Local Order in Polyelectrolytic Solutions Detected by Magnetic Birefringence Measurements on the (Tyr-Glu)$_n$ Copolymer

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Synopsis

The local electrostatic order in polyelectrolytic solutions has been studied by magnetic birefringence measurements on short segments ($n \sim 34$) of the (Tyr-Glu)$_n$ polypeptide in high magnetic fields up to 13 T. We give evidence that only above a critical polymer concentration do small domains exist within which the polymers show a parallel ordering. This structural order disappears suddenly at ionic strengths above about 5×10^{-2} *M* and above a melting temperature of about 50°C.

INTRODUCTION

A great number of biopolymers involved in organized biological systems are polyelectrolytes. In order to understand their supermolecular assembly, it is important to investigate—on a model system—the role of long-range electrostatic interactions on the interchain arrangement.[1]

Recently, the existence of a periodic arrangement of highly charged spherical polystyrene particles in solution has been detected.[2] This order has been attributed to purely electrostatic repulsion. However, if the particles have a rodlike shape, ordering of this sort is observed only at rather high concentrations, where, in addition to the coulombic repulsion, van der Waals and steric interactions have to be taken into account. (Such work is reviewed in Ref. 1.) Solutions of flexible polyelectrolytes also seem to show some order, as concluded from recent neutron scattering experiments.[3,4] Theoretical treatments of the electrostatic problem[5] have investigated lattice models. A hexagonal lattice built up of parallel rods, a cubic lattice built up of perpendicular neighboring rods, and an "isotropic" gel are all expected to have very similar electrostatic energies. Therefore, it seems difficult to predict theoretically which kind of periodic electrostatic order should be present in a polyelectrolytic solution.

Birefringence measurements on various polymers, biopolymers, and liquid crystals in high magnetic fields have demonstrated that this technique is a very sensitive tool for determining the orientational molecular correlation in solution. For example, both the correlation length over which a liquid crystalline order extends in a polymeric cluster and the persistence

Biopolymers, Vol. 18, 101–111 (1979)

0006-3525/79/0018-0101$01.00

length, b, of polymers[6,7] can be determined. However, it has not yet been explicitly pointed out that the diamagnetic anisotropy responsible for the magnetic orientation of such a polymeric cluster—and hence responsible for the magnetic birefringence—is very sensitive to the nature of the lattice. We shall show that lattices of rodlike polymers that are oriented in parallel, on the one hand, and perpendicularly on the other, behave very differently in a magnetic field. There exists a large magnetic birefringence for the former, but none at all for the latter.

Furthermore, an orientational study of polyelectrolytes in *magnetic* fields yields, in principle, more unambiguous results than those obtainable by *electric* orientation measurements, since in a diamagnetic solution no measurable magnetic field screening effects by the solvent occur. In addition, the electric charge distributions of macromolecules and solvent are not disturbed by the magnetic field. Finally, no ohmic heating takes place; hence, an orientational study of the molecules in complete thermal equilibrium with the electrolytic solvent is possible. Therefore, we think that this magnetic method is very well adapted to the problem of local order in polyelectrolytes.

In this paper we present a magnetic birefringence study on the $(\mathrm{Tyr\text{-}Glu})_n$ copolymer and its monomer at various concentrations, degrees of dissociation, temperatures, and ionic strengths.

PRINCIPLES

Consider first a rodlike polymer, consisting of n monomers of length l_0 and having an overall diamagnetic anisotropy $n(\chi_\parallel - \chi_\perp)$ with respect to the rotational symmetry axis. The $\chi_\parallel$ and $\chi_\perp$ are the monomeric diamagnetic susceptibilities referred to the same symmetry axis. Following the definitions given in Ref. 6, this rod shows a degree of alignment

