(3) Brant, D. A.; Dimpfl, W. L. *Macromolecules* **1970**, *3*, 655.
(4) Kurata, M.; Stockmayer, W. H. *Fortschr. Hochpolym. Forsch.* **1963**, *3*, 196.
(5) Burchard, W. In "Solution Properties of Natural Polymers"; The Chemical Society: London, 1968; p 135.
(6) Friebolin, H.; Frank, N.; Keilich, G.; Siefert, E. *Makromol. Chem.* **1976**, *177*, 845.
(7) Colson, P.; Jennings, J. J.; Smith, I. C. P. *J. Am. Chem. Soc.* **1974**, *96*, 8081.
(8) Allerhand, A. *Pure Appl. Chem.* **1975**, *41*, 247.
(9) Allerhand, A.; Doddrell, D. *J. Am. Chem. Soc.* **1971**, *93*, 2777.
(10) Selenew, J. W.; Glaskow, W. I. *Plaste Kautschuk.* **1976**, *23*, 571.
(11) Lelievre, J.; Mitchell, J. *Stärke* **1975**, *27*, 113.
(12) Hennig, H. J.; Lechert, H. *Stärke* **1974**, *26*, 232.
(13) Fedin, E. I.; Tsitsishvili, V. G.; Grinberg, V. Ya.; Bakari, T. I.; Tolstoguzov, V. B. *Carbohydr. Res.* **1975**, *39*, 193.
(14) Levy, G. C.; Nelson, G. L. "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists"; Wiley-Interscience: New York, 1972; p 161.
(15) Saito, H.; Miyata, E.; Sasaki, T. *Macromolecules* **1978**, *11*, 1244.
(16) Saito, H.; Ohki, T.; Sasaki, T. *Biochemistry* **1977**, *16*, 908.
(17) Saito, H.; Ohki, T.; Takasuka, N.; Sasaki, T. *Carbohydr. Res.* **1977**, *58*, 293.
(18) (a) Pfannemüller, B.; Husemann, E. *Makromol. Chem.* **1961**, *49*, 214. (b) Husemann, E.; Fritz, B.; Lippert, R.; Pfannemüller, B.; Schupp, E. *Ibid.* **1958**, *26*, 181.
(19) Jones, A. A.; Stockmayer, W. H. *J. Polym. Sci., Polym. Phys. Ed.* **1977**, *15*, 847.
(20) Valeur, B.; Jarry, J.-P.; Gény, F.; Monnerie, L. *J. Polym. Sci., Polym. Phys. Ed.* **1975**, *13*, 667.
(21) Bendler, J. T.; Yaris, R. *Macromolecules* **1978**, *11*, 650.
(22) Jones, A. A.; Robinson, G. L.; Gerr, F. E., *ACS Symp. Ser.* **1979**, *No. 103*, 271.
(23) Hall, C. K.; Helfand, E. *J. Chem. Phys.* **1982**, *77*, 3275.
(24) Skolnick, J.; Helfand, E. *J. Chem. Phys.* **1980**, *72*, 5498.
(25) Viovy, J. L., private communication, 1983.
(26) Viovy, J. L., Frisch, H. L., private communication, 1983.
(27) Viovy, J. L.; Monnerie, L.; Brochon, J. C. *Macromolecules* **1983**, *16*, 1845.
(28) Wittebort, R. J.; Szabo, A. *J. Chem. Phys.* **1978**, *69*, 1722.
(29) Riddick, J.; Bunger, W. B. "Organic Solvents", 3rd ed.; Wiley-Interscience: New York, 1970; p 466.
(30) Burchard, W. *Makromol. Chem.* **1963**, *64*, 110.
(31) Casu, B.; Reggiani, M.; Gallo, G. G.; Vigevani, A. *Tetrahedron* **1966**, *22*, 3061. "Solution Properties of Natural Polymers"; The Chemical Society: London, 1968; p 217.
(32) Walton, A. G.; Blackwell, J.; Carr, S. H. "Biopolymers"; Academic Press: New York, 1973; pp 63, 499.

