Articles

Optically Active Polyelectrolytes with Variable Hydrophobicity. 2. Effects of pH-Induced Chromophore and Conformation Changes on Chiroptical Properties of Acrylic Acid/(+)-*N*-(*sec*-Butyl)-*N*-methylacrylamide Copolymers

Christian Braud and Michel Vert*

Laboratoire de Chimie Macromoléculaire, ERA CNRS No. 471, Faculté des Sciences et des Techniques, Université de Rouen-Haute Normandie, 76130 Mont-Saint-Aignan, France.

Received February 23, 1978

ABSTRACT: CD of COP 62 and COP 90.5 (two random copolymers of acrylic acid and (+)-*N*-(*sec*-butyl)-*N*-methylacrylamide having respectively 62 and 90.5% of acid monomeric units) was studied in water at different values of the degree of ionization, α, of these polyelectrolytic macromolecules. As acidic groups progressively ionized, COP 90.5 behaved like a normal polyelectrolyte while COP 62 showed a transition from compact conformations ($\alpha < 0.33$) to more or less extended ones for higher values of α. Differences in conformational behavior of these two nonstereoregular optically active polyelectrolytes were not reflected in the chiroptical properties. Both copolymers showed similar CD spectra mainly due to chiral amide repeating units. Small variations observed as α varied arose from the ionization reaction $COOH \rightarrow COO^-$. The possibility for some of the carboxylic chromophores being optically active when belonging to repeating units adjacent to chiral amide ones is discussed.

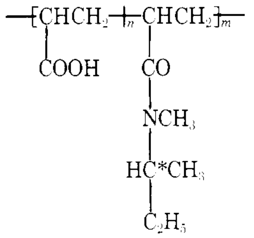
pH-induced chiroptical property changes of biopolymers (poly(α-amino acids), polynucleotides , . . .) have been correlated with secondary structures in solution and used for studying conformational transitions, either between different ordered macromolecular conformations or from ordered to desordered ones as the so-called helix-to-coil transition of poly(α-amino acids).[1]

For the last 30 years, optically active polymers of other types were synthetized using different methods.[2] The interpretation of their chiroptical properties raised two different problems. The first one was to correlate optical activity in a given medium to chirality and macromolecular conformations, especially when ordered secondary structures are likely, as for highly stereoregular polymers.[3] The second one was to explain chiroptical property changes when parameters like temperature, solvent, salt concentration, etc., are modified. Resulting conformation changes can account for the perturbation of optically active chromophores but usually chemical modifications have also to be considered.[4] The latter can become predominant if no ordered secondary structure exists in solution as for macromolecules of low stereoregularity.[4] Relative effects of both sources of chromophore perturbations have to be considered to explain optical activity changes of polymers undergoing a chemical reaction, which usually causes simultaneous conformational and chemical modifications.

A special case is found for some polyelectrolytes which show a particular conformational behavior when strongly interacting hydrophobic groups are present within the polymer backbone or side chains. For low values of the degree of ionization α, compact disordered conformations are stabilized by short-range interactions which balance the electrostatic repulsions of ionized groups.[5] Beyond a certain value of α, compact conformations are no longer stable and a compact coil-to-extended coil transition is observed as α increases.[5] Whether this particular polyelectrolytic behavior is able to perturb chiroptical properties, and can hence be followed by ORD and CD, is unknown. Some contributions to these properties were found for an optically active polysulfonamide-type polycondensate of L-lysine and 1,3-benzenedisulfonyl chloride.[6] However, these were correlated with solvent effects on aromatic chromophores and not to changes in the macromolecular conformation.

In order to better understand chiroptical properties of optically active polyelectrolytes, we have undertaken investigations on a series of more or less hydrophobic optically active polyelectrolytes.