$$\beta = n(\chi_\parallel - \chi_\perp)H^2/kT \tag{1a}$$

in a magnetic field, H, at temperature, T, when $\beta \ll 1$ and k is the Boltzmann constant. In the case of a semiflexible polymer having a persistence length b, or in the case of a cluster built up of n_0 rods aligned parallel to each other, n has to be replaced by $2b/l_0$ or by n_0n, respectively. The magnetic orientability increases with increasing local parallel order. For illustration, we calculate that, at room temperature and even in high magnetic fields of 10 T and taking as monomeric $(\chi_\parallel - \chi_\perp)$ the very high diamagnetic anisotropy of a benzene ring [$(\chi_\parallel - \chi_\perp) \sim 60 \times 10^{-6}$ emu/mol[7]], one obtains $\beta \sim 1\%$ for the parallel arrangement of the rods, assuming $n = 30$ and $n_0 = 10$. Therefore, the magnetic alignment of such a short-range polyelectrolytic structure will have only a small effect. Even the highest magnetic fields available ($\sim$ 20 T) do not seem to be strong enough to increase significantly the size of such an ordered domain; rather, they are just sufficient to orient slightly the domains already existing in zero field so that a measurement of β becomes possible.

In contrast, if there is perpendicular order of at least three neighboring polymer segments or rods, as in the cubic lattice proposed,[5] the cluster is magnetically isotropic and there can obviously be no orientation, i.e., $\beta = 0$.

A very sensitive measurement of β can be made by the magnetic birefringence $\Delta n = n_{\parallel} - n_{\perp}$, where $n_{\parallel}$ and $n_{\perp}$ are the refractive index of the sample for light of wavelength, λ_0, propagating perpendicular to H and being linearly polarized parallel or perpendicular to H, respectively, because Δn is proportional to β and to the polymeric concentration c, if contributions from the solvent can be neglected (the magnetic birefringence of H_2O is too small to measure):

$$\Delta n \propto \beta c$$

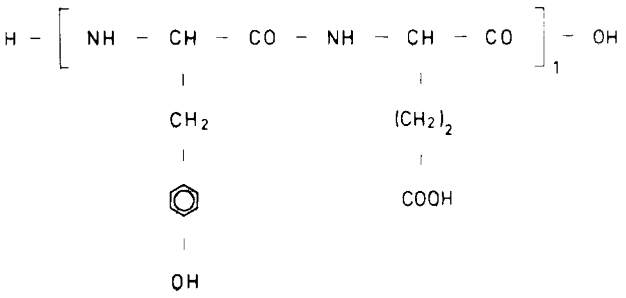
When introducing the Cotton-Mouton constant, CM, as a measure for the molecular order[6,7]

$$\Delta n = CM\, \lambda_0 H^2 \tag{1b}$$

the effective number of monomeric units of the polyelectrolyte correlated parallel to each other is given by the ratio of polymeric to monomeric Cotton-Mouton constants measured with respect to the same concentration of monomers.

MATERIALS AND METHODS

We studied the monomer having the chemical structure



in the partially neutralized state (neutralization of the carboxyl groups by sodium hydroxide) in aqueous solution. This sample was obtained commercially (SERVA ref. 52535).

The polymer $(\text{Tyr-Glu})_n$ was prepared by Trudelle[8] using a method proved to be racemization-free. The number-average molecular weight was 10,000 ($n \sim 34$) as revealed by osmometry; this corresponds to a total length (if fully extended) of about 250 Å.

$(\text{Tyr-Glu})_n$ was studied in aqueous solutions at different degrees of neutralization, α' (adjusted by the subsequent addition of sodium hydroxide), α' being defined as the fraction of OH^- ions added per monomeric unit. Both the effective degree of dissociation of the polymer (i.e., its effective surface charge density responsible for the strength of electrostatic repulsion between monomers) and the total ionic strength, μ, of the solvent (responsible for screening of the surface charges) vary simultaneously with α' because $(\text{Tyr-Glu})_n$ is a weak acid. The effective degree of dissociation, $\alpha\phi$, is defined as the effective number of charges per COOH group, α being the chemical degree of dissociation (fraction of free charged sites per monomeric unit) and ϕ being the osmotic coefficient defined and discussed by Oosawa[9] and Katchalsky and coworkers.[10,11] $\alpha\phi$ and μ are related by[9]

$$\mu = \alpha\phi c_0 + \mu_s \qquad (2)$$

where c_0 is the equivalent monomer concentration and μ_s is the ionic strength of added salt.