Poly(methacrylic acid) Derivatives. 8. Effects of Solvent- and pH-Induced Conformational Transition on the Chiroptical Properties of Hydrophilic–Hydrophobic Poly(*N*-methacryloyl-L-alanine-*co*-*N*-phenylmethacrylamide) Copolymers

Joelle Morcellet-Sauvage, Michel Morcellet,* and Claude Loucheux

Laboratoire de Chimie Macromoleculaire, LA No. 351 du CNRS, Université des Sciences et Techniques de Lille, 59655 Villeneuve d'Ascq Cédex, France. *Received May 4, 1983*

ABSTRACT: CD spectra of poly(*N*-methacryloyl-L-alanine) (PNMA), of its model molecule *N*-isobutyryl-L-alanine (NIBA), and of a random copolymer of *N*-methacryloyl-L-alanine and *N*-phenylmethacrylamide (P50) were recorded in water at different values of the degree of neutralization $\bar{\alpha}$ of the carboxylic functions. Upon ionization, PNMA behaves like a normal polyelectrolyte and undergoes a progressive expansion of the macromolecular conformation. On the contrary, P50 shows a transition from a compact conformation at $\bar{\alpha} < 0.3$ to a more expanded conformation at $\bar{\alpha} > 0.7$. These differences in the conformational behavior of PNMA and P50 were not reflected in the chiroptical properties. The changes in the CD spectra upon neutralization are mainly attributed to the perturbation of all the electronic transitions (carboxyl, amide, and aromatic chromophores) of both kinds of residues due to the ionization of the carboxyl group.

Introduction

During the past decades, it has been widely demonstrated that the chiroptical properties of poly(α-amino acids), proteins, polynucleotides, etc. are very sensitive to the existence of secondary structures in solution and they have been used to study conformational transitions such as the helix-to-coil transition.[1] More recently, other kinds of synthetic optically active polymers have been synthesized and studied.[2-4] In some cases, these polymers are highly stereoregular and the presence of ordered structure is likely. The chiroptical properties may then be interpreted in terms of macromolecular conformation.[5]

Nevertheless, in most cases, synthetic optically active polymers are stereoirregular and no ordered structure can be predicted. Chiroptical properties are then mainly dependent on the effect of external factors, e.g., solvent, temperature, added salt, ionization, etc.,[3,4] on the different chromophores present in the macromolecule. When dealing more especially with polyelectrolytes, an interesting case is that of hydrophobic–hydrophilic polyelectrolytes in which the balance between the hydrophobic cohesive interactions and the repulsive electrostatic interactions governs the conformation taken by the polyelectrolyte in aqueous solution.[6-8] At low values of the degree of ionization $\bar{\alpha}$ and when the amount and/or the size of the hydrophobic groups is sufficient, the cohesive interactions are predominant and the macromolecule takes a compact, tightly coiled conformation in which the hydrophobic groups are clustered in microdomains without contact with water. As $\bar{\alpha}$ increases, the repulsive electrostatic interactions also increase, leading to the unfolding of the compact conformation. This well-known compact → coil conformational transition may also be induced by addition of an organic solvent to the aqueous solution in order to weaken the hydrophobic interactions.

In recent years, a number of papers have been devoted to the study of such hydrophobic polyelectrolytes, having a chiral center in their side chain, in order to determine whether or not their special conformational behavior is reflected in the chiroptical properties (optical rotatory

0024-9297/84/2217-0452$01.50/0

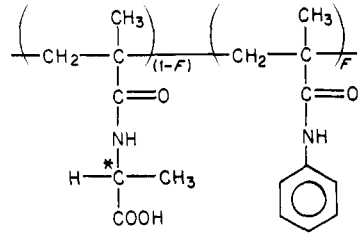
dispersion (ORD) and circular dichroism (CD)). Braud and Vert[9] reported the case of optically active acrylic acid/*N*-*sec*-butyl-*N*-methylacrylamide random copolymers, for which the hydrophobicity depends on the content of the hydrophobic amide repeat unit. Copolymers with less than 20% of hydrophobic groups behave like "normal" polyelectrolytes, while those with greater hydrophobic character take a compact conformation at low $\bar{\alpha}$ values. The CD spectra of different copolymers were studied and no particular CD feature was found to be connected with the existence of the compact conformation.[9] More recently, a similar study was reported by Villiers et al. concerning a series of alternating maleic acid/optically active alkyl vinyl ether copolymers, the conformations of which depend on the length of the alkyl side chain.[10] In this case also, no correlation was found between the conformational behavior and the pH-induced optical activity changes.

