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ELECTRIC BIREFRINGENCE OF IONIZED POLYPEPTIDES IN SOLUTION AND THE EFFECT OF HIGH ELECTRIC FIELDS ON THE HELIX–COIL TRANSITION

Koshiro YOSHIOKA, Kazuo KIKUCHI and Mitsuo FUJIMORI
Department of Chemistry, College of General Education, University of Tokyo,
Komaba, Meguroku, Tokyo, Japan

Electric birefringence and circular dichroism measurements have been made on solutions of two poly(L-lysine) homologs. The specific Kerr constant and the molar ellipticity at 222 nm of poly(L-α, γ-diaminobutyric acid hydrochloride) in methanol/water mixtures underwent an abrupt change between 75 and 80 vol% methanol at 25°C, corresponding to a solvent-induced helix-coil transition. On the helix side of the transition region, i.e., between 78 and 80 vol% methanol, anomalous birefringence transients indicative of field-induced helix-to-coil transition were observed at high fields. In the case of poly(L-ornithine hydrobromide) in methanol/water mixtures, a helix–coil transition was induced between 93 and 98 vol% methanol and anomalous birefringence transients were observed between 96 and 98 vol% methanol. The double logarithmic plots of the steady-state specific birefringence versus the square of field strength for various solvent compositions and polymer concentrations could be superimposed on one another by horizontal and vertical shifts, except for the range where anomalous birefringence transients were observed. This enabled us to estimate the threshold field strength.

1. Introduction

Electro-optical methods have found wide use in study of the ordered conformations and conformational changes of biopolymers [1]. When an electric field is applied to a macromolecular solution, the macromolecules possessing electrical anisotropy are preferentially oriented by the field, resulting in anisotropy of the optical properties of the solution. The anisotropy of the refractive index caused by the electric field is called electric birefringence or Kerr effect. If the applied electric field induces a conformational change of the macromolecules in addition to preferential orientation, such a field effect will be detected as a change in electro-optical properties. This effect was reported especially for polynucleotides such as DNA [2–4], RNA [5], poly(A) [6,7] and poly(A)·2poly(U) [8], and its biological implications were discussed [2, 8]. We have been concerned with the field effect on the helix-coil transition of synthetic polypeptides.

In the case of non-ionic polypeptides such as poly(γ-benzyl L-glutamate), $[\mathrm{Glu(OBzl)}]_n$, application of an electric field should shift the helix–coil equilibrium towards the helical conformation, since this conformation possesses a much larger overall permanent dipole moment than the coil form. As is well known, $[\mathrm{Glu(OBzl)}]_n$ undergoes a solvent-induced helix–coil transition in mixtures of dichloroethane and dichloroacetic acid. Schwarz and Schrader [9] measured electric birefringence of the same system in the helix–coil transition region at 15 kV/cm and observed an appreciable enhancement of the steady-state birefringence, in comparison with the theoretical calculation neglecting the field effect. This was ascribed to the displacement of the helix–coil equilibrium induced by high electric fields. Subsequently, Watanabe and Yoshioka [10] obtained more direct evidence for the field-induced coil-to-helix transition of $[\mathrm{Glu(OBzl)}]_n$.

The situation is quite different and complicated in the case of ionized polypeptides which serve as the simplest model of ordered biopolyelectrolytes. Previously we carried out electric birefringence measurements on poly(L-lysine hydrobromide), $(\mathrm{Lys.\ HBr})_n$, in methanol/water mixtures at various solvent compositions [11,12]. This polypeptide undergoes a solvent-induced helix-coil transition between 87 and 90 vol% methanol at neutral pH [13]. Joubert, Lotan and Scheraga [14] showed in a nuclear magnetic resonance study that the ε-amino groups of poly(L-lysine) are charged in 90 vol% methanol at pH 6, even though the conformation is helical. We observed anomalous birefringence transients between 90 and 95 vol% methanol above a certain threshold field strength: the birefringence passed through a maximum and began to decrease slowly before the applied rectangular pulse terminated, reaching a steady state. A distinct difference between the responses to low and high fields was noticed in the proximity of the helix-coil transition region. These results were interpreted as indicating that a helix-to-coil transition is induced by high electric fields.

