d, $-CH_2O-$), 7.23–8.05 (7 H, m, aromatic H). Anal Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 71.36; H, 6.85.

3-(1-Naphthyl)-*n*-propanol (3). To suspensions of lithium aluminum hydride (7.1 g) in THF, 14.6 g of β-(1-naphthyl)propionic acid was added dropwise. The product obtained after refluxing for 16 h was separated and distilled at 151–152 °C (0.4 mm) (10.7 g, 83.1%) (lit.[20] 125–127 °C (0.01 mm)).

3-(1-Naphthyl)-*n*-propyl Acetate (MN-II). The alcohol **3** was acetylated by the same method as the preparation of MN-I: bp 182 °C (0.2 mm); ir (neat) 1740 cm^{-1}. Anal. Calcd for $C_{15}H_{16}O_2$: C, 78.92; H, 7.06. Found: C, 79.05; H, 6.98.

Dimer Model Compounds: DN-2, DN-4, and DN-8 were prepared by reactions of **3** with succinyl chloride, adipoyl chloride, and sebacyl chloride, respectively, in benzene and recrystallized from *n*-hexane. DN-2: mp 58–59 °C; ir (KBr disk) 1730 cm^{-1}; NMR ($CDCl_3$) δ 1.90 (4 H, m $-CH_2CH_2CH_2C_{10}H_7$), 2.63 (4 H, s, $-C(=O)CH_2CH_2C(=O)-$), 4.33 (4 H, t, $CH_2C_{10}H_7$), 7.40–8.30 (14 H, m, aromatic H). Anal. Calcd for $C_{30}H_{30}O_4$: C, 79.27; H, 6.65. Found: C, 79.22; H, 6.47. DN-4: mp 66–67 °C; ir (KBr disk) 1732 cm^{-1}. Anal. Calcd for $C_{32}H_{34}O_4$: C, 79.64; H, 7.10. Found: C, 79.41; H, 7.15. DN-8: mp 52–53 °C; ir (KBr disk) 1740 cm^{-1}. Anal. Calcd for $C_{36}H_{42}O_4$: C, 80.26; H, 7.86. Found: C, 80.46; H, 7.69.

Solvents. THF and ethyl acetate were purified by accepted procedures.

Polycondensation. (i) An equimolar mixture of **2** and a dimethyl or diethyl ester of dicarboxylic acid together with a small amount of catalyst was heated gradually in a stream of nitrogen bubbled through the reaction mixture with such a rate that the reaction temperature reached 150–160 °C after 3 h. When the viscosity of the reaction mixture began to increase, the vessel was gradually evacuated to the final pressure of 0.02–0.05 mm during a period of 10 h. The polycondensate was reprecipitated from THF–*n*-hexane.

(ii) An equimolar mixture of **2** and sebacyl chloride was reacted without catalysts or acid acceptors. The evolved hydrogen chloride was removed by bubbling nitrogen through the reaction mixture. The reaction conditions were the same with (i) except for a shorter reaction period of 4–6 h.

Determination of the Degree of Polycondensation. The number of repeating units in a polymer was estimated by GPC using a Toyo Soda HIC-801A (eluting solvent; THF). The molecular weight–count number relation was calibrated for the monomer and dimer model compounds. The determined molecular weight of polymers agreed well with the values determined by means of vapor pressure osmometry.

Fluorescence Spectroscopy. The fluorescence spectra were measured by a Hitachi MPF-4 fluorescence spectrometer. In the concentration region of the present measurements, any corrections for the reabsorption of fluorescence by solutes were not required. As a measure of excimer formation, the ratio of fluorescence intensity at 400 nm (excimer emission, F_e) to the value at 324 nm (monomer emission, F_m) was determined.

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Properties of Monomeric Paramyosin Using Transient Electric Birefringence Techniques[1]

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ABSTRACT: Paramyosin samples obtained from the chowder clam, *Mercenaria mercenaria*, by different extraction techniques were studied using transient electric birefringence techniques. The proteins remain monomeric (unaggregated) in 1 mM buffer solution at pH 3.1 to 3.8 and near pH 10. At pH 3.2, the molecules obtained by different extraction techniques exhibit rotational diffusion constants that indicate a 5% difference in length between them, with the probable native form of paramyosin being the longer species. This difference in rotational diffusion constant disappears at higher pH, and, in addition, a large difference in dipole moment between the molecules observed at pH 3.2 also disappears at high pH. These results are used to hypothesize that the rodlike native paramyosin molecules have one or two partly flexible portions on their ends; at one end of each molecule this portion probably contains excess basic amino acids which are charged at low pH to account for the higher dipole moment of this form of paramyosin at these low pH values. At pH 3.2, these portions of the macromolecule are not flexible and act as stiff parts of the rodlike molecules, but they gradually become flexible at higher pH. Possible mechanisms for this change in flexibility are discussed.

In addition to the myosin and actin that are found in most muscle tissue, molluscan muscles contain large amounts of another fibrous protein, paramyosin. In these muscles, the paramyosin is located in large filaments that are analogous to the myosin-containing filaments found in skeletal muscle. The role of paramyosin in these muscles is thought to be purely structural by some[3] while others have proposed that it plays a major role in the "catch contractions" peculiar to molluscan muscle.[4,5] The "catch contraction" is characterized by the ability of these muscles to contract for long periods of time with the utilization of little or no energy.

It has been shown that paramyosin is a rod-shaped protein with a two-chain α-helical coiled coil structure.[6,7] The two chains, which appear to be identical,[8] probably both run in the same direction giving rise to a molecular polarity. The length of the molecule determined by light scattering[9] and electron microscopy[10] is in the range of 1200 to 1300 Å with some differences between species. The molecular weight lies in the range of 190 000 to 220 000 also with some interspecies differences.[10]

It has been known for some time that different procedures for extracting paramyosin from molluscan muscle result in samples whose properties are appreciably different. One such procedure is the ethanol extraction procedure as developed by Bailey[11] and modified by Johnson et al.[4] In this technique, the paramyosin, along with actin, myosin, and tropomyosin, is extracted from the muscle using a 0.6

M KCl solution. The extracted proteins are then precipitated in 95% ethanol to denature the actin and myosin without affecting the paramyosin which is then redissolved in 0.6 M KCl, pH 7, and is then further purified and characterized. Of particular interest is the solubility behavior of the paramyosin which shows a very sharp precipitation region near pH 7 which is strongly dependent upon the pH and ionic strength.[4] The needle-like paracrystals that precipitate have characteristic banding patterns as seen by electron microscopy which reflect the arrangement of the aggregated molecules.[10] Another extraction procedure is the acid extraction procedure, in which the actin and myosin are removed from the sample by precipitation in acetic acid.[12] The molecular properties of the resulting paramyosin sample appear to be the same as those of ethanol-extracted paramyosin, but the acid-extracted paramyosin shows a lower solubility than that of the protein obtained by the ethanol technique.[12] In addition, the paracrystals precipitated from the acid extracts are longer and more laterally aggregated than those obtained by the ethanol method.