In a previous paper,[11] we studied the chiroptical properties of stereoirregular random copolymers of *N*-methacryloyl-L-alanine (chiral unit) and *N*-phenylmethacrylamide (achiral unit). These copolymers behave like normal polyelectrolytes in water when the mole fraction *F* of the hydrophobic achiral residue is lower than 15%. Beyond this value a compact conformation exists and a compact–coil conformational transition may be induced by increasing the pH.[8] In methanol no compact conformation exists, even for the more hydrophobic copolymers.[8,12] Despite these different types of conformational behavior, the chiroptical properties are rather similar in water and methanol.

In this paper we report a detailed study of the effect of the conformational transition of one of these copolymers on its chiroptical properties. Both the pH-induced and the methanol-induced transition will be considered. Only the results of CD measurements will be given here.

Experimental Section

Samples. The samples of poly(*N*-methacryloyl-L-alanine) (PNMA), the model molecule, *N*-isobutyryl-L-alanine (NIBA), and the *N*-methacryloyl-L-alanine/*N*-phenylmethacrylamide copolymers are the same as those used in the previous paper.[11] The copolymer used in this study will be referred to hereafter as P50, F = 0.50 being the mole fraction of the *N*-phenylmethacrylamide residue.



Solutions. PNMA, NIBA, and the copolymer P50 are readily soluble in methanol. PNMA and NIBA are also soluble in water. Copolymers are not directly soluble in water when *F* is higher than 0.15.[8] Thus in the case of P50 the sample is first dissolved in an aqueous sodium hydroxide solution and then percolated through a cation-exchange column in the acid form (Amberlite IR 120). For the study of the methanol-induced changes, methanol was added to a stock aqueous solution of the sample. The composition of the mixture is given as the volume percent of methanol. For the study of the pH-induced changes, the aqueous solutions were neutralized to various degrees of neutralization $\bar{\alpha}$ with aqueous 0.1 N NaOH ($\bar{\alpha}$ = equivalents of NaOH per equivalent of COOH) and then adjusted to the desired concentration (from 5×10^{-3} to 2×10^{-2} M).

Measurements. pH values were measured with a Radiometer pH M 65 pH meter.

CD spectra were recorded with a Jobin-Yvon Mark III dichrograph flushed with dry nitrogen. Quartz cells with optical path lengths of 0.1, 0.5, and 1 mm were used. The molar ellipticities $[\theta]$ were expressed in the usual units (deg·cm^2·dmol^{-1}), using for the calculation the mean residue weight M_r of the sample under study.

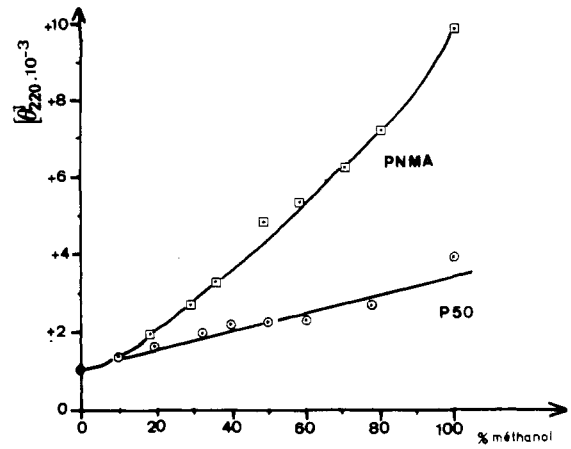


Figure 1. Variation of the molar ellipticity at 220 nm for PNMA and P50 in water–methanol mixtures.

The compact-to-random coil conformational transition of P50 was previously studied by a number of methods, among which were potentiometric and conductimetric titrations.[8,13] In some figures the variation of F_r vs. $\bar{\alpha}$ is plotted, where F_r is the fraction of the polymer in the random coil conformation. For P50, most of the transition occurs between $\bar{\alpha} = 0.3$ and $\bar{\alpha} = 0.7$.

Results and Discussion

The effect of methanol or ionization on the chiroptical properties of PNMA, NIBA, and P50 was studied in order to see whether the conformational transition of P50 can be detected by measurements of optical activity.

(A) Effect of Methanol. In water, PNMA takes a random coil conformation. Upon addition of methanol, no conformational transition occurs.[8] For P50, on the contrary, a compact conformation exists in water and a conformational transition to a more expanded coil occurs on adding methanol. This transition is completed at 57 vol % methanol.