In order to demonstrate the universality of this field effect, we have extended our investigation to two homologs of poly(L-lysine), namely poly(L-α, γ-diaminobutyric acid) and poly(L-ornithine). The former has two less methylene groups and the latter has one less methylene group per residue in the side chain as compared to poly(L-lysine). We measured transient electric birefringence of poly(L-α, γ-diaminobutyric acid hydrochloride), $(A_2bu \cdot HCl)_n$, and poly(L-ornithine hydrobromide, $(Orn \cdot HBr)_n$, in methanol/water mixtures and obtained experimental results similar to those for $(Lys \cdot HBr)_n$ [15,16]. This convinced us of the occurrence of the field-induced helix-to-coil transition in ionized polypeptides.

2. Materials and methods

$(A_2bu \cdot HCl)_n$ was generously supplied by Dr. S. Kubota of the University of California. The limiting viscosity number of this sample, measured in 0.2 M NaCl at 25°C, was 105 cm^3/g. $(Orn \cdot HBr)_n$ was purchased from Sigma Chemical Company (Lot No. 25C-5023, molecular weight given as 122 000). Methanol was of reagent grade, and distilled twice prior to use. Stock solutions were prepared by dissolving the dried polypeptide in double-distilled water and then adding an appropriate amount of methanol. The solvent composition was expressed in terms of volume percentage of methanol. The polymer concentration was expressed as g/dm^3 (= kg/m^3).

The apparatus, hitherto used for electric birefringence measurements [17], has been improved by replacing the cathode follower circuit with the operational amplifier system. The optical system consisted of a 90-W ultra-high pressure mercury lamp, an interference filter transmitting light of 546 nm, a pair of Glan–Thompson prisms, a Kerr cell and a photomultiplier. The Kerr cell was a quartz spectrophotometer cell and contained two platinum electrodes of 0.988 cm length supported by Teflon block at a separation of 0.284 cm. The electric field was applied to a solution in the cell in the form of single rectangular pulses. The pulse duration was from 150 to 500 μs and the maximum pulse amplitude was 7 kV. The resulting birefringence signal was displayed and photographed on the screen of an Iwasaki type-DS-6016 dual-beam oscilloscope, together with the pulse attenuated by a calibrated voltage divider.

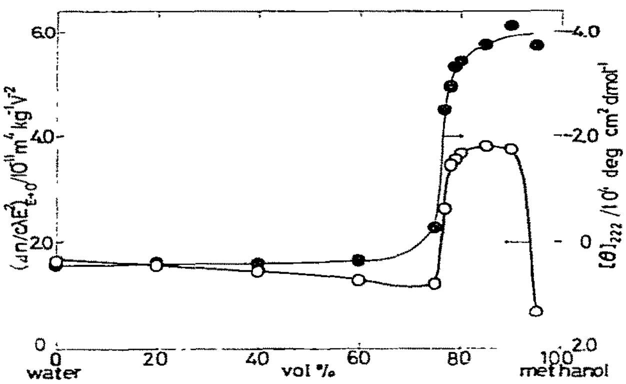

Fig. 1. Specific Kerr constant and molar ellipticity at 222 nm of $(A_2bu \cdot HCl)_n$ in methanol/water mixtures at 25°C, as functions of methanol content. Polymer concentration, 0.19 g/dm^3.

Circular dichroism (CD) spectra were obtained with a JASCO J-20 recording spectropolarimeter under constant nitrogen flush. The CD data were expressed in terms of molar ellipticity, $[\theta]$, in deg cm^2 $dmol^{-1}$, based on the molecular weight of the amino acid residue.