When $\alpha' \leq 1$, i.e., at pH values below complete neutralization of COOH, α' is almost equal to α and ϕ stays near unity. Consequently, μ increases almost linearly with α', and the Debye screening length, λ_D, decreases almost proportionally to $(\alpha')^{-1/2}$. When the COOH group is stoichiometrically neutralized ($\alpha = 1$), the Katchalsky charge parameter[10,11]

$$\lambda = (\alpha/l_0)\,(e^2/\epsilon kT)$$

is 0.96 (e being the electric charge and ϵ, the dielectric constant of water). This is very near to the threshold value $\lambda = 1$ beyond which the effective ionization $\alpha\phi$ becomes constant because of counter-ion condensation.[9-11] Therefore, further addition of NaOH, which is needed in excessive amounts ($\alpha' \gg 2$) in order to dissociate the weakly acidic OH group (i.e., in order to obtain $\alpha = 2$), leads to a decreasing Debye screening length ($\mu_s \neq 0$) at almost constant effective polymeric charge density $\alpha\phi e$. In this case ($1 \leq \alpha \leq 2$), the exact degree of the dissociation of the OH group must be determined separately—for instance, by uv spectroscopy, as proposed by McKnight and Auer.[12] Our uv data and the determination of α are given in Fig. 1. At complete dissociation of both the COOH- and OH group, λ was 1.92. All solutions were centrifuged at 15,000 × g for 10 min and prepared by subsequent dilution.

The uv absorption spectra were recorded using a Beckman ACTA III spectrophotometer and quartz cells of 1-cm optical path. By studying the uv spectra at different concentrations and at $\alpha = 1$, a constant value of the extinction coefficient, ϵ, is found, showing, in agreement with Trudelle,[13] that under the solvent conditions investigated no measurable stacking of the tyrosine groups exists.

The measurement of Δn was made by using a combined compensation–modulation technique as described earlier (Ref. 6 and manuscript in preparation). The resolution in Δn was nearly 10^{-10}. The magnetic field up to 13.0 T was produced by a Bitter-type solenoid with a radial optical access of 4-mm diameter, recently developed at the Service National des Champs Intenses, Grenoble.[14] This new coil allowed us to use a sample length of 3 cm and a sample volume of only about 200 μl.