Figure 1 shows the variation of the molar ellipticity at $\lambda = 220$ nm as a function of the composition of the solvent mixture. At 220 nm are located the $\pi_1 \rightarrow \pi^*$ transition of the amide chromophore and the $n \rightarrow \pi^*$ transition of the carboxyl chromophore in the *N*-methacryloyl-L-alanine residue.[11] As shown by Figure 1, $[\theta]_{220}$ increases monotonically both for PNMA and for P50. Thus, the methanol-induced transition of P50 has no significant influence on its chiroptical properties.

(B) Effect of Ionization in Water. As stated above, PNMA behaves as a normal polyelectrolyte in water, and the ionization of the side-chain carboxyl groups only results in a stretching of the macromolecular conformation in the absence of added salt. For P50, an increase of the degree of neutralization $\bar{\alpha}$ yields a pH-induced transition, which occurs between $\bar{\alpha} = 0.3$ and $\bar{\alpha} = 0.7$.[13]

Figures 2–5 show the CD spectra of NIBA, PNMA, and P50 at different values of the degree of neutralization $\bar{\alpha}$. For NIBA (Figure 2), the ionization process induces a strong red shift of the positive 215-nm band, which is related to the amide $\pi_1 \rightarrow \pi^*$ and carboxyl $n \rightarrow \pi^*$ transitions.[11] The negative 195-nm band (amide $\pi_2 \rightarrow \pi^*$ or $n \rightarrow \sigma^*$ transition) is less affected by ionization. As shown previously[11] the effect of ionization is maximum around 207 nm, which is the location of the $n \rightarrow \pi^*$ transition of the carboxyl chromophore.[10,14-16]

For PNMA (Figure 3) the effect of ionization is less important: the red shift of the 220-nm band is only 7 nm

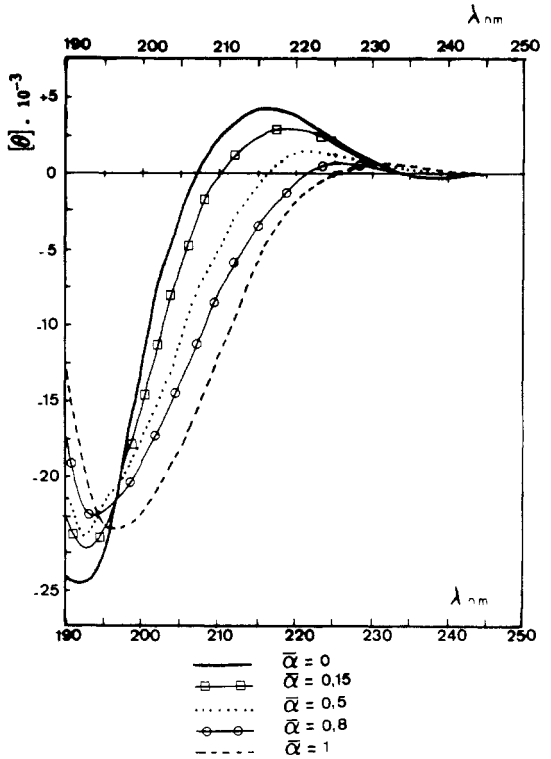


Figure 2. CD spectra of NIBA at different degrees of neutralization $\bar{\alpha}$ as indicated.

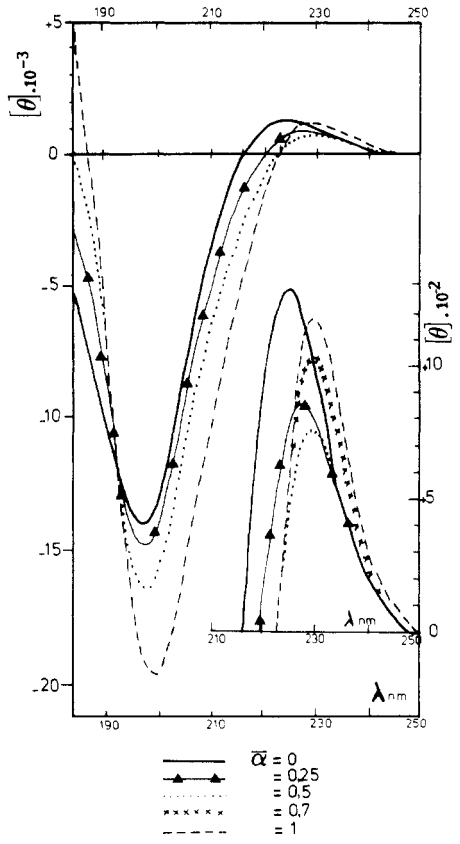


Figure 3. CD spectra of PNMA at different degrees of neutralization $\bar{\alpha}$ as indicated.