3. Results and discussion

The molar ellipticity at 222 nm of $(A_2bu \cdot HCl)_n$ in methanol/water mixtures is plotted against the volume percentage of methanol in fig. 1. This corresponds to one of the two CD minima characteristic of α-helix and serves as a measure of the helix content. In this figure is also plotted the specific Kerr constant, B/c, against the solvent composition. This constant is defined by

$$B/c = \lim_{E\to 0} \Delta n/\lambda c E^2 , \qquad (1)$$

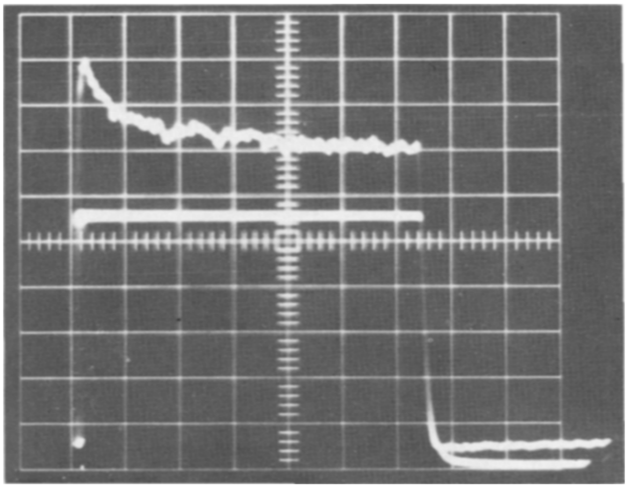

Fig. 2. Oscillogram of anomalous birefringence signal for (Orn · HBr)$_n$ (c = 0.14 g/dm^3) in 97 vol% methanol. Field strength, 15.5 kV/cm. Time scale, 50 μs per large division on the horizontal axis.

where Δn is the steady-state electric birefringence, λ is the wavelength of light *in vacuo*, c is the mass concentration of the solute, and E is the field strength.

Both of these quantities undergo an abrupt change between 75 and 80 vol% methanol, accompanied by a solvent-induced helix-coil transition. The specific Kerr constant for the "coil" (charged coil) is considerably large, in contrast with non-ionic polypeptides such as [Glu(OBzl)]$_n$, and decreases gradually with an increase in methanol content. The increase in specific Kerr constant upon transition from "coil" to helix is rather small. On the helix side, the specific Kerr constant undergoes a sharp drop in the vicinity of 95 vol% methanol.

In the case of (Orn · HBr)$_n$ in methanol/water mixtures, the molar ellipticity at 222 nm and the specific Kerr constant underwent an abrupt change between 93 and 98 vol% methanol at 25 °C. This corresponds to a solvent-induced helix-coil transition, as reported by Tseng and Yang [18].

On the helix side of the transition region, i.e., between 78 and 80 vol% methanol for (A$_2$bu · HCl)$_n$ and between 96 and 98 vol% methanol for (Orn · HBr)$_n$, anomalous birefringence transients were observed at high fields as in the case of (Lys · HBr)$_n$. A typical oscillogram is shown in fig. 2. It takes rather long time (of the order of 0.1 ms) to reach the plateau region which is regarded as the steady state. The appearance