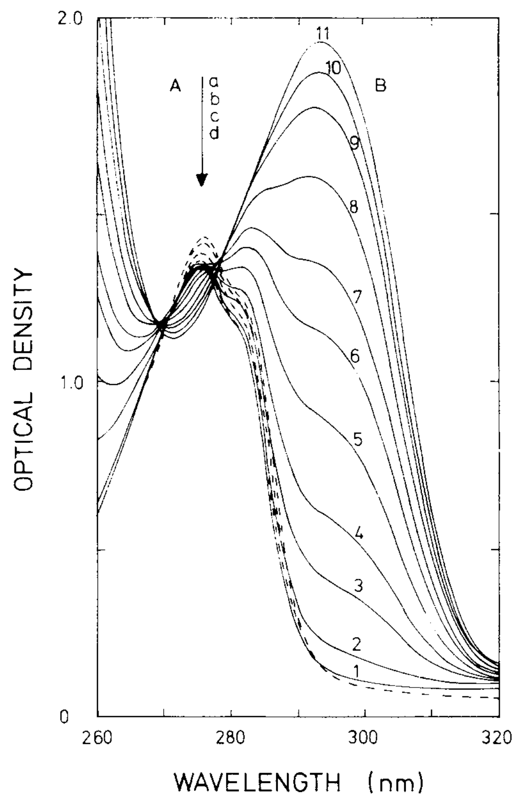


Fig. 1. The uv spectra of (Tyr-Glu)$_n$ during titration with NaOH. (A) Neutralization of the glutamic carboxyl group (---), the degree of neutralization, $\alpha' = \alpha$, being determined from pH measurements: (a) $\alpha' = 0$, (b) $\alpha' = 0.2$, (c) $\alpha' = 0.4$, (d) $\alpha' = 0.8$. (B) Neutralization of the tyrosine OH-group (—), the total degree of dissociation, α, is defined for $\lambda_0 = 293.5$ nm by the relation $\alpha = 1 + [(\mathrm{OD}^{\alpha} - \mathrm{OD}^{\alpha=1})/(\mathrm{OD}^{\alpha=2} - \mathrm{OD}^{\alpha=1})]$, OD^{α} being the optical density at a degree of dissociation α. We used extinction coefficients $\epsilon_{293.5}^{\alpha=1} = 150\ \mathrm{cm}^{-1} \times$ (residues Tyr/l.)$^{-1}$ and $\epsilon_{293.5}^{\alpha=2} = 2010\ \mathrm{cm}^{-1} \times$ (residues Tyr/l.)$^{-1}$. We deduce the following: (1) $\alpha = 1$, (2) $\alpha = 1.05$, (3) $\alpha = 1.15$, (4) $\alpha = 1.25$, (5) $\alpha = 1.4$, (6) $\alpha = 1.54$, (7) $\alpha = 1.64$, (8) $\alpha = 1.78$, (9) $\alpha = 1.89$, (10) $\alpha = 1.95$, (11) $\alpha = 2$.

EXPERIMENTAL RESULTS

Dependence on Concentration, Degree of Neutralization, and Ionic Strength

Monomer and polymer solutions were studied over a concentration range from 5 to 100 mg/ml. In all measurements we observed a linear relation between the birefringence Δn and H^2, as illustrated in Fig. 2, showing that even in high magnetic fields only a small alignment of the polyelectrolyte takes place. We estimate that β for the most favorable case investigated [i.e., curve (c) in Fig. 2] is only of the order of 0.1% at $H = 12$ T.

The *monomeric* Cotton-Mouton constant CM_{mono} is $8.4 \times 10^{-7}\ \mathrm{T}^{-2}\ \mathrm{cm}^{-1}$ at $c = 50$ mg/ml and hence CM_{mono}/c_0 per aromatic ring (c_0 being the concentration of aromatic rings) is $8.5 \times 10^{-27}\ \mathrm{T}^{-2}\ \mathrm{cm}^2\ \mathrm{ring}^{-1}$. This is near

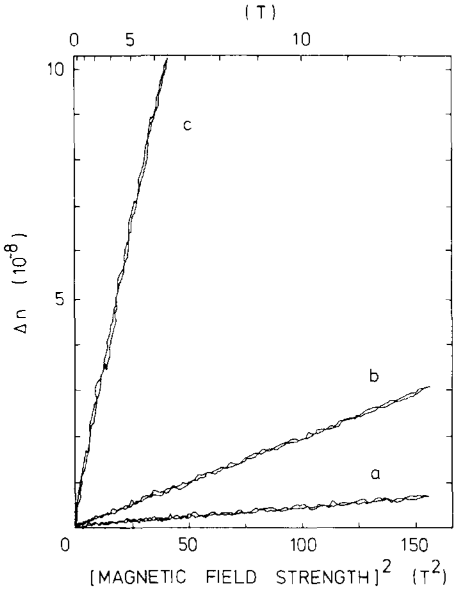


Fig. 2. Magnetic birefringence from monomeric and polymeric (Tyr-Glu) at t = 22°C and λ_0 = 6328 Å. (a) monomer at c = 50 mg/ml; (b) polymer at c = 25 mg/ml, α = 1; (c) polymer at c = 50 mg/ml, α = 1.

to that of benzene ($CM/c_0 = 6.7 \times 10^{-27}$ T^{-2} $cm^2/ring^{-1}$).[7] This result is not surprising because, in general, for small aromatic molecules the total diamagnetic anisotropy is mainly determined by the phenyl group and all contributions from other bond anisotropies are relatively small. Figure 3 shows that CM_{mono}/c_0 is independent of c up to very high concentrations, demonstrating that there is no measurable interaction between monomers under the given solvent conditions.