The difference observed in the paramyosin obtained by the two different extraction methods was explained by Stafford and Yphantis.[13] They found, by SDS gel electrophoresis of paramyosin extracted from the adductor muscle of the chowder clam, that the acid-extraction procedure produced a protein with a molecular weight 5% greater than that obtained from the ethanol extraction method. They were able to show, moreover, that when EDTA was included in all of the steps of the ethanol extraction, the protein obtained was the same as that isolated by the acid procedure, and that, furthermore, the reduced form of this molecule, α_R-paramyosin, is in the native form. The protein extracted by the unmodified ethanol extraction method was called β-paramyosin.

Although they were able to measure a 5% difference in the molecular weight of α- and β-paramyosin by gel electrophoresis, Stafford and Yphantis were unable to confirm this difference by ultracentrifugation. In addition, the amino acid contents of the two forms of the protein were essentially identical except for a small difference in the serine content. Stafford and Yphantis concluded that β-paramyosin is a degraded form of the native reduced α-paramyosin. They speculated that α-paramyosin has an "extra piece" on one end which is attacked by a metalloenzyme released during the extraction procedure. This metalloenzyme becomes inoperative in acid solution or in the presence of EDTA.

Since very small changes in the structure of the large paramyosin molecule appear to have such a great effect upon its properties, it was of interest to obtain more information on these structural differences. Transient electric birefringence was selected as a suitable technique for these studies, since it provides a sensitive measure of the size of the species present in solution as well as a measure of their dipole moments and other electrical properties.

The transient electric birefringence technique is described at length in a recent monograph[14] and three recent reviews of the theoretical and experimental aspects of transient electric birefringence of macromolecules in solution.[15-17]

When an electric field is applied to a solution of polar or electrically anisotropic molecules they tend to be oriented by the field. If plane polarized light, with its plane of polarization at 45° to the applied field, is then passed through the solution of the partially oriented molecules, components of the light beam polarized parallel and perpendicular to the applied field travel along the same path, but at

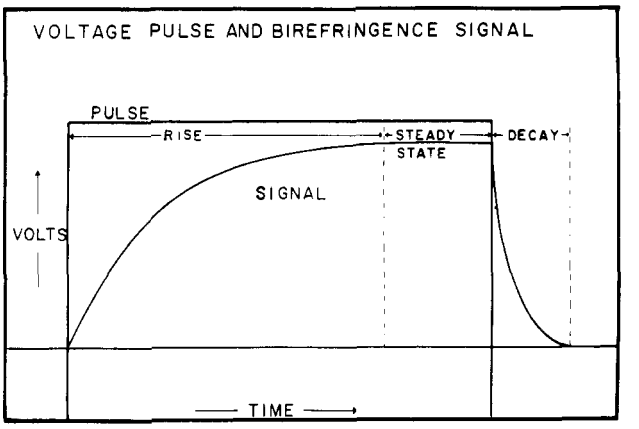

Figure 1. Schematic diagram of voltage pulse and birefringence signal.

different velocities. This leads to a difference in the optical path length for the two components:

$$\delta = \frac{2\pi l(n_{\parallel} - n_{\perp})}{\lambda} \tag{1}$$

where δ is the retardation, in radians, of the component parallel to the field with respect to the component perpendicular to the field, l is the path length through the solution, λ is the wavelength of the incident radiation in vacuo, and $n_{\parallel}$ and $n_{\perp}$ are the refractive indices of the solution parallel and perpendicular to the direction of the electric field. Their difference $\Delta n = n_{\parallel} - n_{\perp}$ is called the birefringence of the solution.

When a rectangular electric field pulse is applied to a solution of macromolecules, the observed birefringence signal is composed of three distinct regions; the rise, the steady state, and the field free decay (see Figure 1). When a rectangular pulse is applied and then reversed in polarity, the same three regions are observed plus a transient at the time of field reversal if the molecule contains a permanent dipole (see Figure 2).

At low electric field, the steady state birefringence of the solution is described in terms of a specific Kerr constant K_{sp},

$$K_{sp} = \Delta n / C_v n E^2 \tag{2}$$

where C_v is the volume fraction of orientating macromolecule in the solution, n is the refractive index of the solution, and E is the electric field. If the macromolecule is in the form of an elongated particle with dipole moment along the long axis, then

$$K_{sp} = \frac{2\pi(g_1 - g_2)}{15n^2}(P + Q) \tag{3}$$

where

$$P = \left[\frac{\mu}{kT}\right]^2 \text{ and } Q = \frac{(\alpha_1 - \alpha_2)}{kT} \tag{4}$$

and μ is the apparent permanent dipole moment, α_1 and α_2 are the excess electrical polarizabilities along the long axis of the molecule and perpendicular to this axis, respectively, k is the Boltzmann constant, and T is the absolute temperature. In a conducting solution, the Q term may involve the conductivity of the solvent and the surface conductivity of the polyelectrolyte,[18] but the exact form of this term will not be considered in this paper.

The term $(g_1 - g_2)$ is the optical anisotropy of the orienting molecule. For prolate ellipsoids with large axial ratios, the value of the optical anisotropy can be expressed as[19]

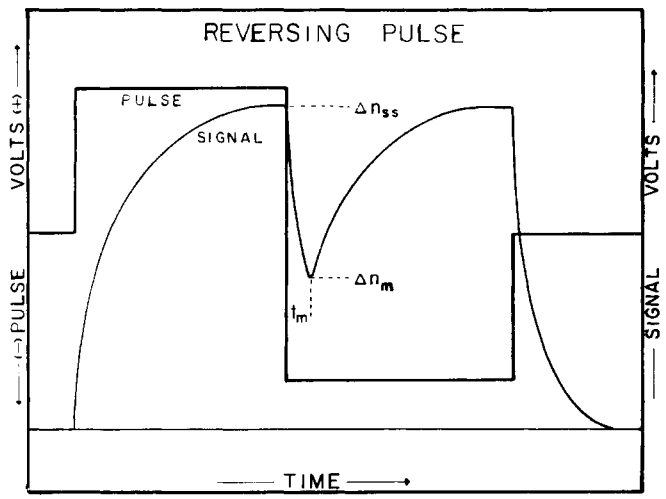

Figure 2. Schematic diagram of reversing voltage pulse and birefringence signal.

$$g_1 - g_2 = \frac{2\pi(n_1^2 - n_2^2) + (n_1^2 - n_0^2)(n_2^2 - n_0^2)/n_0^2}{4\pi[2\pi + (n_2^2 - n_0^2)/n_0^2]} \quad (5)$$

where n_1 and n_2 are the refractive indices along the major and minor axes of the ellipsoid, respectively, and n_0 is the refractive index of the solvent. Since the optical anisotropy of a macromolecule can be expected to remain constant with changes in pH and ionic strength of the solution unless there is a change in conformation, the specific Kerr constant can be directly related to the dipolar properties of the molecule.