(compared to 17 nm for NIBA) and the intensity does not change appreciably. This difference is probably due to the fact that the $\pi_1 \rightarrow \pi^*$ amide transition is located at higher wavelength for PNMA than for NIBA[11] whereas the n $\rightarrow$ π^* carboxyl transition is at the same wavelength as that for NIBA (the effect of ionization is maximum at 207 nm,

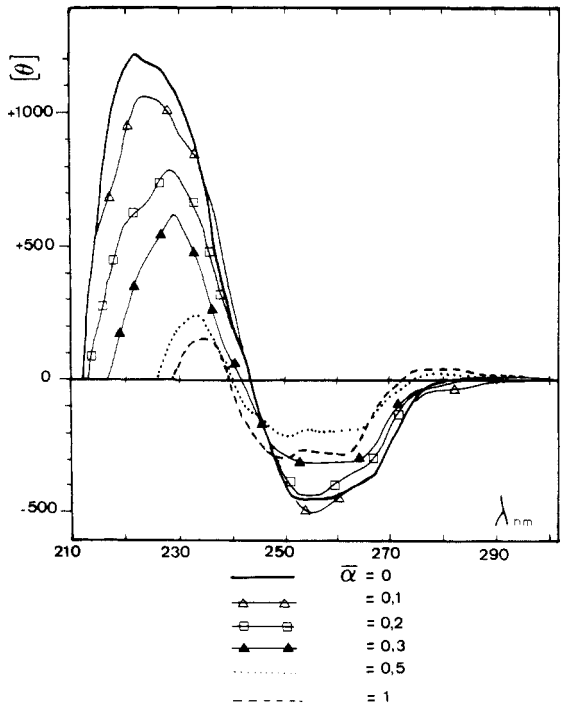


Figure 4. CD spectra of P50 between 210 and 310 nm at different degrees of neutralization $\bar{\alpha}$ as indicated.

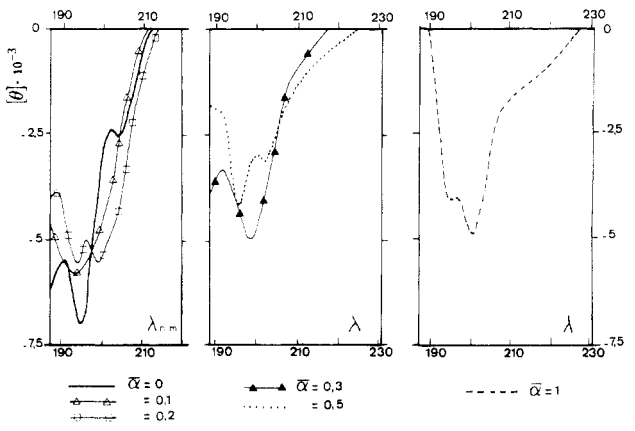


Figure 5. CD spectra of P50 between 190 and 230 nm at different degrees of neutralization $\bar{\alpha}$ as indicated.

too[11]). In addition, the electrostatic interactions, which are important in ionized PNMA, probably play a major role.

In the case of P50 (Figures 4 and 5), the ionization process also induces a red shift (13 nm) and a strong decrease of the intensity of the 200-nm band (as for NIBA). The 255-nm band, which has been attributed to induced optical activity in the ^1L_A transition of the aromatic chromophore,[11] remains at the same wavelength but its intensity decreases to half its initial value at $\bar{\alpha} = 0$. The 190-nm band is slightly red shifted and its intensity decreases, especially between $\bar{\alpha} = 0$ and $\bar{\alpha} = 0.2$ (Figure 5).

Figure 6 shows the difference spectra between P50 at $\bar{\alpha}$ and P50 at $\bar{\alpha} = 0$. The electronic transitions that are perturbed by ionization appear clearly in this figure: first, the perturbations in the aromatic chromophore at 255 nm (^1L_A transition) and 200 nm (^1B transition); second, the electronic transitions of the amide chromophore near 220 nm ($\pi_1 \rightarrow \pi^*$ transition) and 190–195 nm ($\pi_2 \rightarrow \pi^*$ or n $\rightarrow$ σ^* transition); third, the electronic transitions of the carboxyl chromophore, which are, in the case of P50, overlapped by the presence of the amide (n $\rightarrow$ π^* transition near 207 nm (as shown with NIBA and PNMA)[11] and π