For the polymer, it is only at concentrations below 25 mg/ml, for $\alpha = 1$, that CM_{poly}/c_0 is independent of concentration, as expected when no interchain interaction occurs. At these low concentrations, we find $CM_{poly}/c_0 = 6.2 \times 10^{-26}$ T^{-2} cm^2 $ring^{-1}$, a value which is about 7 times higher than CM_{mono}/c_0. This observed birefringence corresponds, under the given solvent conditions, to 3.5 monomer units for the persistence length, b, and to 7 monomer units for the distance over which the phenyl groups along the backbone are correlated. This means that our experiments do not allow us to make firm conclusions about whether the (Tyr-Glu)$_n$ chain is in a more fully extended rodlike conformation.

At higher concentrations (> 25 mg/ml), well above the critical concentration $c^{++} \simeq 2.5$ mg/ml defined by de Gennes[5] and calculated for the *fully extended* molecule, a dramatic increase of the specific Cotton-Mouton constant with increasing concentration is observed under the given solvent conditions.

c^{++} corresponds to the transition from the dilute regime to the semidilute regime where

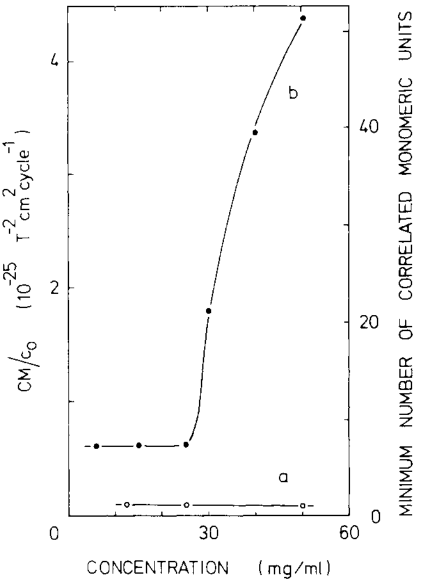


Fig. 3. Concentration dependence of the Cotton-Mouton constant normalized to the concentration of aromatic rings, c_0, of (a) (Tyr-Glu), $\alpha = 1$, $t = 22°C$, and of (b) $(\text{Tyr-Glu})_n$, $\alpha = 1$, $t = 22°C$. At the right scale, $(CM_{\text{poly}}/c_0)/(CM_{\text{mono}}/c_0)$ is given.

spheres having a diameter of the polymeric rod length nl_0, *i.e.*, a volume of $(4\pi/3)(nl_0/2)^3$, begin to overlap.

This may be attributed to the formation of domains of parallel chain segments. If the length of each chain segment is assumed to be identical to twice the magnetic persistence length $b = 3.5$ monomers, the number of ordered chain segments, n_0, in each domain must be larger than 10 at the highest concentration measured—where a tendency for saturation of the ordering process is observed (Fig. 3b). (n_0 would only be about 1.5, if we assumed the extreme case of fully extended rods and a completely parallel order of the tyrosine groups.)

The rapid and large increase of the Cotton-Mouton constant cannot be accounted for by a sudden increase of the persistence length of only *single* polymers. The magnitude of the increase requires that neighboring polymers interact and assume a parallel orientation.

The experiment has been performed with the polymer at different degrees of charge neutralization (Fig. 4). We discuss first the low-concentration range ($c \ll c^{++}$), where CM/c_0 is independent of c for all α values investigated. We observe a strong drop of CM/c_0 with increasing α. In principle, for $\alpha \leq 1$, two competing processes which might influence the persistence length of the polymer have to be considered. First, b should strongly *decrease* with an increase in the screening of the polymeric charges (i.e., with increasing $\mu \cong \alpha$). This process has been demonstrated theoretically, as well as experimentally, by the strong dependence of the poly-