The orientation of a particle in an electric field is opposed by random Brownian motion. When the electric field is removed, this disorienting effect results in the decay of the birefringence to zero as the particles become disoriented by the process of free rotational diffusion. For a dilute system of monodisperse particles, the field free decay of the birefringence is exponential[20,21]

$$\Delta n/\Delta n_0 = e^{-t/\tau} \quad (6)$$

where Δn is the magnitude of the birefringence at time t and Δn_0 is the birefringence at $t = 0$, when the field is removed. The relaxation time τ is defined as the period of time in which the birefringence decays to $1/e$ of its value at $t = 0$. For a rigid elongated particle, the relaxation time τ is related to the rotational diffusion constant for rotation about the major axis, θ, by the expression

$$\theta = 1/6\tau \quad (7)$$

The rotational diffusion constant and thus the relaxation time can be related to the size and shape of the rotating particle through several equations depending upon the model chosen. In this paper, all forms of paramyosin are considered as rigid cylinders with axial ratios much greater than 1. For this model, an equation developed by Broersma[22] is used:

$$\theta = \frac{3kT}{8\pi\eta a^3}\{\ln(2a/b) - 1.57 + 7(1/\ln(2a/b) - 0.28)^2\} \quad (8)$$

where $2a$ and $2b$ are the length and diameter of the cylinder, respectively, and η is the viscosity of the solvent.

When a molecule is oriented in an electric field and that field is suddenly reversed in polarity, the presence of a permanent dipole requires that the molecule rotate to reorient itself in the new field. This reorientation of the molecule gives rise to a transient in the birefringence observed for the system at the time of field reversal. Tinoco and Yamaoka[23] have shown that for elongated ellipsoids of revolution at low field strengths, the transient in the birefringence upon reversal of the field depends upon the ratio P/Q according to the expression

$$(\Delta n/\Delta n_0) = 1 + \frac{3(P/O)}{(P/Q) + 1}(e^{-6\theta t} - e^{-2\theta t}) \quad (9)$$

Equation 9 shows that if $P/Q = 0$ (i.e., the orientation of the molecule is due to only induced dipole moments) the birefringence will remain unchanged upon reversal of the field.

When a permanent dipole moment directed along the major axis of the orienting particle is present, the depth of the transient observed on reversal of the field is related to the ratio P/Q through the expression

$$P/Q = \frac{1 - (\Delta n_m/\Delta n_0)}{0.1547 + (\Delta n_m/\Delta n_0)} \quad (10)$$

where Δn_m is the magnitude of the birefringence at the minimum in the transient (see Figure 2).

Experimental Section

Materials. The chowder clams, *Mercenaria mercenaria*, used as a source of paramyosin in the experiments were obtained fresh from a local fish market. The various forms of paramyosin were all extracted from the white portion of the adductor muscles. β-Paramyosin was extracted from the muscle tissue by the ethanol extraction method of Bailey[11] as modified by Johnson et al.[4] All of the reagents used in the extraction were of analytical reagent grade except for doubly distilled deionized water. Each protein sample was recrystallized at least three times. When the ratio of the uv absorbance at 280 and 260 nM, measured on a Cary 14 UV-Visible spectrophotometer, was greater than two, the absence of contaminating nucleotides in the samples was assumed. Further evidence for the purity of the paramyosin was provided by polyacrylamide gel electrophoresis in the presence of the detergent sodium dodecyl sulfate (SDS). Only a single protein peak was observed for each sample. The β-paramyosin was stored in 0.6 M KCl, 0.01 M potassium phosphate buffer, pH 7 (high ionic strength buffer, HISB), at 4 °C. None of the samples used in these experiments was stored for more than 1 week.

The α-paramyosin with reduced sulfhydryl groups, α_R-paramyosin, was isolated using the ethanol extraction method with the addition of 10 mM potassium EDTA and 0.5 mM dithiothreitol to all of the steps, following Stafford.[24] The purity of the α_R-paramyosin was monitored in the same way as the β-paramyosin; storage was similar, except that 10 mM potassium EDTA and 0.5 mM dithiothreitol were included in the solutions.

The one sample of α_O-paramyosin studied was prepared by the oxidation of an aliquot of α_R-paramyosin in the presence of 0.5 mM free Cu^{2+} as described by Cowgill.[13] The oxidation was carried out for 24 h at room temperature to assure complete oxidation. The sample was recrystallized and stored in the same way as β-paramyosin.

In preparation for a series of electric birefringence measurements, a sample of paramyosin stock solution was diluted volumetrically to the desired protein concentration with the HISB. Aliquots of this solution were placed in dialysis sacks and dialyzed at 4 °C against a 1 mM buffer solution at the desired pH for 24–28 h using five changes of buffer solution. Buffers used in these experiments included citrate in the range pH 3–4, and 3-(cyclohexylamino)propanesulfonic acid, CAPS, a Good's buffer,[25] Polysciences, Inc., Warrington, Pa., for pH 10 and higher. After dialysis, each sample was centrifuged at 18 000 rpm for 20 min to clarify the solution. Prior to an electric birefringence measurement, the concentration of each sample was determined spectrophotometrically from the tyrosine–tryptophan absorbance at 277 nM. An extinction coefficient $E_{1\,cm}^{1\%}$ of 3.125 determined for β-paramyosin by Gaffin[26] was used in all low pH measurements. At high pH, above 10, it was necessary to correct the absorption measurements for the shift in peak absorbance due to the titration of the tyrosine residues; the maximum correction in this work was 5%. The pH of each paramyosin solution was measured on a Beckmann Expandomatic SS-2 pH meter before and after each birefringence run.

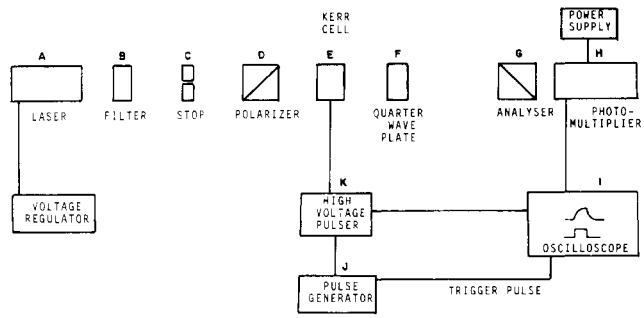

Figure 3. Schematic diagram of the transient electric birefringence apparatus.

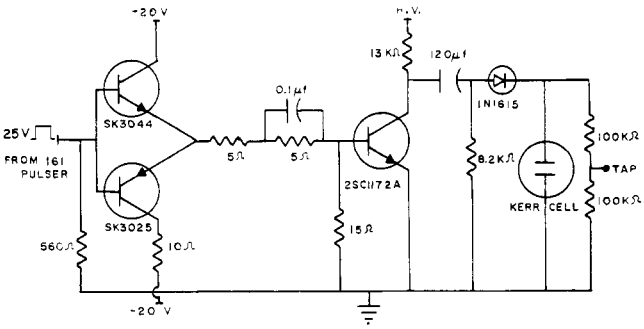

Figure 4. Circuit diagram of the transistorized pulsing unit.

