useful for detecting conformational change of PMA, and PyM has moderate solubility in water, which allows us to easily prepare samples of aqueous solutions.

Figure 1 shows the fluorescence and excitation spectra of PyM in an aqueous solution. In the excitation spectra, the absorption ranging from 300 to 350 nm is assigned to the $S_2 \leftarrow S_0$ allowed transition. As $S_1 \leftrightarrow S_0$ transition is forbidden, the $S_1 \leftarrow S_0$ absorption bands appear at the shoulder of the strong $S_2 \leftarrow S_0$ transition.[40] The emission ranging from 360 to 460 nm is assigned to $S_1 \rightarrow S_0$ fluorescence of PyM in the monomeric state. The excimer fluorescence[41] is not observed in this system. As seen in Fig. 1, the fluorescence spectrum of PyM monomer is fine-structured, and consists of five prominent bands. The band 1 is assigned to 0–0 and the others to vibronic bands. Similar to the case of pyrene,[42—44] the intensity of band 1 is very sensitive to the polarity of the environments, but that of band 3 is not. Thus, an intensity ratio between the bands 1 and 3 (so-called I_1/I_3) can be used as a measure of the polarity of the environments of the probe.[45]

We measured the fluorescence spectra of PyM in aqueous PMA solutions at various pHs, and plotted the I_1/I_3 as a function of pH (Fig. 2). At low pH (pH 1—4), the I_1/I_3 values were low and almost constant. The low values of I_1/I_3 indicate a lower polarity of the environments of the probe. This observation indicates that PMA has a contracted coil structure in the lower pH region, and that PyM is effectively incorporated into the PMA coil, which has a polarity lower than that of the surrounding aqueous phase. As the pH was increased, we noted a drastic increase in I_1/I_3 values around pH 5. The changes of the fluorescence parameter suggested that PMA polymer chains undergo a conformational change from the contracted coil to the open structure; i.e. PyM is transferred from PMA coils to the polymer–water interface (or into the aqueous phase) as a result of the PMA conformational change. Similar observations were reported for

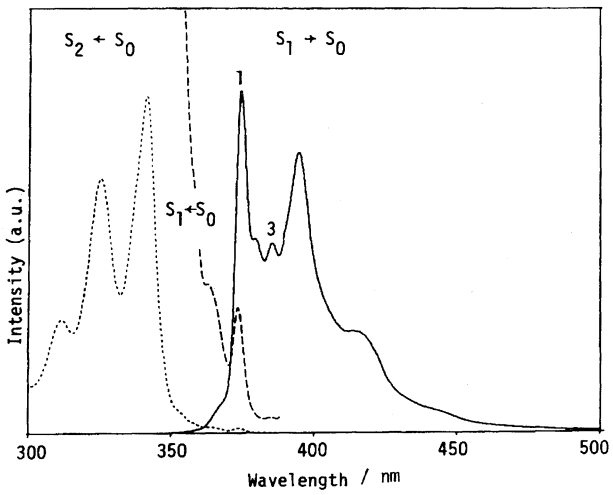

Fig. 1. Fluorescence (—) and excitation (···; - - -) spectra of PyM in an aqueous solution. Concentration of PyM is 10 μM. The dashed line is an expanded trace of the excitation spectrum in the $S_1 \leftarrow S_0$ absorption region.

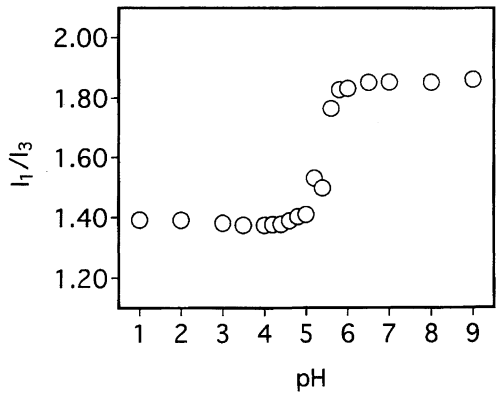

Fig. 2. pH dependence of I_1/I_3 of PyM in aqueous solutions of PMA. Concentrations of PMA and PyM are 1.0 g dm^{-3} and 10 μM, respectively.