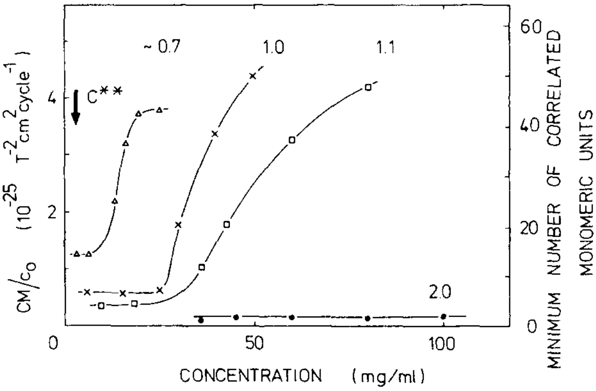


Fig. 4. Concentration dependence of the specific Cotton-Mouton constant for different degrees of dissociation: (▲) $\alpha \sim 0.7$, (x) $\alpha = 1$, (□) $\alpha = 1.1$, (●) $\alpha = 2$. c^{++} is the critical concentration calculated for extended rods.

meric radius of gyration on μ (see, for example, Ref. 15) and by recent Cotton-Mouton measurements on the highly acidic polyelectrolyte DNA.[6] Second, b should *increase* due to an increase of $\alpha\phi$. Since, under the described conditions of our experiment, $\alpha\phi$ is almost saturating when going from $\alpha = 0.7$ to $\alpha = 1.0$ and is constant for $\alpha \gtrsim 1$, we expect the second process to be largely overcompensated by the first one. This is in good agreement with our observation.

In addition, we expect that simultaneously lowering the local rigidity, b, and λ_D should enhance the concentration, c^{++}. This also agrees with the observed increase in the concentration at which onset of the local order occurs when going to higher α values. At very high α' values, no ordering at all can be observed up to the highest polymer concentrations investigated due to strong electrostatic screening; the polymer behaves like a very flexible chain having a persistence length comparable to the monomeric length, l_0. The breakdown of local polymer order and rigidity with increasing ionic strength is further demonstrated by the data shown in Fig. 5.

Furthermore, we recorded neutron small-angle scattering-intensity profiles from a solution of 40 mg/ml (solvent D_2O, $\alpha = 1$) that showed a small peak at scattering vectors q^+ corresponding to a preferential Bragg spacing $d = 2\pi/q^+ = 70$ Å between the chains in solution. This is compared with a distance of 47 Å between the axis of two fully extended molecules calculated for the cellular model of Katchalsky and coworkers.[10,11] The data are very similar to those obtained recently on poly(glutamic acid).[3]

It is interesting to note that, for each α value used in this study, the persistence length, b, is comparable to the mean distance, d^{++}, between ordered chains at the concentration, c^{++}, and that both b and d^{++} are only slightly larger than λ_D.

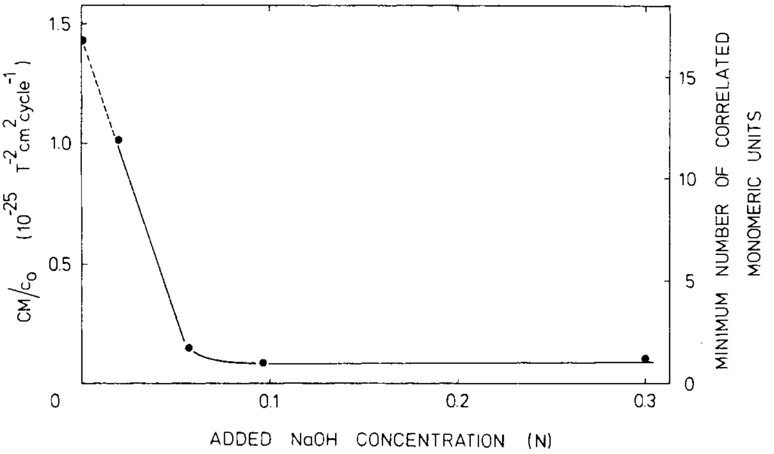


Fig. 5. Ionic strength dependence of the specific Cotton-Mouton constant of $(\text{Tyr-Glu})_n$, c = 48 mg/ml, t = 22°C, initial α = 1.1. The ionic strength is varied by subsequent addition of NaOH (2M); corresponding addition of NaCl produced precipitation.