Birefringence Apparatus. A schematic diagram of the apparatus used in these experiments is shown in Figure 3. The light source was a 1 mW Spectra Physics Model 132 Helium–Neon laser (A), λ 632.8 nM. The beam of laser light was initially passed through a red filter (B) to eliminate stray wavelength components and to decrease the intensity, and then through a collimating stop (C). The collimated beam was then plane polarized at 45° to the plane of the horizontal electric field by a Glan-Thompson prism. From the polarizer the plane polarized beam was passed through the sample chamber (E) which consisted of a 1-cm Pyrex spectrophotometer cell selected for the absence of any detectable strain birefringence. The cell was thermostated at 20 ± 0.2 °C for most measurements; the temperature was raised to 60 °C for thermal stability measurements.

In the cell the light beam passed between two platinum bars which served as the electrodes. These electrodes were held parallel, 2.30 ± 0.05 mm apart, by a special bakelite electrode holder which also served as the top to the cell. The electrode holder, which was fitted with plug receptacles for connection to the pulsing unit, was held firmly in position by two guides in the cell mount which also served to position the electrodes in the center of the cell.

The light beam emerging from the Kerr cell passed through a 632.8 nM quarter wave plate (F), with its slow axis oriented at 135° with respect to the plane of the electric field, and then through an analyser (G), another Glan-Thompson prism identical with the polarizer, but mounted in a rotating holder which could be read to 0.05°. The photomultiplier was an EMI 9558 B. When used with a 3 kΩ anode resister the circuit time constant was 2.4 μs, which allows birefringence time constants above 11.2 μs to be determined accurately (Appendix A). The oscilloscope was a Tektronix 547 Dual Trace Oscilloscope for obtaining simultaneous voltage pulse and birefringence signals which were photographed on Polaroid transparencies.

Two different types of orienting waveforms were used in this work: a single rectangular pulse and a reversing rectangular pulse. The single rectangular pulse was produced by two different units: a medium range (0-1000 VDC) transistorized pulser constructed in this laboratory and a high-voltage unit (0-4400 VDC) constructed from a stacked combination of two commercially available Cober 605P High Power Pulse Generators. The reversing pulses were generated by the same two Cober 605P pulsers with different polarities.

The transistorized pulsing circuit was essentially a capacitor discharge circuit (Figure 4) in which a 120 μF capacitor was discharged across the electrodes of the Kerr cell. The switching circuit which produced the rectangular pulses was centered upon a Toshiba 2SC1172A power transistor that would operate normally at currents of up to 5 A. This circuit was triggered by a 25-V pulse of the desired duration produced by a Tektronix Type 161 Pulser triggered by a Tektronix Type 162 Waveform Generator.

Table I
pH Dependence of the Electric Birefringence Properties of Paramyosin, 20 °C, 1 mM Citrate Buffer pH 3.1–3.8

pH	Concn, mg/ml	$K_{sp} \times 10^5$, esu	τ, μs
		α_R-Paramyosin	
3.14	0.49	4.45 ± 0.09	23.5 ± 0.9
3.21	0.42	5.13 ± 0.17	23.8 ± 0.7
3.30	0.69	3.85 ± 0.05	23.4 ± 0.5
3.29	0.45	3.71 ± 0.12	19.9 ± 0.4
3.48	0.45	3.17 ± 0.07	19.8 ± 0.6
3.78	0.45	2.83 ± 0.07	19.4 ± 0.4
		α_O-Paramyosin	
3.2	0.47	4.38 ± 0.04	25.5 ± 1.0
		β-Paramyosin	
3.10	0.50	1.96 ± 0.02	18.3 ± 0.2
3.20	0.64	1.64 ± 0.03	20.0 ± 0.9
3.22	0.72	1.32 ± 0.02	21.1 ± 2.1
3.27	0.74	1.35 ± 0.02	20.4 ± 1.0
3.43	0.75	1.32 ± 0.02	20.2 ± 1.3
3.59	0.75	1.31 ± 0.02	19.8 ± 0.6
3.78	0.65	1.27 ± 0.02	24.7 ± 2.7

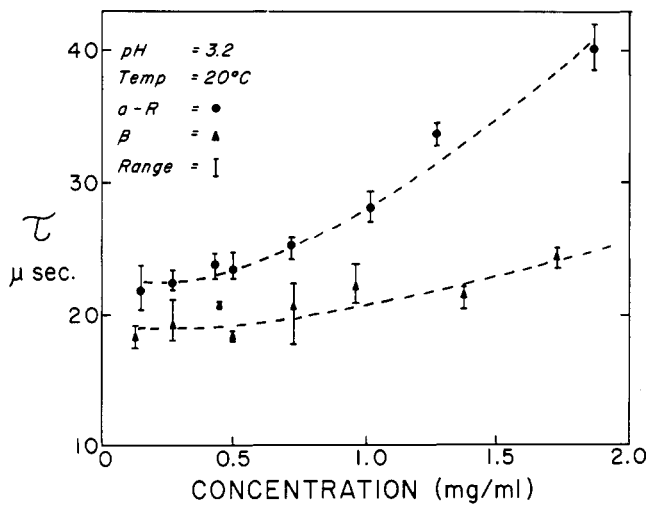

Figure 5. Relaxation time, τ, vs. concentration for α_R- and for β-paramyosin in 1 mM citrate buffer.

Results

The values of the specific Kerr constants shown in the tables were obtained from a least-squares fit of the low-field values of δ vs. E^2. The precision of these values is represented by the mean square deviation from the mean, approximately 10% in most cases. The precision of tabulated values of the relaxation times is also about 10%, but is stated in terms of average deviation from the mean of at least three measurements on each sample.

Single Pulse Experiments. The pH dependence of the field free relaxation time and the specific Kerr constants obtained for solutions of all forms of paramyosin in 1 mM citrate buffer over the pH range of 3–4 are shown in Table I. In this pH region, the molecule appears to be monomeric and shows no apparent aggregation up to pH 4 where it

Table II
pH Dependence of the Electric Birefringence Properties of Paramyosin, 20 °C, 1 mM CAPS Buffer

pH	Concn, mg/ml	$K_{sp} \times 10^{-5}$, esu	τ, μs
		α_R-Paramyosin	
10.00	0.44	5.92 ± 0.16	20.0 ± 1.1
11.27	0.42	7.82 ± 0.18	19.7 ± 0.5
		α_O-Paramyosin	
9.90	0.35	5.90 ± 0.11	20.2 ± 0.1
		β- Paramyosin	
9.65	0.88	7.13 ± 0.12	21.2 ± 0.9
10.10	0.72	5.92 ± 0.09	20.7 ± 1.0
10.30	0.58	7.61 ± 0.17	19.8 ± 0.2

Table III
Electric Birefringence Properties of Paramyosin from Reversing Pulse Measurements, 20 °C

Buffer	pH	Concn, mg/ml	P/Q
		α_R-Paramyosin	
1 mM citrate	3.10	0.49	2.24 ± 0.22
1 mM citrate	3.29	0.45	2.18 ± 0.01
1 mM citrate	3.71	0.45	2.10 ± 0.10
1 mM CAPS	10.00	0.44	14.5 ± 2.4
1 mM CAPS	11.27	0.42	∞
		β-Paramyosin	
1 mM citrate	3.13	0.50	0.729 ± 0.075
1 mM citrate	3.20	0.64	0.808 ± 0.055
1 mM citrate	3.78	0.28	0.474 ±0.010
1 mM CAPS	10.30	0.58	13.04 ± 2.72

forms a thick gel. Gelation may occur at lower pH after prolonged pulsing. None of these gels were investigated further. The relaxation time of α_R-paramyosin decreased from 23.5 μs at pH 3.14 to 19.4 μs at pH 3.80. Over the same pH range, the specific Kerr constant also decreased from a value of 4.5×10^{-5} esu[27] at pH 3.14 to 2.8×10^{-5} esu at pH 3.80.