One type of such a polyelectrolyte has been found in random copolymers of acrylic acid and (+)-*N*-(*sec*-butyl)-*N*-methylacrylamide (COP *Y*), the latter repeating units introducing optical activity and variable hydrophobicity according to the polymer composition ($Y = 100n/(n + m)$):



$$-\!\!\left[\underset{\underset{COOH}{|}}{C}HCH_2\right]_n\!\!-\!\!\left[\underset{\underset{\underset{\underset{\underset{C_2H_5}{|}}{HC^*CH_3}}{|}}{\underset{NCH_3}{|}}}{\underset{CO}{|}}}{C}HCH_2\right]_m\!\!-$$

COP *Y*

0024-9297/78/2211-0448$01.00/0

The synthesis of these copolymers[7] and their polyelectrolytic behavior as a function of the hydrophobic group content[8,9] were reported previously. Copolymers having less than 20% acrylamide repeating units behaved like poly(acrylic acid) while copolymers with more than 20% of these hydrophobic groups showed compact conformations at low values of the degree of ionization.[8,9]

In this paper, we report a comparison of pH-induced optical-activity changes of copolymers having 62% (COP 62) and 90.5% (COP 90.5) of acrylic acid repeating units. The former shows compact-coil conformations for values of the degree of ionization lower than 0.33 while the latter behaves normally, i.e., without compact conformation at low α values.

Since both copolymers have similar chiral repeating units derived from the optically active (+)-*N*-(*sec*-butyl)-*N*-methylacrylamide, we expected to be able to distinguish the effect, if any, of the particular conformational behavior of COP 62 on optical activity by comparing chiroptical properties of both copolymers at different α values.

We first investigated the optical activity of COP 62 and COP 90.5 by ORD. However, very weak optical rotations were found for both in the visible part of the spectrum. Under these conditions, accurate ORD data would have needed larger quantities of each copolymer than those available. Accordingly, only CD measurements were carried out in detail and are reported here.

Experimental Section

Materials. COP 62 and COP 90.5 were synthesized via an acidic hydrolysis of (−)-homopoly[*N*-(*sec*-butyl)-*N*-methylacrylamide] according to a process previously described.[7]

Measurements. Acidic solutions of both copolymers ($C_{\text{cop}} \simeq 5 \times 10^{-3}$ M) were prepared from alkaline ones by successively percolating them through anion and cation exchange columns.[8] These stock solutions were neutralized to selected degrees of neutralization, $\bar{\alpha}$, with a 0.1 N KOH solution ($\bar{\alpha}$ = [equiv of KOH]/[equiv of COOH]) and then diluted to $C_{\text{cop}} = 2 \times 10^{-3}$ M. Corresponding pH values were measured by using a Radiometer PHM 52 pH meter while CD spectra were simultaneously recorded by using a Jasco J 40 B dichrometer at room temperature. Values of the degree of ionization were deduced from $\alpha = \bar{\alpha} + ([\text{H}^+] - [\text{OH}^-])/[\text{COOH}]$. CD data at λ 200 nm were obtained by setting the monochromator at this wavelength and not from CD spectra. Molar ellipticities were expressed in the usual units according to $[\theta] = (\theta)\bar{M}_r/lc$, where (θ) is the measured ellipticity (in degree) for a solution at concentration c (in g/100 cm^3) for path length l (in dm) and $\bar{M}_r$ is the mean residue weight (98.2 for COP 62 and 78.5 for COP 90.5).

Results and Discussion

CD spectra of COP 62 and COP 90.5 ($C_{\text{cop}} = 2 \times 10^{-3}$ M) in water were recorded at different pH values.

Although only 9.5% of the chiral amide repeating units remain within the COP 90.5 polymer chain, it was possible to get data as accurate as those obtained for COP 62 where 38% of the chiral units are present. Indeed, the n → π^* absorption band of the COOH chromophores in the 190–210-nm spectral range is about 50–60 times weaker than that of the $\pi \rightarrow \pi^*$ amide one located at the same location of the spectrum.[8,10] Accordingly, the lower molar absorption coefficient of COP 90.5 as compared to that of COP 62 allowed us to balance the decrease of the amide unit content (and thus that of optical activity) by an increase of path length, keeping convenient signal-to-noise ratios.