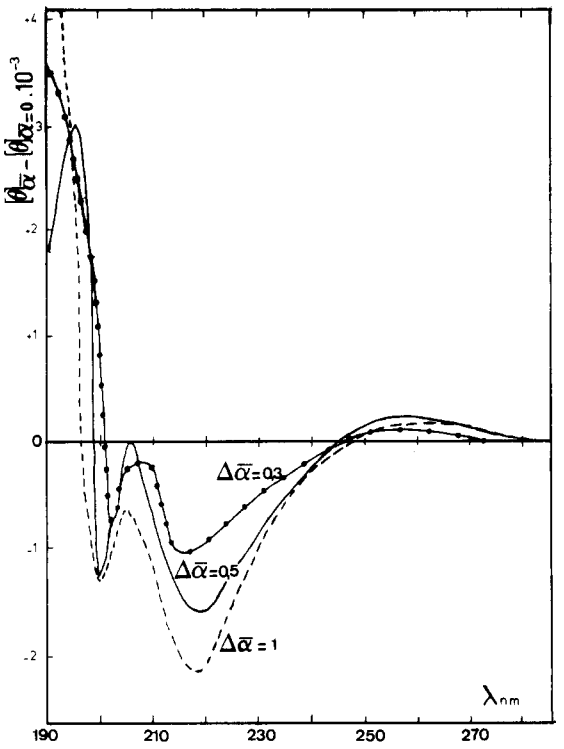


Figure 6. Difference CD spectra $\Delta[\theta] = f(\lambda)$ for P50: $\Delta[\theta] = [\theta]_{\bar{\alpha}} - [\theta]_{\bar{\alpha}=0}$.

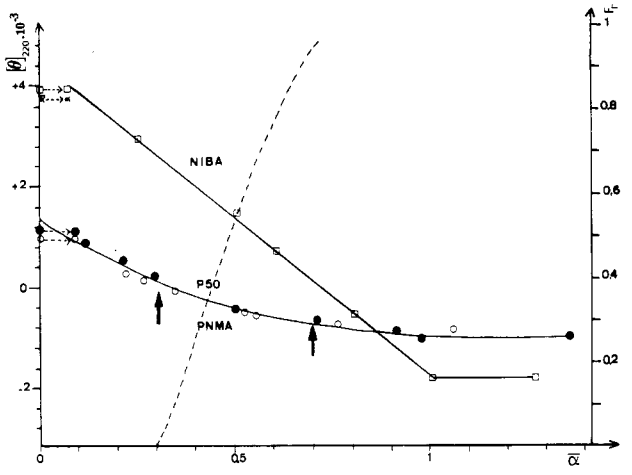


Figure 7. Variation of the molar ellipticity at 220 nm as a function of $\bar{\alpha}$ for NIBA (□), PNMA (○), and P50 (●). Dotted arrows around $\bar{\alpha} = 0$ indicate that $[\theta]$ values recorded in pure water (no added base) actually correspond to $\bar{\alpha} = 0.08$ due to the autodissociation of the carboxyl groups. Dotted line: Variation of the fraction of random conformation (F_r) for P50 (thick arrows indicate the beginning and the end of the conformational transition).

→ π* transition below 195 nm). Therefore, all the optically active electronic transitions of the copolymer are perturbed by ionization.

The change of the molar ellipticity $[\theta]$ when the carboxyl groups are ionized is shown in Figures 7–9 for NIBA, PNMA, and P50 at fixed wavelengths. The molar ellipticity of NIBA at 220 nm (Figure 7) decreases linearly between $\bar{\alpha} = 0.1$ and $\bar{\alpha} = 1$. The deviation from linearity below $\bar{\alpha} = 0.1$ is due to autoionization of the weak acid. Beyond $\bar{\alpha} = 1$, i.e., during the addition of an excess of sodium hydroxide, the ionization state of the molecule does not change and its optical activity remains constant. The behavior of NIBA is very similar to that of *N*-pivaloyl-D-alanine and *N*-isobutyryl-L-asparagine, for which it has been shown[17-19] that the molar rotation at 589 nm also