pyrene/PMA systems by Thomas and coworkers.[1,18)]

Figure 3 represents fluorescence decay curves of PyM at pH 4 and pH 6 in aqueous PMA solutions. At a glance we notice a significant decrease in the fluorescence lifetime on going from pH 4 to 6. We tried to analyze the decay curves in terms of a series of exponential functions, and realized that at least two exponential functions are necessary:

$$I(t) = I(0)[A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)], \quad (1)$$

where A_i and τ_i ($i = 1, 2$), respectively, denote the preexponential factor and the lifetime of the ith component. $I(0)$ is the fluorescence intensity at time $t = 0$. We obtained $\tau_1 = 108$ ns and $\tau_2 = 295$ ns for the decay curve at pH 4. Relative quantum yields ($A_i\tau_i$) are 5.4 and 94.6% for the short and long lifetime components, respectively. The short lifetime can be assigned to the species exposed to the aqueous phase, and the long lifetime to that located in the polymer coils.[18)] From the relative quantum yields we know that most of the PyM molecules are accommodated in the PMA polymer coils. At pH 5, where the conformation of PMA seems to be in a transient state, we obtained a double-exponential decay curve with a comparable distribution: $\tau_1 = 114$ ns ($A_1\tau_1 = 32.4\%$) and $\tau_2 = 272$ ns ($A_2\tau_2 = 67.6\%$). This indicates that about 30% of the probe molecules are exposed to water in the partly unwound PMA chains. The decay curve at pH 6 is quite in contrast to that at pH 4: $\tau_1 = 120$ ns ($A_1\tau_1 = 92.5\%$) and $\tau_2 = 434$ ns ($A_2\tau_2 = 7.5\%$). As the short lifetime can be assigned to the species which is exposed to water, this result shows that PMA chains take an almost perfectly extended form at pH 6.

To obtain an overview of the dependence of the lifetime on pH, we used the averaged lifetime[46)] defined by the following equation:

$$\langle\tau\rangle = \sum A_i\tau_i^2 / \sum A_i\tau_i. \quad (2)$$

In Fig. 4a we plot $\langle\tau\rangle$ against pH. We note sharp change of the lifetime around pH 5 on going from lower to higher pH. The large $\langle\tau\rangle$ values at low pH suggests that the motion of PyM is hindered so that nonradiative processes of the probe are suppressed, leading to the increase in the lifetime of fluorescence. This is in accord with that PyM is