As suggested in the title of this paper, the chiroptical property changes we are dealing with are pH-induced. However, the pH is not a suitable variable to study these changes because it depends on a number of factors, especially polymer concentration, presence of salt, temperature, etc. A meaningful representation is obtained when the degree of ionization, α, or for weak acid as COP *Y*'s the degree of neutralization, $\bar{\alpha}$, is considered.

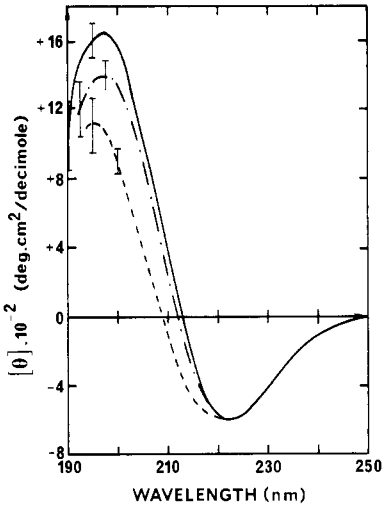


Figure 1. CD spectrum of COP 62 ($C_{\text{cop}} = 2 \times 10^{-3}$ M in water) as a function of the degree of neutralization $\bar{\alpha}$ (KOH): $\bar{\alpha}$ = 0 (—); $\bar{\alpha}$ = 0.5 (– • –); $\bar{\alpha}$ = 1.0 (- - -).

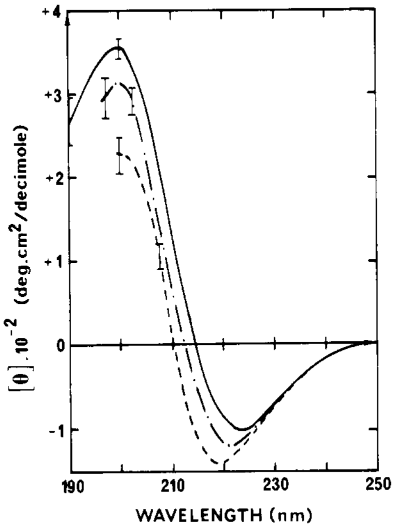


Figure 2. CD spectrum of COP 90.5 ($C_{\text{cop}} = 2 \times 10^{-3}$ M in water) as a function of the degree of neutralization $\bar{\alpha}$ (KOH): $\bar{\alpha}$ = 0 (—); $\bar{\alpha}$ = 0.5 (– • –); $\bar{\alpha}$ = 1.0 (- - -).

Accordingly, Figures 1 and 2 show CD spectra of COP 62 and COP 90.5 in aqueous solution at different values of the degree of neutralization $\bar{\alpha}$.

(a) CD at $\bar{\alpha}$ = 0. At $\bar{\alpha}$ = 0, i.e., when no alkaline reagent was added, both copolymers showed similar CD curves with a negative maximum at 223–224 nm and a positive one at 198–200 nm. The presence of two cotton effects of opposite sign accounted for the low optical rotations observed in the visible.[11] On the other hand, optical activity of COP 90.5 seemed to be much lower than that of COP 62.

Both magnitude and shape of these curves deserve some comment before considering the effects of ionization. In Figures 1 and 2, ellipticities were expressed in terms of mean residue weights as is usual for macromolecules composed of different monomeric units (such as proteins).[12] This means that the whole macromolecule (including chiral acrylamide and achiral acid repeating units) was considered as the optically active species. Another way of representation is to consider amide repeating units as the only optically active species, i.e., referring c and $\bar{M}_r$ to amide repeating units, acrylic acid repeating units being considered as independent of the amide residues and optically inactive. In this case, optical activity of COP *Y* must be proportional to the amide content, and

Table I
Molar Ellipticity $[\theta]_{220}$ of COP 62 and COP 90.5 Referred to Amide Monomeric Units

	λ, nm	Positive max $[\theta]$, 10 × deg cm^2/mol × 10^{-3}	λ, nm	Negative max $[\theta]$, 10 × deg cm^2/mol × 10^{-3}
COP 62	198	+6.2 ± 0.5	222	−2.3 ± 0.2
COP 90.5	198	+6.6 ± 0.3	222	−1.9 ± 0.1

molar ellipticities must be constant. Actually, molar ellipticities of COP *Y* expressed in the second way were found to be of the same order of magnitude but no longer independent of the copolymer composition as shown in Table I for COP 62 and COP 90.5.