The concentration dependence of the field free relaxation time of α_R-paramyosin at pH 3.1–3.3 is shown in Figure 5. The observed concentration dependence of the relaxation time cannot be attributed to the small variations in pH, but is probably due to intermolecular interactions. When the data were extrapolated to C → O, a relaxation time of 22.0 μs was obtained. In contrast to the relaxation time, the specific Kerr constant of α_R-paramyosin remained essentially constant over the entire range of concentrations.

At and above pH 10, α_R-paramyosin also exists only in the monomeric form. High pH data on all forms of paramyosin are shown in Table II. The relaxation time of the species present at pH 10, 20.0 ± 1.1 μs, was close to that observed for the molecule at pH 3.8. The specific Kerr constant of the molecule in this pH region 7 ± 1 × 10^{-5} esu showed some scatter, but was invariably higher than the values measured at lower pH. When the pH was increased above pH 10, no significant changes in the relaxation time or specific Kerr constant were noted.

The oxidized form of α-paramyosin, α_O-paramyosin, was similar to α_R-paramyosin both at low and at high pH. The relaxation time of β-paramyosin remained essentially constant over the range of pH 3.2–3.6 with small variations at higher and lower pH. The specific Kerr constant for β-paramyosin in this pH range is much smaller than that of the

Table IV
Temperature Dependence of the Electric Birefringence Properties of Paramyosin, 1 mM Citrate Buffer pH 3.1–3.2, 0.50 mg/ml

T, °C	pH	$K \times 10^5$, esu	τ, μs	$\tau_{20°}$, μs
		α_R-Paramyosin		
20.0 ± 0.2	3.14	4.45 ± 0.09	23.5 ± 0.7	23.5 ± 0.7
25.1 ± 0.2	3.10	4.01 ± 0.07	20.0 ± 0.6	23.1 ± 0.6
30.0 ± 0.2	3.12	3.92 ± 0.05	18.0 ± 0.7	23.4 ± 0.8
35.0 ± 0.2	3.13	3.70 ± 0.10	16.9 ± 0.2	24.7 ± 0.3
40.3 ± 0.5	3.17	3.00 ± 0.07	13.7 ± 0.4	22.5 ± 0.6
45.1 ± 0.5	3.18	2.83 ± 0.10	14.0 ± 0.3	25.5 ± 0.6
49.7 ± 0.5	3.21	2.71 ± 0.95	12.5 ± 0.5	25.0 ± 0.9
		β-Paramyosin		
20.0 ± 0.2	3.10	1.96 ± 0.02	18.3 ± 0.2	18.3 ± 0.2
25.0 ± 0.2	3.10	1.77 ± 0.2	16.3 ± 0.2	18.6 ± 0.3
30.0 ± 0.2	3.12	1.38 ± 0.05	14.8 ± 0.8	19.2 ± 1.1
35.0 ± 0.2	3.15	1.54 ± 0.02	14.1 ± 0.5	20.6 ± 0.7
39.9 ± 0.2	3.17	1.27 ± 0.11	12.7 ± 0.3	20.8 ± 0.5
45.1 ± 0.5	3.18	1.31 ± 0.01	11.7 ± 0.8	21.3 ± 1.5
48.9 ± 0.5	3.20	1.25 ± 0.02	11.2 ± 0.4	22.4 ± 0.9

α_R form; from pH 3.3 to 3.8 the value remained constant at 1.3×10^{-5} esu, although it was as large as 1.9×10^{-5} esu at pH 3.1.

In contrast to α_R-paramyosin, β-paramyosin relaxation times show only a moderate concentration dependence (see Figure 5). Extrapolation of the data to C → O yielded a relaxation time of 18.5 μs. At high pH, 10–10.3, β-paramyosin has a relaxation time and a specific Kerr constant which are essentially the same as those for both α_R- and α_O-paramyosin.

Reversing Pulse Experiments. α_R- and β-paramyosin each exhibited a transient in the birefringence signal with field reversal indicating the presence of a permanent dipole moment in both forms of the molecule. No reversing pulse experiments were performed upon the α_O-paramyosin. The value of P/Q obtained from these measurements in the pH 3–4 range and near pH 10 are shown in Table III. In 1 mM citrate buffer at pH 3.1, α_R-paramyosin had a P/Q value of 2.24. This value remained constant, within error, as the pH was increased to 3.7. In contrast, β-paramyosin had a P/Q of 0.73 at pH 3.1 and showed a 40% decrease when the pH was increased to 3.8. At pH 10, the P/Q values of the two forms of the molecule were similar, with values of 14 ± 2. When the pH of a sample of the α_R-paramyosin was increased to 11.3, P/Q approached infinity indicating a purely permanent dipole moment orientation mechanism. These data appear to indicate that, at and above pH 10, both α_R- and β-paramyosin have similar, if not identical, charge and size.

Thermal Stability Studies of α_R- and β-Paramyosin. The thermal stability of samples of α_R- and β-paramyosin at pH 3.1–3.2 was studied by measurement of the field-free relaxation times over the temperature range 20–50 °C. The results of these experiments are shown in Table IV. The values in the last column are the field-free relaxation times corrected to 20 °C by the relation

$$\tau_{20°,w} = \frac{(\eta_{20\,°C,w})(T)}{293.15(\eta_{T,w})}\tau \qquad (9)$$

where τ = observed relaxation time; $\eta_{20\,°C,w}$ = viscosity of water at 20 °C; $\eta_{T,w}$ = viscosity of water at the temperature of measurement, T; T = temperature of measurement (K).

These experiments showed that both α_R- and β-paramyosin are extremely stable toward thermal denaturation at pH 3. Within the error of measurement, no change was

seen in the dimensions of α_R- or β-paramyosin over the entire 20–50 °C temperature range. It was also noted that the difference in the relaxation times (and thus the difference in length) of the two forms of paramyosin also remained unchanged.

Discussion

The Broersma equation (eq 8) relating the birefringence relaxation time to the axial ratio of a rigid cylinder was used to obtain a measure of the length of the molecule in all cases where the field-free relaxation curve indicated the presence of only monomeric paramyosin. In order to obtain a unique value for the length of the molecule it was also necessary to assume a diameter for the rigid cylinder. Diameters ranging from 15 to 22 Å have been calculated for the coiled coils of paramyosin;[6,9,12,28] in this work we used the most frequently reported value of 20 Å which also seems to be common to other fibrous muscle proteins such as myosin and tropomyosin.[9,29] Furthermore, the Broersma equation is rather insensitive to the diameter of a long cylinder, and thus the lengths calculated using the maximum and minimum reported diameters differ by only a few percent.