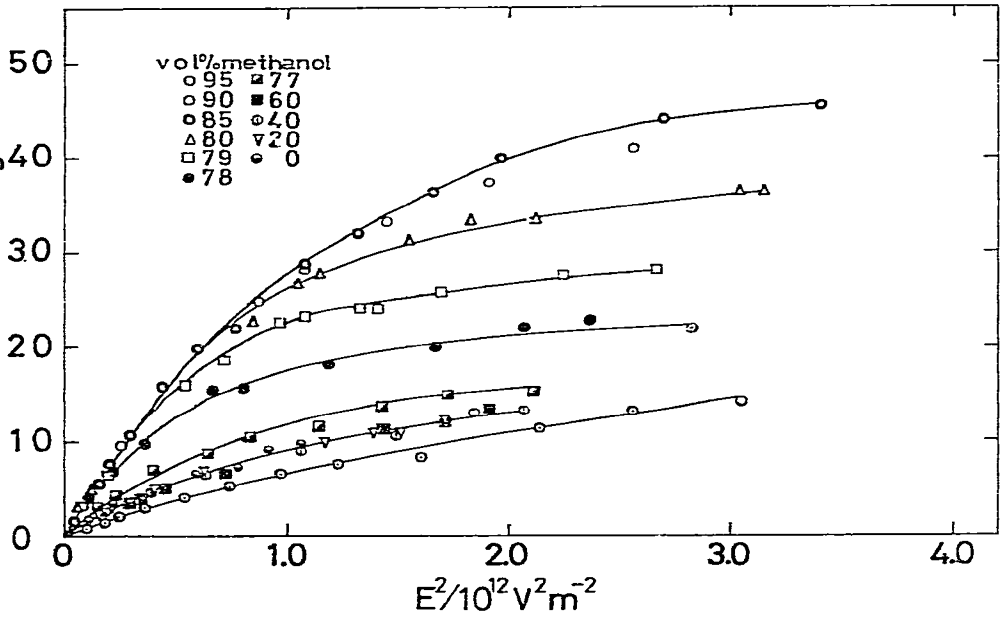

Fig. 3. Plots of $\Delta n/\lambda c$ versus E^2 for (A$_2$bu · HCl)$_n$ in methanol/water mixtures at 25 °C. Polymer concentration, 0.19 g/dm^3. Solvent compositions are given in the figure.

Table 1
Values of B/c, A and $(B/c)/A$ for $(\mathrm{A_2bu \cdot HCl})_n$ in methanol/water mixtures at 25 °C ($c = 0.19\ \mathrm{g/dm^3}$)

methanol vol%	conformation	B/c	A	$(B/c)/A$
		$10^{-11}\ \mathrm{m^4\ kg^{-1}\ V^{-2}}$	$10\ \mathrm{m^2\ kg^{-1}}$	$10^{-13}\ \mathrm{m^2\ V^{-2}}$
40	"coil"	1.46	1.8	8.1
85	helix	3.78	5.3	7.1
95	helix	0.68	~5	~1.3

of anomalous birefringence transients is attributable to the field-induced helix-to-coil transition. However, the initial, fast step of the transition cannot be detected by our technique, because it is concealed behind the build-up process of electric birefringence.

In fig. 3, the steady-state specific birefringence, $\Delta n/\lambda c$, of $(\mathrm{A_2bu \cdot HCl})_n$ at various solvent compositions is plotted against the square of field strength. Kerr's law holds at low fields and the specific Kerr constant can be calculated from the initial slope of these plots. Deviations from Kerr's law arise at higher fields. When a pronounced saturation is noticed, it is possible to obtain the saturated specific birefringence, A, by extrapolating a $\Delta n/\lambda c$ versus $1/E^2$ plot to $1/E^2 = 0$.

$$A = \lim_{E\to\infty} \Delta n/\lambda c\ . \tag{2}$$

For a dilute solution of rigid, axially symmetric macromolecules, the electric birefringence is expressed by the equation

$$\frac{\Delta n}{\lambda c} = \frac{2\pi}{n\rho\lambda}(g_1 - g_2)\Phi(E) = A\Phi(E)\ , \tag{3}$$

where n is the refractive index of the solution, ρ is the density of the solute, $g_1 - g_2$ is the optical anisotropy factor, and Φ is the orientation factor which is a function of the field strength and depends on the electrical properties of the macromolecule [19,20]. In this case the saturated specific birefringence is proportional to the optical anisotropy factor. From eqs. (1) and (3), we obtain

$$\frac{1}{A}\frac{B}{c} = \lim_{E\to 0} \frac{\Phi(E)}{E^2}\ . \tag{4}$$

The values of B/c, A and $(B/c)/A$ for helical and "coil" conformations of $(\mathrm{A_2bu \cdot HCl})_n$ are listed in table 1. Thus, the optical anisotropy factor for the helix is about three times as large as that for the "coil". On the other hand, the orientation factor for the "coil" in 40 vol% methanol is somewhat larger than that for the helix in 85 vol% methanol at the same field strength. The large decrease in the specific Kerr constant for the helix in 95 vol% methanol would be ascribed to a strong binding of counterions to the polyion in a medium of low dielectric constant.