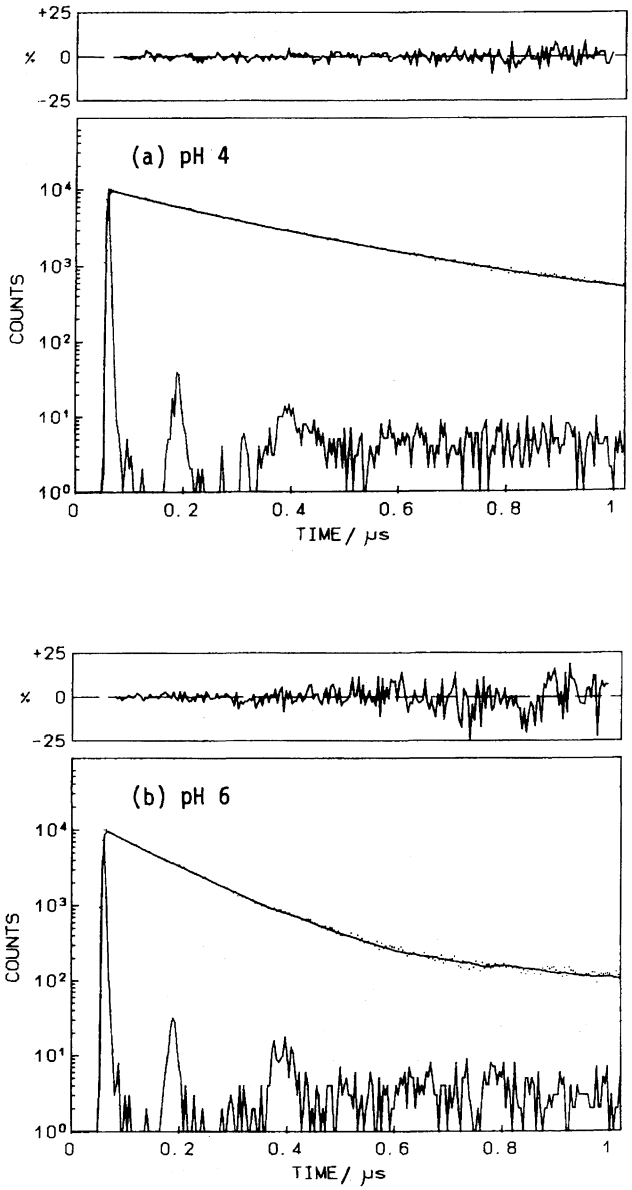

Fig. 3. Fluorescence decay curves of PyM in aqueous solutions of PMA at pH 4 (a) and pH 6 (b). Concentrations of PMA and PyM are 1.0 g dm^{-3} and 10 μM, respectively. PyM is excited at 325 nm and its fluorescence is collected through a Toshiba UV-37 cut-off filter.

effectively incorporated into the PMA coil at low pH. The drastic decrease in $\langle\tau\rangle$ around pH 5 suggests that PyM is transferred from viscous PMA coils to nonviscous aqueous environments. In Fig. 4b we show the pH dependence of $\langle\tau\rangle$ of PyM in the presence of a fluorescence quencher, iodide ion (I^-). A sharper change of $\langle\tau\rangle$ is seen in this case. As the quencher does not much penetrate into the coils of PMA due to its ionic nature, a quenching of PyM fluorescence is less effective at low pH. When PMA undergoes the conformational change, PyM is exposed to the aqueous phase, resulting in effective quenching (i.e. shortening of $\langle\tau\rangle$) by I^-. Thus, the change of the lifetime in Fig. 4 is further evidence for the conformational change of PMA around pH

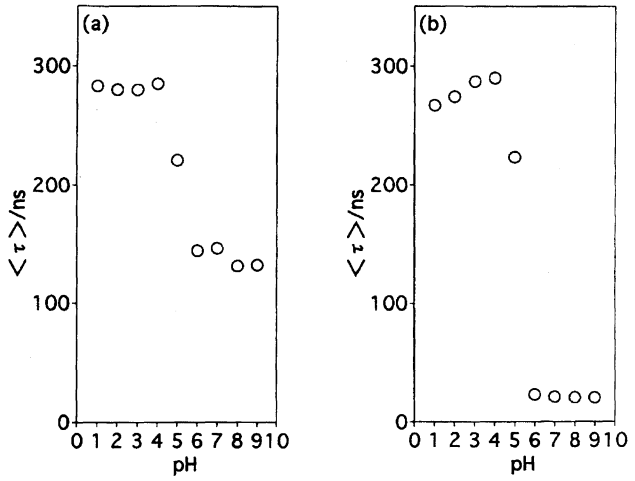

Fig. 4. pH dependence of the fluorescence lifetime of PyM in aqueous solutions of PMA in the absence (a) and presence (b) of NaI. Concentrations of PMA, PyM, and NaI are 1.0 g dm^{-3}, 10 μM, and 20 mM, respectively.

5.

Another useful fluorescence parameter for probing polymer conformations is the degree of polarization (P),[47,48] which is defined by:

$$P = (I_{vv} - GI_{vh})/(I_{vv} + GI_{vh}), \quad (3)$$

$$G = I_{hv}/I_{hh}, \quad (4)$$

where I_{vv} and I_{vh}, respectively, denote the intensities of the vertically- and horizontally-polarized emission when the sample is excited with vertically-polarized light; and I_{hv} and I_{hh}, the intensities of a vertically- and horizontally-polarized component when the sample is excited with horizontally-polarized light.[46] According to Weber and coworkers,[49,50] P is related to the viscosity (η) of the environments of the probe as follows:

$$1/P = 1/P_0 + (1/P_0 - 1/3)(kT\tau/\eta V), \quad (5)$$

where P_0 is the degree of polarization in rigid media, k the Boltzmann constant, T absolute temperature, τ the fluorescence lifetime, V the effective volume of the probe. The degree of polarization reflects the rotational mobility of the probe under consideration, and Eq. 5 clearly shows that P is large if the motion of the probe is hindered during its lifetime. Therefore, it is expected that P is large when the probe is incorporated into polymer coils, but is small when the probe is exposed to aqueous phase. We observed the polarization spectrum of PyM in aqueous solutions of PMA at pH 9 and 3 (Fig. 5). As seen from Fig. 5, P is almost zero at pH 9. This value corresponds to free rotation of the probe molecules in nonviscous solvents, indicating that the probe is located at the flexible polymer chains with a stretched form or the probe is in the aqueous phase. On the other hand, P has a value of about 0.02 at pH 3, indicating a hindered rotation due to incorporation into the polymer coils. These observations also support the conclusion that PMA takes a contracted form at pH 3, but an expanded form at pH 9.

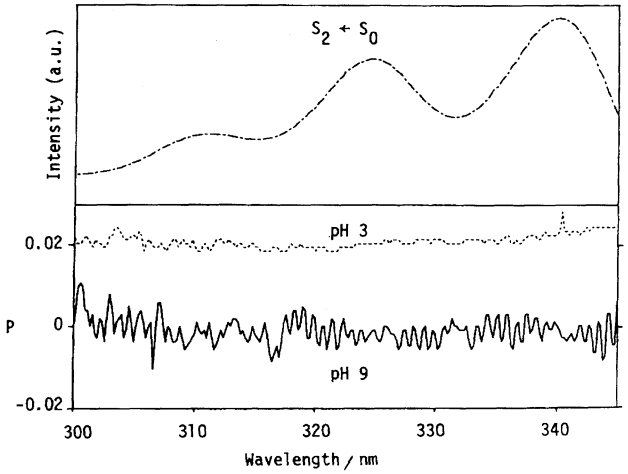

Fig. 5. Fluorescence polarization spectra of PyM at pH 9 (—) and pH 3 (···) in aqueous solutions of PMA. Concentrations of PMA and PyM are 1.0 g dm^{-3} and 10 μM, respectively. The $S_2 \leftarrow S_0$ excitation spectrum (–·–) is also shown for comparison. The fluorescence is monitored at 395 nm.

FTIR Studies on the Ionization of the Carboxyl Groups. FTIR-ATR spectra of PMA in aqueous solutions were observed at various pHs. The representative spectra are shown in Fig. 6. In Fig. 6a (at pH 4), we note a prominent band at 1702 cm^{-1}. This band can be assigned to a C=O stretching mode of the unionized form of the carboxyl group.[51] The C=O stretching band is so strongly affected by the ionization of the carboxyl group that we can use it as a marker band for monitoring the ionization of the carboxyl group. As pH of the solution is increased, the C=O stretching band gradually loses its intensity and shifts its location to higher wavenumbers. The high frequency shift of the C=O stretching band may be ascribed to the breakdown of hydrogen bonds as a result of the ionization of the carboxyl group. Concomitant with the decrease of the intensity of the C=O stretching band, a new band appears at around 1545 cm^{-1} (Fig. 6b). The new band is assigned to a COO^- antisymmetric stretching mode of the ionized form of the carboxyl group.[51]

Here we can extract the information on PMA conformation from the band profile of the COO^- antisymmetric mode. Painter et al. investigated Na^+ and Ca^{2+} ionomers of poly(ethylene-*co*-methacrylic acid) by FTIR spectroscopy.[33] These ionomers, known to have an aggregated structure from forming clusters of carboxylates, showed a split band profile of the asymmetric stretching vibration of the COO^- group in the region of 1520—1580 cm^{-1}. This is due to the strong interaction of COO^- anions which are close to each other in the ionomers. In Fig. 6b, however, we do not observe any splitting of the COO^- band in the 1520—1580 cm^{-1} region. This implies a great reduction of the strong interaction between the COO^- groups in the short range. In other words, the simple band profile in Fig. 6b indicates the absence of such aggregated structure, due to the expanded overall conformation of PMA.