This composition dependence of the molar ellipticity, $[\theta]$, means that the two types of monomeric units in COP *Y* are not chiroptically independent. A similar conclusion was previously drawn from optical rotations of COP *Y* in methanol.[13]

As for the bisignate shape of COP 62 and COP 90.5 CD curves in water at $\bar{\alpha} = 0$, it may seem amazing to find two cotton effects of opposite sign and of rather high rotational strengths in a spectral range where the only amide $\pi \rightarrow \pi^*$ electronic transition is supposed to occur in the absence of rigidity and high stereoregularity. Actually, similar bisignate curves were also found for (−)-poly[*N*-(*sec*-butyl)-*N*-methylacrylamide] in organic solvents.[14] In this case, exciton coupling being reasonably excluded on account of low stereoregularity, it was suggested that cotton effects of opposite sign are correlated to uncoupled amide chromophores, far-UV electronic transitions being shifted to higher wavelengths because of the N,N disubstitution.[14] Similarities between polyacrylamide and COP *Y* CD curves confirm the absence of coupling between amide chromophores. Indeed, the introduction of acid units between coupled amide ones would have given rise to completely different homopolymer and copolymer CD patterns especially with disappearance of the bisignate shape for the copolymers. Consequently, CD spectra of COP 62 and COP 90.5 in water at $\bar{\alpha} = 0$ mainly result from disubstituted amide chromophores disymmetrically perturbed by chiral centers in side chains. As proposed to account for optical activity of COP *Y* in methanol,[13] in water some neighboring effects of different repeating units contribute to the total optical activity which however mostly depends on amide chromophores. Because of these neighboring effects, COP *Y* macromolecules, and not solely amide units, had to be considered as optically active species. In the following discussion, ellipticities are reported in the first way discussed above.

(b) Variation of CD Spectra vs. $\bar{\alpha}$. Increasing the degree of neutralization from 0 to 0.5 and 1.0 by adding KOH only gave rise to slight changes in CD spectra as shown in Figures 1 and 2. Bisignate CD curves observed at $\bar{\alpha} = 0$ remained for higher $\bar{\alpha}$ values, again confirming the absence of coupling between amide chromophores for the same reason as that mentioned above. The positive maximum decreased as $\bar{\alpha}$ increased and variations were similar for both copolymers. The only difference appeared at the level of the negative maximum which was practically unchanged for COP 62 (Figure 1) while it slightly increased to negative values for COP 90.5 (Figure 2).

The decrease of the positive band located at 198–200 nm as $\bar{\alpha}$ increased could have different origins especially a change in magnitude or/and in position of the corresponding cotton effect or a change in magnitude or/and in position of a small induced CD band overlapped by large amide bands as suggested elsewhere to explain CD spectra of COP *Y* in methanol.[10] For the latter possibility, the induced CD band would correspond to the $n \rightarrow \pi^*$ electronic transition of carboxylic chromophores located at about 205 nm.[15] It would be perturbed during ionization by the changes of COOH to COO$^-$ chromophores. As a matter of fact, it is difficult to clear up this point on account of the presence of large amide CD bands at the same position in the spectrum. For a series of alternating maleic acid/chiral alkyl vinyl ether copolymers where there was no overlap of COOH absorption bands at 200–220 nm, optically active $n \rightarrow \pi^*$ CD bands were found and attributed to perturbation of COOH chromophores by chiral centers in vicinal ether units.[16] This induction of optical activity suggests that some of the COOH chromophores in COP *Y* macromolecules are optically active especially those close to chiral amide units. The particular conformational behavior of COP 62 as compared to that of COP 90.5 seemed not to affect the shape of CD spectra significantly. CD depends mainly on cotton effects of amide chromophores whatever the ionization state of the two polyelectrolytes.