The double logarithmic plots of $\Delta n/\lambda c$ versus E^2 for various solvent compositions can be superimposed on one another by shifting them horizontally along the abscissa and vertically along the ordinate except for the range where anomalous birefringence transients were observed, as demonstrated in fig. 4. This figure represents the double logarithmic plots of $(\Delta n/\lambda c)/A$ versus $(15/A)(B/c)E^2$ for $(\mathrm{A_2bu \cdot HCl})_n$ in methanol/water mixtures. The two parameters, A and B/c, related to the shift factors were adjusted so as to produce the best superimposition [21]. The initial slope of these plots was chosen to be equal to 1/15 for the sake of convenience. Hence the electric birefringence of these solutions can be expressed by the equation involving two parameters, A and B/c,

$$\frac{1}{A}\frac{\Delta n}{\lambda c} = \Phi\left(\frac{15}{A}\,\frac{B}{c}E^2\right)\ , \tag{5}$$

where the functional form of the orientation factor Φ is independent of the solvent composition and, moreover, the polymer concentration [21]. Downward deviations from the common, solid curve take place above some critical values of $(15B/Ac)E^2$ in 78, 79 and 80 vol% methanol. The corresponding field strength can be regarded as the threshold field strength.

The solid curve in fig. 4 represents the theoretical curve for molecular orientation due primarily to a permanent dipole moment. In this case the orientation factor is given by

$$\Phi(E) = 1 - \frac{3(\coth bE - 1/bE)}{bE}$$

$$= \tfrac{1}{15}\, b^2E^2 - \tfrac{2}{315}\, b^4E^4 + \tfrac{1}{1575}\, b^6E^6 - \ldots\ , \tag{6}$$