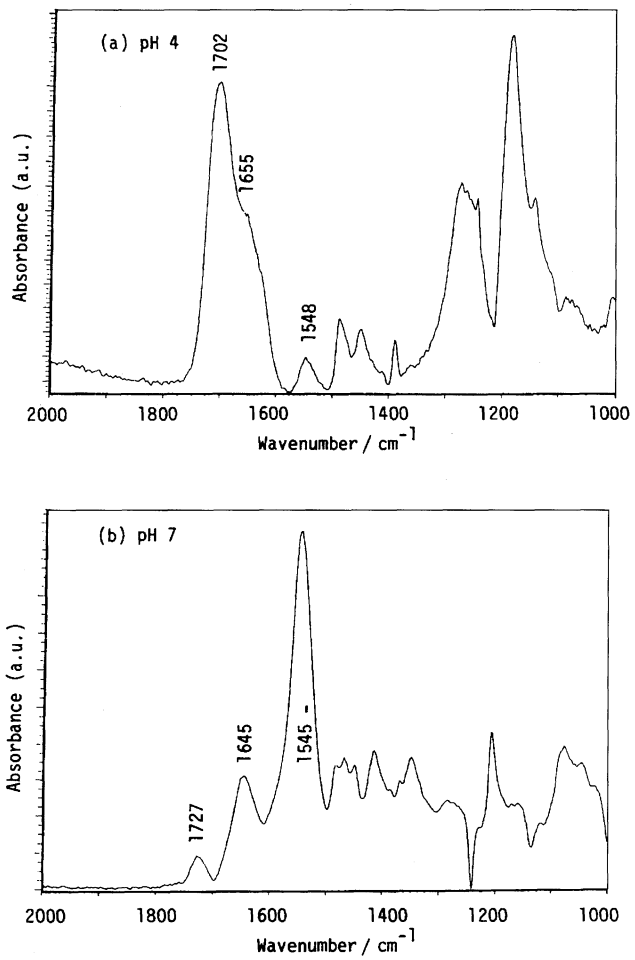

Fig. 6. FTIR-ATR spectra of PMA in aqueous solutions at pH 4 (a) and pH 7 (b). Concentration of PMA is 10 g dm^{-3}.

In Fig. 7, we plot the intensity of the C=O stretching band for the COOH form as a function of pH, together with the intensity of the COO^- antisymmetric stretching band for the COO^- form. By monitoring the intensity of the COO^- band, we can get information on the dissociation behavior of the carboxyl group. This seems to be more straightforward and reliable than the traditional method based on potentiometric titration.[4—6] At pH 3 the intensity of the band for the COO^- species is almost zero, indicating the absence of this species. As the pH is increased, the band for the COO^- group gradually increases and that for the COOH group decreases. These behaviors of the C=O and COO^- bands clearly show the progressive change of the carboxyl groups from the COOH to COO^- forms.[52] Thus, we conclude that the ionization of the carboxyl groups of PMA takes place progressively over the pH range from 3 to 8.

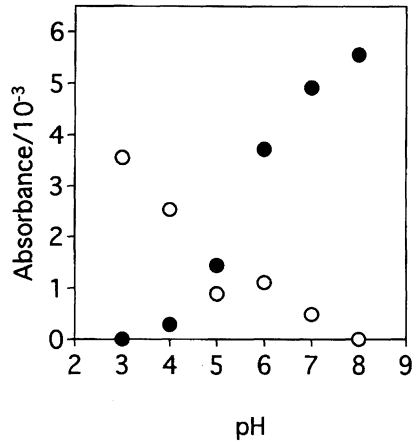

Fig. 7. pH dependence of the intensities of the COOH (○) and COO^- (●) bands of PMA in aqueous solutions.

In this study we did not monitor hydrogen-bond formation of COOH groups of PMA. By a band resolution technique,[37] we can follow the appearance and disappearance of the hydrogen bond. However, the ATR spectra obtained in this study are too weak to have enough quality for band resolution. If we resolve the COOH band around 1700 cm^{-1} into the components of free and hydrogen-bonded species, we can obtain some information on the nature of interaction which is mainly responsible for the sharp conformational change of PMA (see the third paragraph of Introduction section). Such investigation will be done.