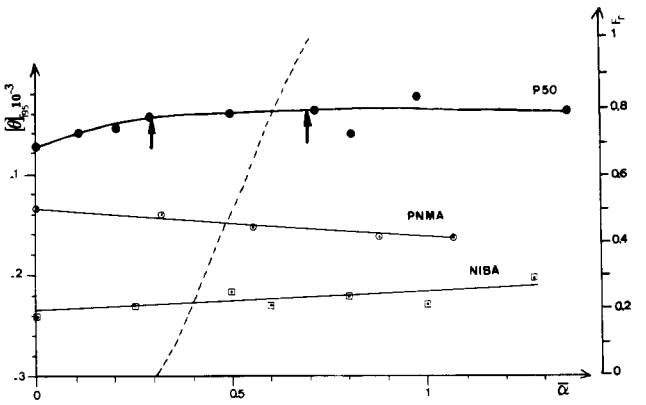


Figure 8. Variation of the molar ellipticity at 195 nm as a function of $\bar{\alpha}$ for NIBA (□), PNMA (○), and P50 (●). Dotted line: Variation of F_r, the fraction of random conformation, vs. $\bar{\alpha}$ for P50 (thick arrows indicate the beginning and the end of the conformational transition).

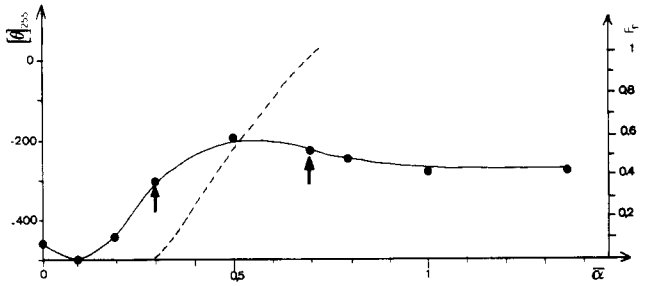


Figure 9. Variation of the molar ellipticity at 255 nm as a function of $\bar{\alpha}$ for P50. Dotted line: Variation of F_r, the fraction of random conformation, vs. $\bar{\alpha}$ for P50 (thick arrows indicate the beginning and the end of the conformational transition).

changes linearly with $\bar{\alpha}$. This is explained by the contribution of two independent optically active species to the molar rotation or molar ellipticity. The molar ellipticity of NIBA at 195 nm is almost constant (Figure 8).

For PNMA, $[\theta]_{195}$ decreases linearly between $\bar{\alpha} = 0$ and $\bar{\alpha} = 1$. At 220 nm, $[\theta]$ decreases monotonously and nonlinearly down to $\bar{\alpha} = 1$. No variation is observed beyond $\bar{\alpha} = 1$, although the conformation of the polymer continues to change because of the decrease in electrostatic interactions due to the screening effect of excess ions. Thus, the changes between $\bar{\alpha} = 0$ and $\bar{\alpha} = 1$ cannot be attributed to the macromolecular expansion but result from the perturbation of the chromophores by ionization. Results obtained with P50 are striking. The variation of $[\theta]_{220}$ is exactly the same as for PNMA, though P50 undergoes a conformational transition between $\bar{\alpha} = 0.3$ and $\bar{\alpha} = 0.7$ (arrows in Figure 7). We are thus led to conclude that, in the case of our polymers, the optical activity is completely independent of the conformational behavior. The same conclusion may be drawn from the variation of $[\theta]_{195}$ with $\bar{\alpha}$ (Figure 8).

At 220 and 195 nm, the main contributions to the optical activity of P50 are those of the carboxyl and amide chromophores, i.e., mainly electronic transitions of the chiral hydrophilic unit. It is thus interesting to examine whether the electronic transitions of the aromatic chromophore are more sensitive to the conformational changes since the hydrophobic side chains of the aromatic residue are engaged in intramolecular interactions when the compact conformation is stable. Figure 9 shows the effect of ionization on the molar ellipticity at 255 nm, the wavelength where the main contribution is the induced optical activity in the ^1L_A transition of the aromatic chromophore. $[\theta]_{255}$ increases up to $\bar{\alpha} = 0.5$ and then remains nearly constant. The conformational transition of P50, which occurs be-

tween $\bar{\alpha} = 0.3$ and $\bar{\alpha} = 0.7$ (arrows in Figure 9), has no direct influence on its optical properties. $[\theta]_{255}$ also remains constant beyond $\bar{\alpha} = 1$.