The Broersma equation was thus used to calculate the length of α_R- and β-paramyosin at pH 3.1 from the relaxation time extrapolated to C → O. These relaxation times gave calculated lengths of 1220 ± 40 and 1150 ± 20 Å for α_R- and β-paramyosin respectively. Figure 5 shows that there was considerable concentration dependence for the relaxation time of α_R-paramyosin at this pH, and a small concentration dependence for that of β-paramyosin. All our data were included in Figure 5 so that the reader may decide for himself whether our extrapolations are meaningful. We feel that these data show definite differences between α_R- and β-paramyosin.

Because of these concentration dependences a simple calculation was done to estimate at what concentration the relaxation time would be affected by the collisions of the molecules during rotation. It was assumed that each rotating molecule sweeps out an excluded volume equivalent to that of a sphere with a diameter equal to the length of the paramyosin molecule. It was further assumed that at the maximum concentration, above which collisions would occur, the spheres of excluded volume could be represented as in a closest packed form where they ideally occupy 72% of the total volume. From these assumptions and the calculated lengths of α_R- and β-paramyosin, the number of molecules per centimeter under these conditions was calculated. The weight to volume concentration was then determined using Avogadro's number and the molecular weights of 210 000 for α_R-paramyosin and 200 000 for β-paramyosin reported by Stafford.[24] The hypothetical values of concentration at which molecular collisions would begin to affect rotational diffusion calculated from this model were 0.27 and 0.32 mg/ml for α_R- and β-paramyosin, respectively. Although the model used was crude, Figure 5 does indeed show that the relaxation times of both forms of paramyosin become almost constant at a concentration of about 0.3 mg/ml. However, the rapid increase in the relaxation time with concentration of α_R-paramyosin relative to that of β-paramyosin cannot be attributed to the 5% difference in the length of the two species. It is possible that this difference in concentration dependence may reflect a higher charge on the α_R form; molecules with a higher charge would be expected to interact more strongly than molecules with a lesser charge.

Relaxation times measured at pH values greater than 3.1 were obtained at concentrations of 0.45 ± 0.05 mg/ml for α_R-paramyosin and 0.70 ± 0.05 for β-paramyosin. From Figure 5 it can be seen that the relaxation times measured at these concentrations are probably not very different from the extrapolated values and will accurately reflect the relative difference in molecular size of the two species.

As the pH was increased from 3.1 to 3.8, the relaxation time and subsequently the calculated length of the α_R-paramyosin decreased from a value of 1250 Å to 1170 Å at the higher pH. At the same time the calculated length of β-paramyosin remained essentially constant at 1180 Å over the same pH range. It is important to note that at pH 3.8 and above pH 10 the relaxation times and thus the calculated lengths of these two forms of paramyosin are identical within the error of measurement. The greater length of α_R-paramyosin at least at pH 3.1 appears to correspond to the "extra piece" hypothesized by Stafford[24] to be responsible for the 5% increase in the molecular weight relative to the β form that he observed by polyacrylamide gel electrophoresis. The significance of the decrease in relaxation time and, therefore, of calculated length of α_R-paramyosin at pH >3.2 will be discussed below in connection with the data on Kerr constants and dipole moments.

The calculated length of the β-paramyosin monomer in the pH 3–4 region is markedly smaller than the value obtained by electron microscopy (1260 Å)[10] and light scattering (1330 Å)[9] although a length of 1200 Å has been obtained from flow birefringence studies of β-paramyosin from *M. mercenaria*.[30] Although there are no reported values for the length of α_R-paramyosin, it was interesting to note that the calculated length of 1220 Å at pH 3.1 is within the range of the 1257 ± 35 Å electron microscopic value obtained for *M. mercenaria* paramyosin (possibly the α form) by Cohen et al.[10]

At pH 3.1, the specific Kerr constants of α_R- and β-paramyosin exhibit a concentration independent difference of 3.1×10^{-5} esu that cannot be attributed solely to the difference in the length of the two forms, but which probably reflects a difference in their dipole moments. Indeed, high-field birefringence and reversing pulse experiments indicate that α_R-paramyosin has a significantly larger permanent dipole moment than β-paramyosin at this pH.

The ratio of the permanent to induced dipole moments, P/Q, at pH 3.1 obtained from the reversing pulse experiments was 2.24 ± 0.22 for α_R-paramyosin and 0.73 ± 0.08 for β-paramyosin. If it is assumed that the value of Q is the same for both α_R- and β-paramyosin, then it would appear that the permanent dipole moment of the former is three times as great as the latter. The assumption that Q is constant is probably valid since both forms of the molecule were measured under identical conditions of ionic strength and pH and the relaxation times indicated that the molecules have no great differences in their size and shape.

Equation 3 shows that the specific Kerr constant is directly related to the sum of the permanent and induced dipole moment terms. Thus it should be possible to calculate these quantities using the values of P/Q determined from the reversing pulse experiments and the value of the optical anisotropy, $g_1 - g_2$. The optical anisotropy of β-paramyosin was determined from flow birefringence experiments by Taylor and Cramer[30] as 4.5×10^{-3}. However, a value of 5.36×10^{-3} may be calculated using values of n_1 (1.591) and n_2 (1.621),[30] the refractive index of water (1.333) as n_0, and an axial ratio, p, of 57 determined from the measured length and diameter[31] using eq 5. Therefore, one would not expect the optical anisotropies of α_R- and β-paramyosin to differ significantly; the same value was used in the calculations for both forms of the molecule. Typical specific Kerr constants of 4.45×10^{-5} esu for α_R-paramyosin and 1.34×10^{-5} esu for β-paramyosin at pH 3.1 were used in eq 3 to obtain $P + Q$. Using the P/Q values from

the reversing pulse experiments, permanent dipole moments of 6300 and 2700 D were calculated for α_R- and β-paramyosin, respectively.

As the pH was increased from 3.1 to 3.8, the specific Kerr constant of the α_R-paramyosin showed a decrease from 4.5 $\times 10^{-5}$ to 2.8 $\times 10^{-5}$ esu. Over this same pH range the β-paramyosin showed a much smaller change from 1.9 $\times 10^{-5}$ to 1.3 $\times 10^{-5}$ esu. In contrast to these data, the P/Q value for α_R-paramyosin at pH 3.7 was 2.1 (compared to 2.2 at pH 3.1) while the value for β-paramyosin dropped to 0.47 at pH 3.8 from an initial value of 0.73 at pH 3.1. These results were surprising since one would expect that both the K_{sp} and P/Q would change in a similar manner since they both reflect changes in the dipole moments. When the above data are combined to calculate the dipole moments, assuming that the value of $g_1 - g_2$ remains constant at 5.36 $\times 10^{-3}$, values of 4950 and 2300 D were obtained for the α_R and β from, respectively. While these values reflect the observed behavior of the specific Kerr constants they do not agree with the change predicted by the P/Q values. At the present time there is no obvious explanation of this discrepancy. However, one may speculate that it may be connected with the change in the size and shape of α_R-paramyosin as the pH changes, to be discussed below, or it may be connected with obvious inadequacies in the theoretical treatment used in this discussion; i.e., the birefringence transient observed in the reversing pulse method may be affected by a time-dependent dipole moment caused by migration of ions along the molecule.