Influence of Temperature

The solution was also heated at a rate of 2°C/min. Starting from conditons of intermediate order, we observed a remarkable drop of CM (Fig. 6). At high temperatures, we obtain a CM value corresponding to the persistence length of one isolated chain for $c < c^{++}$. Therefore, we interpret the temperature $t \simeq 51°C$ as the "melting" temperature of the ordered domains under the solvent conditions used. Furthermore, by cooling the solution to room temperature again, we observed a very pronounced hysteresis; e.g., up to 3 hr after the heating cycle, we still observed the high-temperature value of CM, whereas about 4 days later the initial CM value had been almost restored.

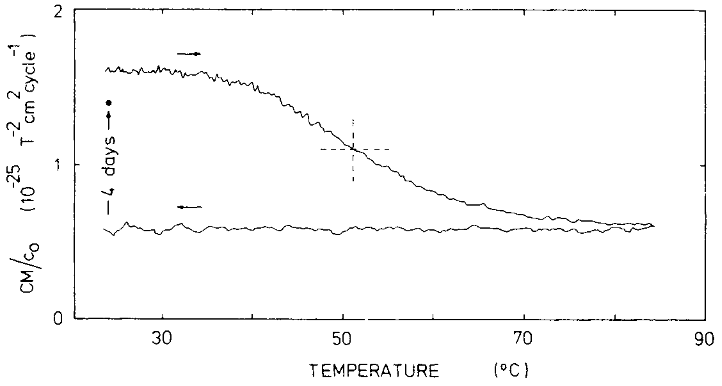


Fig. 6. Temperature dependence of the specific Cotton-Mouton constant of $(\text{Tyr-Glu})_n$ (α = 1.1, c = 48 mg/ml, dT/dt = 2°/min) measured continuously by the method described in the text and in Ref. 6, but using in addition a magnetic field modulation at 4 Hz and of amplitude 0.125 T superimposed on a steady field of 5.64 T. A detailed description of this technique will be given in a subsequent paper.

After some attempts with other materials, we note that the (Tyr-Glu)$_n$ polymer seems to be particularly well suited for magnetic orientation studies of this kind, probably because of the rather weak mobility of its aromatic groups in the ionized state.

CONCLUSION

The polypeptide (Tyr-Glu)$_n$ is very convenient material for a systematic magnetic orientation study of the conformation of polyelectrolytes under various solvent conditions. The aromatic ring seems to be a good label, having a mobility, with respect to the chain backbone, that is sufficiently weak to be used in determining the persistence length and local "crystallinity" under various degrees of dissociation, ionic strengths, and temperatures.

Our magnetic birefringence measurements demonstrate the existence of small domains in which chain segments of neighboring polymers are ordered. This result is consistent with our neutron scattering data, but, in addition, it enables us to determine the mean size of an ordered domain and demonstrates that within each domain the polymer segments are ordered parallel. A domain seems to contain at least 10 correlated polymers. This quantitative estimate is reliable if in our experiments the persistence length of the polymer backbone is identical to the persistence length of the phenyl groups along the backbone. The order sets in only above a certain critical polymer concentration and is strongly dependent on ionic strength and temperature. The high critical concentration needed for the onset of local order is enhanced by an increasing ionic strength, corresponding to a decrease in the persistence length. Furthermore, we have observed a "melting" temperature of 51°C and a pronounced hysteresis effect.

We think that more systematic kinetic studies of this kind and inelastic light-scattering experiments could give new information about the formation mechanism, stability, and dynamics of the electrostatic polymer lattice.

The experiments were carried out at the Service National des Champs Intenses and at the Max von Laue-Paul Langevin Institut, both in Grenoble, and we acknowledge their technical support. Furthermore, we are very grateful to Dr. Y. Trudelle for the gift of the polypeptide sample, to Dr. Timmins for carrying out the neutron experiments, and to Professors P. G. de Gennes and K. Dransfeld for carefully reading our manuscript and providing critical comments.

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Received October 12, 1977
Returned for Revision January 31, 1978
Accepted April 4, 17, 1978