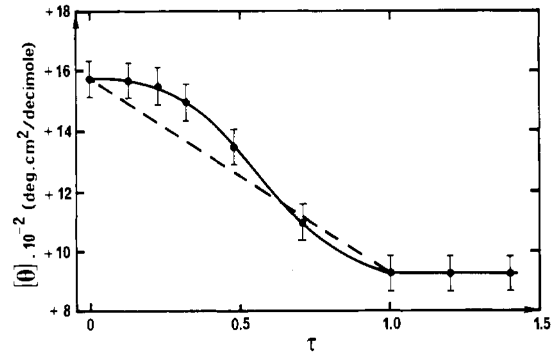


Figure 3. Variation of the COP 62 molar ellipticity $[\theta]$ as a function of τ at λ 200 nm ($C_{\text{cop}} = 2 \times 10^{-3}$ M in water; alkaline reagent, KOH).

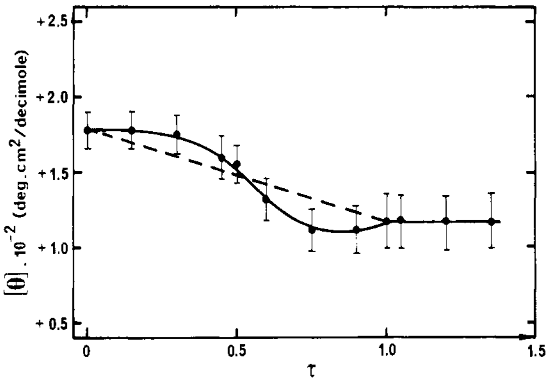


Figure 4. Variation of the COP 90.5 molar ellipticity $[\theta]$ as a function of τ at λ 200 nm ($C_{\text{cop}} = 2 \times 10^{-3}$ M in water; alkaline reagent, KOH).

This fact was confirmed by variations of ellipticity at 200 nm as a function of τ (equiv of added KOH/equiv of COOH initially present) (Figures 3 and 4). τ is equivalent to the usual degree of neutralization $\bar{\alpha}$ for $0 < \tau < 1$. However, its use further allowed us to characterize mixtures containing excess KOH with regard to the polyelectrolyte content ($\tau > 1$). τ is also equivalent to the usual degree of ionization α for $0.05 < \tau < 0.95$ under the selected experimental conditions, i.e., when both self-ionization and hydrolysis of the salt can be neglected.

Comparing Figures 3 and 4, both copolymers showed similar S-shaped variations of ellipticity for $0 < \tau < 1.0$. No variation was observed above $\tau = 1.0$ although macromolecular conformations continue to change because of increasing ionic strength and the well-known screening effect of excess ions.[17] On the other hand, no peculiarity appears in Figure 3 as compared to Figure 4 below $\tau = 0.33$ ($\alpha = 0.33$), i.e., in the range where differences in conformational behavior occur.[8,9] Taking into account the constancy of ellipticity for $\tau > 1.0$ and the absence of difference in the range of τ where the compact coil-to-extended coil transition occurs, it is concluded that optical activity is completely independent of the polyelectrolyte conformational behavior in the case of COP *Y* copolymers.

Inflection points observed at $\tau \simeq 0.5$ for curves of both copolymers (Figures 3 and 4) occurred in a region where no conformational peculiarities were identified. Nonlinear variations of optical activity vs. the degree of neutralization were already found, in the absence of any order-to-disorder transition, for poly-*N*-methacryloyl and poly-*N*-acryloyl derivatives of α-amino acids[18–20] and for phenol–formaldehyde type polycondensates,[21–23] in contrast to the linearity shown by low molecular weight model compounds. Modifications of relative strength of different acid functions for multifunctional polyacids and conformation changes of side chains were proposed to account for the nonlinearity.[21,22]