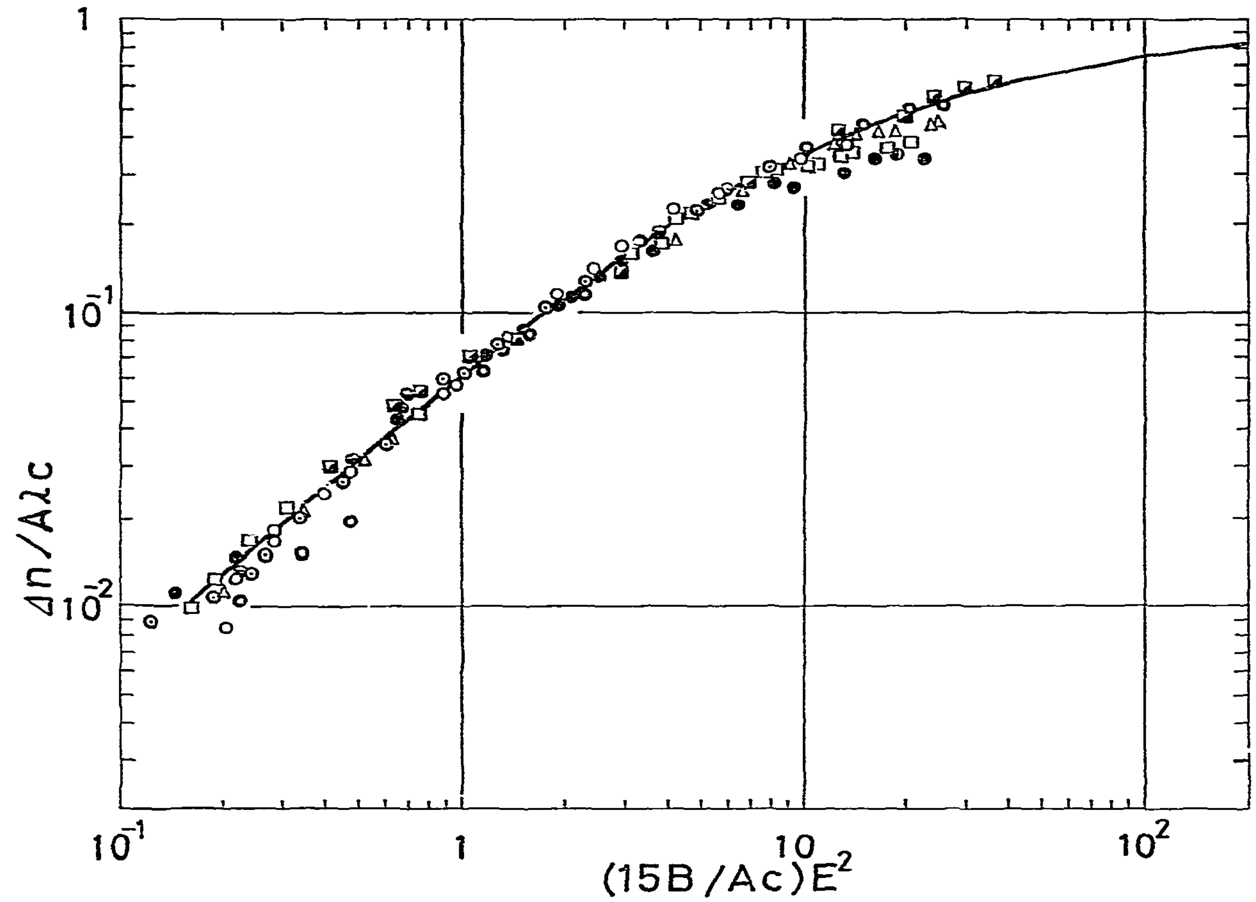

Fig. 4. Double logarithmic plots of $\Delta n/A\lambda c$ versus $(15B/Ac)E^2$ for $(A_2bu \cdot HCl)_n$ in methanol/water mixtures at 25 °C. Polymer concentration, 0.19 g/dm^3. Solvent compositions are given in fig. 3.

with

$$b = \mu'/kT\,, \tag{7}$$

where μ' is the apparent permanent dipole moment along the symmetry axis, k is Boltzmann's constant, and T is the thermodynamic temperature [20].

Thus, the field strength dependence of the birefringence for both helical and "coil" conformations resembles the behaviour for permanent dipole moment orientation over a rather broad range of field strengths. However, it seems unreasonable to ascribe the field-induced orientation of polyelectrolytes solely to the permanent dipole moment in a narrow sense. The apparent permanent dipole moment for $(Lys \cdot HBr)_n$, $(Orn \cdot HBr)_n$ and $(A_2bu \cdot HCl)_n$ in methanol/water mixtures, calculated by use of eqs. (6) and (7), was found to be much larger than expected for uncharged polypeptides. Furthermore, the build-up curve of electric birefringence did not fit the theoretical equation for permanent dipole moment orientation.

Several other explanations for the field strength dependence of the orientation factor in the case of polyelectrolytes have been proposed. They are enumerated below: (i) saturation of the induced dipole moment due to counterion polarization [3,8,22], (ii) quasi-permanent dipole moment due to the counterion fluctuation mechanism [23], (iii) orientation due to anisotropic ion flow [24], and (iv) dispersity in the

Table 2
Threshold field strengths in kV/cm for $(\mathrm{A_2bu \cdot HCl})_n$ in methanol/water mixtures at 25 °C

methanol vol%	polymer concentration, g/dm^3			
	0.047	0.094	0.14	0.19
78	3.3	4.2	5.0	6.0
79	4.6	5.5	6.1	7.4
80	5.0	6.0	7.0	8.1

electric polarizabilities [25]. At the present stage of the theory, however, it is premature to arrive at a final conclusion.

The threshold field strengths for $(\mathrm{A_2bu \cdot HCl})_n$ at different solvent compositions and polymer concentrations, estimated according to the above-mentioned pro cedure, are presented in table 2. As can be seen from this table, the threshold field strength increases with increasing polymer concentration and with increasing methanol content on the verge of the transition region. It is to be noted that the threshold field strength is rather low under favorable conditions.

Similar results were obtained with $(\mathrm{Orn \cdot HBr})_n$ in methanol/water mixtures. The thresold field strength could be determined from the field strength dependence of the birefringence in the same way.

We have also measured the circular dichroism and the electric birefringence of $(\mathrm{Orn \cdot HBr})_n$ in ethanol/water, 2-propanol/water and tertiary butyl alcohol/water mixtures. A solvent-induced helix-coil transition was found in every case. However, anomalous birefringence transients were observed only in ethanol/water mixtures.

Thus far we obtained evidence for the field-induced helix-to-coil transition in four systems: $(\mathrm{Lys \cdot HBr})_n$/water/methanol, $(\mathrm{Orn \cdot HBr})_n$/water/methanol, $(\mathrm{Orn \cdot HBr})_n$/water/ethanol and $(\mathrm{A_2bu \cdot HCl})_n$/water/methanol. These systems have three characteristics in common. Firstly, the helical conformation as well as the coil form is charged. Secondly, counterions are not very strongly bound to the polyion. Thirdly, the solvent-induced helix—coil transition takes place in a strongly cooperative fashion. Undoubtedly this field effect will be closely connected with a large perturbation of the counterion atmosphere around the helical polyion provoked by high fields. The charged coil will be thermodynamically more stable than the charged helix at high fields.