Conclusions

Various fluorescence parameters of PyM have been observed to investigate the pH induced conformational change of PMA in aqueous solutions. From the dependence of the parameters on pH, it is seen that PMA undergoes a sharp conformational change around pH 5. FTIR spectra of PMA in aqueous solutions have been successfully observed by an ATR technique. The band profile of the COO^- antisymmetric stretching vibration in 1520—1580 cm^{-1} region gives evidence for an expanded conformation of PMA in basic conditions. The C=O stretching band for the unionized form and the COO^- antisymmetric stretching band for the ionized form of the carboxyl group of PMA gradually change in their intensities over the pH range from 3 to 8, indicating progressive dissociation of the COOH group in this pH range.

Based on the observation that the fluorescence parameters of PyM drastically change around pH 5 (Figs. 2 and 4), whereas the IR bands gradually change in their intensities at the same pH (Fig. 7), we have concluded that the conformational change of PMA is cooperatively brought about around pH 5. Hydrophobic interaction among methyl groups seems to be important in the cooperative conformational change in PMA since such a sharp conformational change has not been reported for poly(acrylic acid) which has a structure similar to PMA but does not have methyl groups.[1] Breakdown of the delicate balance between the attractive forces due to hydrogen bonding and hydrophobic interaction and the repulsive force due to electrostatic interaction may trigger the cooperative conformational change of PMA.

References

1) T. S. Chen and J. K. Thomas, *J. Polym. Sci.: Part A-1*, **17**, 1103 (1979).