The relative values of the permanent dipole moments of the α_R- and β-paramyosins at pH 3.1 would seem to indicate that the "extra piece" on α_R-paramyosin also introduces a higher charge anisotropy into the molecule. At pH 3.1 this extra charge may be due to the cationic amino acid residues lysine, histidine, and arginine which compose about 18% of the molecule. Assuming a mean residue weight of 109 and a molecular weight of 210 000, the maximum possible charge from these groups is 346. However, only a few of these groups need be present on the "extra piece" as is indicated by the following considerations. The dipole moment that arises from the separation of one positive and one negative charge by the length of a paramyosin molecule (~1200 Å) is about 5800 D, while a single positive charge on one end of the molecule is equivalent, in terms of these experiments, to a dipole moment of about 2900 D (see Appendix B). Therefore, the charge on α_R-paramyosin in excess of that on the β form need not be great to produce the observed difference.

The calculated change in the length of the α_R-paramyosin molecule as the pH is increased from 3.1 to 3.8 indicates that the titration of the molecule in this region leads to a change in its conformation, most probably in the "extra piece". This "extra piece" could, of course, be two extra pieces, one on each end of the molecule. In view of the high dipole moment of α_R-paramyosin, however, only one of these pieces would be expected to exhibit the extra positive charge(s) at pH 3.1.

The change in the conformation of the extra piece(s) could possibly arise from an increase in the flexibility or an unwinding of the coiled coil at the end(s) of the molecule. Even if the 80 Å "extra piece(s)" was (were) rigid but attached by a hinge region, the relaxation time of the small piece, with the hinge flexible, would be too fast to be observed in these experiments and the molecule would appear to have a shorter length. Now the question becomes: what could cause an increase in flexibility of part of the α_R-paramyosin molecules at pH >3.2 to account for the decrease in relaxation time and calculated length? There are at least two possible explanations, both connected with the titration of the terminal carboxyl groups and possibly aspartic acid residues as the pH increases. The presence of a small number of proton bonds between chains, using un-ionized carboxylic acid groups, may be surmised, although a very large number of these is improbable.[32] The stabilization of the two-chain coiled coil structure of paramyosin has been assumed to arise from nonpolar interactions between chains,[32] but the small "extra piece(s)" could well be stabilized in the rigid coiled coil conformation by proton bonds instead. These proton bonds would then be broken when the carboxylic acids are titrated, thus allowing the "extra piece(s)" to become flexible or hinged.

It is also possible that the extra piece(s) are normally flexible, but the charged cationic groups on the extra piece(s) and on the bulk of the molecule at pH 3.1 repel each other and stiffen the segment to produce an apparent increase in length. This type of behavior occurs in low ionic strength solutions containing polyelectrolytes or polyampholytes with excess charge such as polyacrylic acid,[23] which has a randomly coiled conformation at low charge and high ionic strength but shows an increasing chain expansion with increasing charge on the molecule and decreasing concentration of added salt. At very low ionic strengths the charged molecule appears to take on the character of a rigid rod. Comparing the data for α_R-paramyosin with the above observations, it is possible to imagine that as the excess positive charge on the "extra piece" is neutralized by titration of the terminal carboxyl groups and the aspartic acid residues, this part of the molecule becomes more flexible. At pH 3.8 where these charges are fully neutralized, the "extra piece" should lose all of its rigidity and the molecule appear to have the same length as the β form at this and higher pH values. The validity of these or other explanations for our data will have to be checked by other methods. It is obvious that one necessary experiment will involve the preparation of β-paramyosin from α-paramyosin by digestion of the extra piece(s). Attempts to denature the extra piece(s) at pH 3.1 to decrease the relaxation time of α_R-paramyosin to its high pH value are contemplated.

The permanent and induced dipole moments of the α_R- and β-paramyosin were calculated at pH 10 assuming that the optical anistropy factor was the same at pH 10 as it was at pH 3. When the pH 10 values of P/Q and K_{sp} for α_R-paramyosin and for β-paramyosin were used, the calculated permanent dipole moments were both 10 000 D, within experimental error of each other. This permanent dipole moment, which probably arises from the appearance of excess negative charge as the tyrosine, lysine, and arginine groups are titrated, increases above pH 10 until the permanent dipole moment becomes the dominant factor in the orientation mechanism and the P/Q ratio approaches infinity. Such behavior was observed for a sample of α_R-paramyosin at pH 11.3. The observation that the lengths of the two forms of paramyosin are similar at and above pH 10 indicates that the "extra piece" on α_R-paramyosin remains flexible in the presence of an excess negative charge.

Both α_R- and β-paramyosin showed stability toward thermal denaturation at pH 3.1 over the range of 20–50 °C (see Table IV). There was no observed decrease in the relaxation time, corrected to 20 °C, that would indicate unwinding or an increase in flexibility. The fact that the molecule actually showed a slight increase in the corrected relaxation time was attributed to the oversimplified correction for temperature and viscosity that was used (eq 9).

There is no directly comparable data in the literature, but Halsey and Harrington[34] studied the thermal denaturation of β-paramyosin at pH 7 by optical rotatory dispersion. They found that the helicity of the molecule de-

creased slowly at temperatures above 20 °C with sharp cooperative transitions at 44 and 60 °C. In addition, Riddeford and Scheraga[32] found that, in the presence of 5 M guanidine hydrochloride, the first transition dropped to 34 °C at pH 6 and 10 but it remained at 44 °C at pH 2 and 3. These observations were confirmed by Cowgill[35] who determined the same transition temperature at pH 2 from fluorescence measurements. From these observations, that low pH confers an added stability to β-paramyosin, it is not surprising that no change in the relaxation time was observed for the sample of the β form that was studied, even at very low ionic strength. However, it is interesting to note that α_R-paramyosin behaved in the same manner. This would seem to indicate that the disulfide cross-bridges found in β-paramyosin[13] do not confer upon it any extra thermal stability above that of the native α_R form at low pH.

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Appendix A

Assume a signal decaying with time constant τ_1 (the birefringence decay signal) coming into a photomultiplier circuit which has time constant τ_2. The voltage signal $V(t)$ across the photomultiplier anode resistor becomes, if $V(0)$ is the voltage before the signal begins to decay:

$$\frac{V(t)}{V(0)} = \frac{\tau_1}{\tau_1 - \tau_2}\exp(-t/\tau_1) - \frac{\tau_2}{\tau_1 - \tau_2}\exp(-t/\tau_2)$$

which becomes, when $\tau_1 = \tau_2$

$$\frac{V(t)}{V(0)} = (1 + t/\tau_1)\exp(-t/\tau_1)$$

If one plots the logarithm of $V(t)/V(0)$ vs. time, t, one obtains curves that are concave downward and from which it is not possible to obtain τ_1 in a simple way unless $\tau_1 \geq 5\tau_2$. When $\tau_1 \geq 5\tau_2$, the plot mentioned above is a straight line from which τ_1 can be obtained in the usual way.