Conclusion

The optical activity of the model molecule NIBA changes linearly with $\bar{\alpha}$, which is normal for a mixture of two optically independent species, the ionized and un-ionized molecules. For PNMA, on the contrary, the two optically active species are not independent. The optical activity of each species (ionized or un-ionized residue) depends on the state of ionization of the vicinal units[20] and thus on the degree of ionization of the whole macromolecule. Nonlinear variations of $[\theta]$ with $\bar{\alpha}$ are thus observed. From this point of view, the homopolymer of PNMA at a given $\bar{\alpha}$ may be considered to be a statistical copolymer of ionized and un-ionized units. The conformation of the polymer plays no important role, as shown by the fact that the molar ellipticity remains constant for $\bar{\alpha} > 1$ whereas the conformation continues to change.

P50 may be considered as a terpolymer of aromatic achiral units, chiral ionized units, and chiral un-ionized units. In addition to the effects of the neighboring units as in PNMA, an extra optical activity induced by the chiral center in the aromatic residue does exist.[11] Thus, the variation of the molar ellipticity is also nonlinear when P50 is ionized. As no peculiar change is observed in the variation of $[\theta]$ at 220, 195, and 255 nm in the range of $\bar{\alpha}$ values where the conformational transition of P50 occurs, it is concluded that the optical activity is completely independent of the conformational behavior for this random stereoirregular copolymer.

Therefore, the main contribution to the optical activity changes is due to the ionization of the carboxyl group, which affects all the electronic transitions of the polymer (carboxyl, amide, and aromatic chromophores).

Acknowledgment. We are grateful to J. P. Aubert, C. Denis, and M. P. Hildebrand (Institut de Recherches sur le Cancer de Lille, France) for having recorded the CD spectra. Thanks are also due to Dr. M. H. Loucheux-Lefebvre and Dr. M. Vert for very helpful discussions.

Registry No. PNMA, 77349-79-2; NIBA, 81524-47-2; P50, 77349-80-5.

References and Notes

(1) See, for example, G. D. Fasman, Ed., "Poly-α-amino Acids: Protein Models for Conformational Studies"; Marcel Dekker, New York, 1967.
(2) M. Vert and E. Selegny, *J. Polym. Sci., Polym. Symp.*, **No. 42**, 1239 (1973).
(3) M. Vert in "Charged and Reactive Polymers: Polyelectrolytes", Vol. 1, E. Selegny, Ed., D. Reidel Publishing Co., Dordrecht, Holland, 1974, pp 347–370.
(4) M. Vert in "Charged and Reactive Polymers: Optically Active Polymers", Vol. 5, E. Selegny, Ed., D. Reidel Publishing Co., Dordrecht, Holland, 1979, pp 291–316.
(5) P. Pino, F. Ciardelli, and M. Zandomeneghi, *Annu. Rev. Phys. Chem.*, **21**, 561 (1970).
(6) P. L. Dubin and U. P. Strauss, *J. Phys. Chem.*, **74**, 2842 (1970).
(7) G. Muller, J. C. Fenyo, C. Braud, and E. Selegny in "Charged and Reactive Polymers: Polyelectrolytes and Their Applications", Vol. 2, A. Rembaum and E. Selegny, Eds., D. Reidel Publishing Co., Dordrecht, Holland, 1975, pp 15–30.
(8) J. Morcellet-Sauvage, M. Morcellet, and C. Loucheux, *Makromol. Chem.*, **182**, 949 (1981).
(9) C. Braud and M. Vert, *Macromolecules*, **11**, 448 (1978).
(10) C. Villiers, C. Braud, M. Vert, and E. Chiellini, *Macromolecules*, **12**, 103 (1979).
(11) J. Morcellet-Sauvage, M. Morcellet, and C. Loucheux, *Macromolecules*, **16**, 1564 (1983).
(12) J. Morcellet-Sauvage, Thesis, Lille, France, 1982.
(13) J. Morcellet-Sauvage, M. Morcellet, and C. Loucheux, *Makromol. Chem.*, **183**, 831 (1982).
(14) C. Braud and M. Vert, *Polym. J.*, **12**, 785 (1980).
(15) V. Madison and J. Schellman, *Biopolymers*, **9**, 511 (1970).
(16) H. H. Jaffe and M. Orchin in "Theory and Applications of U.V. Spectroscopy"; Wiley, New York, 1962, p 180.
(17) R. K. Kulkarni and H. Morawetz, *J. Polym. Sci.*, **54**, 491 (1961).
(18) H. Morawetz, "Macromolecules in Solution", Interscience, New York, 1965, p 241.
(19) F. B. Brunschtein, *Diss. Abstr.*, **26**, 1931 (1965).
(20) C. Braud and M. Vert, *Polymer*, **16**, 115 (1975).