2) J. L. Koenig, A. C. Angood, J. Semen, and J. B. Lando, *J.*

Am. Chem. Soc., **91**, 7250 (1969).
3) J. B. Lando, J. L. Koenig, and J. Semen, *J. Macromol. Sci.-Phys.*, **B7**, 319 (1973).
4) R. Arnold, *J. Colloid Sci.*, **12**, 549 (1957).
5) M. Mandel and J. C. Leyte, *J. Polym. Sci.*, **56**, S23 (1962).
6) P. Dubin and U. P. Strauss, *J. Phys. Chem.*, **71**, 2757 (1967).
7) P. Dubin and U. P. Strauss, *J. Phys. Chem.*, **74**, 2842 (1970).
8) P. Dubin and U. P. Strauss, *J. Phys. Chem.*, **77**, 1427 (1973).
9) A. Katchalsky and H. Eisenberg, *J. Polym. Sci.*, **6**, 145 (1951).
10) A. Silberberg, J. Eliassaf, and A. Katchalsky, *J. Polym. Sci.*, **23**, 259 (1957).
11) Y. Muroga, I. Noda, and M. Nagasawa, *Macromolecules*, **18**, 1580 (1985).
12) M. Nagasawa, T. Murase, and K. Kondo, *J. Phys. Chem.*, **69**, 4005 (1965).
13) P. J. Kay, D. P. Kelly, G. I. Milgate, and F. E. Treloar, *Macromol. Chem.*, **177**, 885 (1976).
14) M. Mandel, J. C. Leyte, and M. G. Stadhouder, *J. Phys. Chem.*, **71**, 603 (1967).
15) J. C. Leyte, L. H. Zuiderweg, and H. J. Vledder, *Spectrochim. Acta*, **23A**, 1397 (1967).
16) A. M. Liquori, G. Barone, V. Crescenzi, F. Quadrifoglio, and V. Vitagliano, *J. Macromol. Chem.*, **1**, 291 (1966).
17) E. V. Anufrieva, T. M. Birshtein, T. N. Nekrasova, O. B. Ptitsyn, and T. V. Sheveleva, *J. Polym. Sci.: Part C*, **16**, 3519 (1968).
18) D-Y. Chu and J. K. Thomas, *Macromolecules*, **17**, 2142 (1984).
19) D-Y. Chu and J. K. Thomas, *J. Phys. Chem.*, **89**, 4065 (1985).
20) B. Bednar, H. Morawetz, and J. A. Shafer, *Macromolecules*, **18**, 1940 (1985).
21) Y. Wang and H. Morawetz, *Macromolecules*, **19**, 1925 (1986).
22) J. A. Delaire, M. A. J. Rodgers, and S. E. Webber, *J. Phys. Chem.*, **88**, 6219 (1984).
23) R. D. Stramel, C. Nguyen, S. E. Webber, and M. A. J. Rodgers, *J. Phys. Chem.*, **92**, 2934 (1988).
24) K. L. Tan and F. E. Treloar, *Chem. Phys. Lett.*, **73**, 234 (1980).
25) K. P. Ghiggino and F. E. Tan, in "Polymer Photophysics," ed by D. Phillips, Chapman and Hall, London (1985), p. 341.
26) B. Bednar, J. Trnena, P. Svoboda, S. Vadja, V. Fidler, and K. Prochazka, *Macromolecules*, **24**, 2054 (1991).
27) I. Soutar and L. Swanson, *Macromolecules*, **27**, 4304 (1994).
28) V. Crescenzi, F. Quadrifoglio, and F. Delben, *J. Polym. Sci.: Part A-2*, **10**, 357 (1972).
29) F. Yamashita and J. C. T. Kwak, *J. Polymer Sci.: Part B: Polym. Phys.*, **25**, 1395 (1987).
30) H. Morawetz, *Macromolecules*, **29**, 2689 (1996).
31) L. Bardet, G. Cassanas-Fabre, and M. Alain, *J. Mol. Struct.*, **24**, 153 (1975).
32) P. F. Krause, J. E. Katon, J. M. Rogers, and D. B. Philips, *Appl. Spectrosc.*, **31**, 110 (1977).
33) P. C. Painter, B. A. Brozoski, and M. M. Coleman, *J. Polym. Sci.: Part B: Polym. Phys.*, **20**, 1069 (1982).
34) M. Picquart, T. Lefevre, and G. Lacrampe, *Appl. Spectrosc.*, **49**, 1268 (1995).
35) D. Tsiourvas, C. M. Paleos, J. Anastassopoulou, and T. Theophanides, *Appl. Spectrosc.*, **49**, 1311 (1995).
36) M. M. Coleman, J. F. Graf, and P. C. Painter, "Specific Interactions and the Miscibility of Polymer Blends," Technoimic Publishing Inc., Lancaster, PA (1991), p. 328.
37) J. Dong, Y. Ozaki, and K. Nakashima, *Macromolecules*, **30**, 1111 (1997).
38) J. Dong, Y. Ozaki, and K. Nakashima, *J. Polym. Sci: Part B: Polym. Phys.*, **35**, 507 (1997).
39) D. V. O'Connor and D. Phillips, "Time-Correlated Single Photon Counting," Academic Press, London (1984).
40) K. Nakashima and M. Koyanagi, *Photochem. Photobiol.*, **44**, 169 (1986).
41) F. M. Winnik, *Chem. Rev.*, **93**, 587 (1993).
42) A. Nakajima, *Bull. Chem. Soc. Jpn.*, **44**, 3272 (1971).
43) K. Kalyanasundaram and J. K. Thomas, *J. Am. Chem. Soc.*, **99**, 2039 (1977).
44) D. C. Dong and M. A. Winnik, *Photochem. Photobiol.*, **35**, 17 (1982).
45) K. Nakashima, M. A. Winnik, K. H. Dai, E. J. Kramer, and J. Washiyama, *Macromolecules*, **25**, 6866 (1992).
46) J. R. Lakowicz, "Principles of Fluorescence Spectroscopy," Plenum, New York (1986), Chap. 2.
47) K. Nakashima, T. Anzai, and Y. Fujimoto, *Langmuir*, **10**, 658 (1994).
48) I. Soutar, L. Swanson, F. G. Thorpe, and C. Zhu, *Macromolecules*, **29**, 918 (1996).
49) G. Weber, *Adv. Protein Chem.*, **8**, 415 (1953).
50) M. A. Shinitzky, A. C. Dianoux, C. Gitler, and G. Weber, *Biochemistry*, **10**, 2106 (1971).
51) N. B. Colthap, L. H. Daly, and S. E. Wiberley, "Introduction to Infrared and Raman Spectroscopy," 3rd ed, Academic Press, Inc., New York (1990), p. 289.
52) The COOH band loses its intensity almost linearly as pH is increased, whereas the COO^- band acquires the intensity sinusoidally. The reason for the inharmonious changes of the intensities of the two bands is currently unclear.