Appendix B

Expressions for K_{sp} are usually calculated for both insulating and conducting solutions using the assumption that the energy of the orienting solute molecules in a uniform electric field depends only on the induced and on the permanent dipole moments in the molecules relative to some of the properties of the solvent. Sometimes, however, one may have a molecule that also has excess charge somewhere along the molecule. This excess charge, especially if it is located far from the center of mass of a very anisometric molecule, must be taken into account when calculating the relative energies of the molecules at various orientations with respect to the applied electric field. It is these relative energies that define the distribution of orientations at equilibrium with the applied electric field.[19]

Let us compare the energy of a long thin molecule with excess charge q at distance d from its center of mass with the energy of the same molecule which has, in addition, an equal and opposite charge, $-q$, at distance d on the opposite side of the center of mass from charge q, i.e., it has a dipole moment $2qd$. We shall assume that the center of mass of the molecule with excess charge remains fixed while the molecule is rotating. Although this assumption is wrong in detail, one may calculate, making reasonable assumptions about the electrophoretic mobility of paramyosin, that, at the low electric fields associated with the specific Kerr constant of the macromolecule, the center of mass moves about 20 Å or less during 20 μs, the birefringence relaxation time of the monomer. During the same 20 μs, the rotating charged end of the macromolecule moves 200 to 400 Å with respect to the electrodes even if the center of mass of the molecule remains fixed. The error in the calculation of energy introduced by the fixed center of mass assumption should therefore be 10% or less.

A long thin molecule with dipole moment $2qd$, with its long axis oriented at an angle θ with respect to an applied electric field, has an interaction energy $2qdE\cos\theta$ with the electric field, neglecting consideration of the reaction field of the dipole. If $\theta = 0$, i.e., dipole and field vectors in the same direction, is taken as a starting point, then, with respect to this value, the energy at other angular orientations is $2qdE(\cos\theta - 1)$.

A long thin molecule with no dipole moment but with excess charge q at distance d from the center of mass will also have different energies in the uniform electric field as it rotates around its center of mass if the center of mass does not translate along the field lines. This is because the charge q, as the molecule rotates, moves closer and then farther away from the electrode to which it is attracted. If we take angle θ, now, as the angle between the field lines and the vector that starts at the center of mass of the molecule and ends at charge q if q is positive but has the opposite direction if charge q is negative, then, using again as a starting point the energy of the molecule in the field when $\theta = 0$ and noting that the scalar distance *with respect to either electrode* traveled by the charge q as θ changes from 0 is $d(1 - \cos\theta)$, we reach the following conclusion. Since θ is defined to bring q closest to the electrode of like charge when $\theta = 0$, then with respect to the energy at $\theta = 0$, the energy of the molecule at other angular orientations is $qdE(\cos\theta - 1)$. This is exactly half the change calculated for the same molecule with dipole moment $2qd$.

Although the reaction field of the dipole was neglected in this analysis, we shall nevertheless say that an excess charge q, situated a distance d away from the center of mass of a molecule, is equivalent in its effect to a dipole moment in the molecule of the order of magnitude of qd.

Reference and Notes

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Photoisomerization and Fluorescence of Chromophores Built into the Backbones of Flexible Polymer Chains[1]

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ABSTRACT: Three copolyamides were prepared with azobenzene residues in the chain backbone. The photoisomerization in dilute solution was found to have the same quantum efficiency for the polymers and their low molecular weight analogues. This result shows that the "crankshaft-like motion" model for conformational transitions in the backbone of polymer chains cannot apply in dilute polymer solutions. Polymer solutions in dimethyl sulfoxide (DMSO) containing a high concentration of glycol or glycerol exhibited a behavior suggesting the presence of polymers in two different states, representing probably aggregated and molecularly dispersed chain molecules. In glassy films, the quantum yield for photoisomerization was very low for azobenzene residues in polymer backbones, while this quantum yield was reduced by a relatively small factor for the polymer analogues. A polyamide with stilbene residues in the chain backbone exhibited less intense fluorescence in dilute solution than its analogue. In plasticized films, the emission intensity decreased in a similar manner for the polymer and its analogue with increasing plasticizer concentration.

In previous work in this laboratory[2] we compared the rate of hindered rotation around bonds in the backbone of flexible chain polymers and their low molecular weight analogues in dilute solution. Since a conformational transition around a single bond in the backbone of a polymer would involve rapid motion of a large part of the molecular chain through the viscous medium with a prohibitively large dissipation of energy, it had been suggested by Schatzki[3] that two conformational transitions are correlated in time in a "crankshaft-like motion" so that only a short segment of the chain has to be displaced. This concept would, however, seem to lead to an increase in the free energy of activation for the hindered rotation, so that conformational transitions around a given bond in a polymer backbone would be much slower than in a low molecular weight analogue. The experiments referred to above were, therefore, designed to find whether such effects can be observed.

Results obtained in a NMR study of the rate of hindered rotation around the amide bond in piperazine polyamides revealed no significant difference between the behavior of the polymer and its analogues.[2a] Similarly, the thermal cis–trans isomerization of azobenzene residues built into the backbone of a polyamide proceeded at rates comparable to those in analogous small molecules.[2b] (It should be noted that for the purpose of this study there is no difference in principle between cis–trans isomerization around a double bond and hindered rotations around single bonds.) These results indicated that the rotation around the amide bond (or the cis–trans isomerization of the azobenzene residue) does not necessitate a simultaneous conformational transition around another bond in the chain backbone. We assumed, therefore, that the rotation around the amide or azobenzene residue involves many small oscillations of the internal angle of rotation. The distortion of this angle and the relaxation of the distorted angle would be slowed down by incorporation into the polymeric chain by the same factor, so that no effect on the rate at which the transition state is surmounted would be observed.[4] This model predicts then that the internal viscosity of the polymeric chain should have no effect on rates of conformational transition involving high activation energies, ΔE^*, just as the viscosity of a solvent medium has no influence on the rate of bimolecular reactions characterized by high values of ΔE^*. However, just as bimolecular reactions become diffusion controlled if ΔE^* is sufficiently low, so the rate of conformational transitions should become dependent on the rate of distortion of internal angles of rotation if the energy barrier for the transition is small. Under these conditions the restraint imposed by the requirement for two correlated transitions in polymer chains should become observable.

Zimmerman et al.[5] suggested that the photoisomerization of azobenzene involves a thermal reaction between the excited trans and cis states separated by a low-energy barrier. This concept was later confirmed by Fischer and Malkin[6] in their study of the temperature dependence of the quantum yield for the trans–cis photoisomerization which yielded an estimate of 2–3 kcal/mol for the energy barrier separating the two ex-