Actually, nonlinear variation of optical activity vs. composition seems to be a usual feature of copolymers. It was observed for most optically active copolymers already reported in the literature and particularly for COP *Y* in methanol in the absence of any ionization.[13] In the latter case, effects of vicinal units on specific properties of each monomeric unit were identified as being responsible for the nonlinearity and were correlated to the copolymer composition in terms of triads assuming a Bernoullian distribution of repeating units.[13]

A partially ionized polyelectrolyte can be regarded as a copolymer of ionized and un-ionized monomeric units contributing differently to the total optical activity and able to give rise to neighboring effects. For partially ionized COP *Y*, an even more complex situation is found since they can be considered as terpolymers of amide, carboxylic, and carboxylate type units. Accordingly, small variations of the amide unit's molar ellipticity due to vicinal acid units changing to the carboxylate form and the possible presence of some optically active COOH or COO^- chromophores depending on the degree of ionization are two factors which can explain the shape of curves for $0 < \tau < 1.0$ in Figures 3 and 4.

Finally, the main factor affecting COP *Y* optical activity when changing pH is ionization of COOH groups. Contribution from simultaneous conformation changes must be excluded since the compact coil-to-extended coil transition was not reflected in CD spectra and since optical activity remained constant for τ values higher than 1.0, i.e., when conformations continue to change.

References and Notes

(1) G. D. Fasman, Ed., "Poly-α-amino acids. Protein Models for Conformational Studies", Marcel Dekker, New York, N.Y., 1967.
(2) R. C. Schulz in "Encyclopedia of Polymer Science and Technology," Vol. 6, H. Mark, Ed., Interscience, New York, N.Y., 1968.
(3) P. Pino, F. Ciardelli, and M. Zandomeneghi, *Rev. Phys. Chem.*, **21,** 561 (1970).
(4) M. Vert in "Polyelectrolytes", E. Selegny, Ed., D. Reidel, Dordrecht, Holland, 1974, pp 347–370.
(5) G. Muller, J. C. Fenyo, C. Braud, and E. Selegny in "Polyelectrolytes and their Applications", A. Rembaum and E. Selegny Ed., D. Reidel, Dordrecht, Holland, 1975, pp 15–30.
(6) J. Beaumais, J. C. Fenyo, and G. Muller, *J. Polym. Sci., Polym. Chem. Ed.*, **12,** 2305 (1975).
(7) Part 1: C. Braud, M. Vert, and E. Selegny, *Makromol. Chem.*, **175,** 775 (1974).
(8) C. Braud, *Eur. Polym. J.*, **11,** 421 (1975).
(9) C. Braud, *Eur. Polym. J.*, **13,** 897 (1977).
(10) C. Braud and M. Vert, to be published.
(11) M. Vert and E. Selegny, *J. Polym. Sci., Polym. Symp.*, **No. 42,** 1239 (1973).
(12) P. Urnes and P. Doty, *Adv. Protein Chem.*, **16,** 401 (1961).
(13) C. Braud and M. Vert, *Polymer*, **16,** 115 (1975).
(14) C. Braud and M. Vert, *Isr. J. Chem.*, **15,** 39 (1976/77).
(15) H. H. Jaffe and M. Orchin, Ed., "Theory and Applications of Ultraviolet Spectroscopy", Wiley, New York, N.Y., 1962, p 180.
(16) C. Villiers, C. Braud, M. Vert, and E. Chiellini, to be published.
(17) D. T. F. Pals and J. J. Hermans, *Recl. Trav. Chim. Pays-Bas*, **71,** 443 (1942).
(18) R. K. Kulkarni and H. Morawetz, *J. Polym. Sci.*, **54,** 491 (1961).
(19) F. B. Bruschtein, *Diss. Abstr.*, **26,** 1931 (1965).
(20) H. Morawetz, "Macromolecules in Solution", Interscience, New York, N.Y., 1965, p 243.
(21) M. Vert and E. Selegny, *Eur. Polym. J.*, **7,** 1321 (1971).
(22) E. Selegny, M. Vert, and N. Thoai, *C. R. Hebd. Seances Acad. Sci., Ser. C*, **262,** 189 (1966).
(23) J. Beaumais, Thesis of Speciality, Paris, 1969.