We have already proposed a possible mechanism for the field-induced helix-to-coil transition in ionized polypeptides [11]. A certain fraction of counterions will be bound to the charged helix, but mobile in the axial direction. Application of an electric field displaces such counterions along the helix axis, giving rise to an induced dipole moment [26]. Then, the helices are preferentially oriented by the field. As the applied field is enhanced, the average degree of orientation becomes larger, causing the component of the field along the helix axis to increase still more. When the counterions are shifted towards one end of the helix and the increased repulsion between the ionized groups at the other end overcomes the attraction due to the amide hydrogen bonds and other non-bonding interactions, unwinding of the helix will start at that end and propagate along the helix axis. This will occur above a critical field on the verge of the transition region. The mechanism is analogous to that proposed for field-induced conformational changes in polynucleotides [8,27].

Acknowledgements

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References

[1] For a review, see C.T. O'Konski, ed., Molecular electro-optics, Part 2, Applications to biopolymers (Marcel Dekker, New York, 1978).
[2] C.T. O'Konski and N.C. Stellwagen, Biophys. J. 5 (1965) 607.
[3] D.-W. Ding, R. Rill and K.E. Van Holde, Biopolymers 11 (1972) 2109.
[4] M. Pollak and H.A. Glick, Biopolymers 16 (1977) 1007.
[5] A. Revzin and E. Neumann, Biophys. Chem. 2 (1974) 144.
[6] D. Pörschke, Biopolymers 15 (1976) 1917.
[8] E. Neumann and A. Katchalsky, Proc. Natl. Acad. Sci. US 69 (1972) 993.
[9] G. Schwarz and U. Schrader, Biopolymers 14 (1975) 1181.

[10] H. Watanabe and K. Yoshioka, in: Electro-optics and dielectrics of macromolecules and colloids, ed. B.R. Jennings (Plenum, New York, 1979) p. 345.
[11] K. Kikuchi and K. Yoshioka, Biopolymers 12 (1973) 2667.
[12] K. Kikuchi and K. Yoshioka, Biopolymers 15 (1976) 1669.
[13] R.F. Epand and H.A. Scheraga, Biopolymers 6 (1968) 1383.
[14] F. Joubert, N. Lotan and H.A. Scheraga, Physiol. Chem. Phys. 1 (1969) 348.
[15] M. Fujimori, K. Kikuchi, K. Yoshioka and S. Kubota, Biopolymers 18 (1979) 2005.
[16] M. Fujimori, K. Kikuchi and K. Yoshioka, Polymer Preprints, Japan 27 (1978) 342.
[17] K. Yoshioka and H. Watanabe, in: Physical principles and techniques of protein chemistry, Part A, ed. S.J. Leach (Academic, New York, 1969) p. 335.
[18] Y.-W. Tseng and J.T. Yang, Biopolymers 16 (1977) 921.
[19] A. Peterlin and H.A. Stuart, Z. Phys. 112 (1939) 129.
[20] C.T. O'Konski, K. Yoshioka and W.H. Orttung, J. Phys. Chem. 63 (1959) 1558.
[21] K. Kikuchi and K. Yoshioka, J. Phys. Chem. 77 (1973) 2101.
[22] K. Kikuchi and K. Yoshioka, Biopolymers 15 (1976) 583.
[23] M. Sakamoto, H. Kanda, R. Hayakawa and Y. Wada, Biopolymers 15 (1976) 879.
[24] M. Hogan, N. Dattagupta and D.M. Crothers, Proc. Natl. Acad. Sci. US 75 (1978) 195.
[25] M. Tricot, C. Houssier and V. Desreux, Eur. Polym. J. 12 (1976) 575.
[26] M. Mandel, Mol. Phys. 4 (1961) 489.
[27] M. Pollak and R. Rein, J. Theor. Biol. 19 (1968) 333.