A conformation change of single stranded polyriboadenylate induced by an electric field

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ABSTRACT

Absorbance measurements performed with high molecular weight poly A at pH 8 show that the degree of single strand stacking present at high ionic strength is reduced at low ionic strengths. The salt dependence of the poly A conformation is assigned to an electrostatic repulsion between subsequent turns of the single strand "helix" structure. - Electric fields of 5 to 80 kV/cm induce an increase in the poly A absorbance consistent with a decrease in the ion concentration in the environment of the polymer. The increase of the absorbance is a linear function of the field strength suggesting that the conformation change is caused by a dissociation field effect. At increasing ionic strength, threshold values of the electric field strength have to be exceeded in order to induce measurable absorbance changes. - The time required for the conformation change decreases from about 2 μ sec at 10^{-4} M ionic strength to about 0.3 μ sec at high ionic strengths. At low ionic strengths the ion equilibration may influence the rate limiting step, whereas the arrangement of the nucleotide residues into the ordered structure is rate limiting at high ionic strengths.

INTRODUCTION

In the past, the investigation of the influence of an electric field upon biological macromolecules has mainly been concerned with the characterization of orientation effects.¹ These allowed conclusions about the size and shape of the oriented particles, about the orientation of the chromophore with respect to the orientation axis, and about the mechanism of orientation. Compared to the rather detailed investigation of orientation effects, the characterization of conformation changes of biological macromolecules induced by electric fields received relatively little attention. A conformation change in DNA induced by electric fields was suggested by O'Konski and

Stellwagen.² More recently Neumann and Katchalsky³ used short electric impulses to trigger conformation changes from metastable states in a triple stranded poly (A)·2 poly (U) helix complex. Revzin and Neumann⁴ analysed field induced conformation changes in ribosomal RNA containing short double helical sequences.

In the present investigation, it is demonstrated that electric fields induce a conformation change in a simple polyelectrolyte model system: single stranded polyriboadenylate (poly A). This system allows a direct analysis of the field effect as a function of the field strength under a variety of solvent conditions. As a result of this analysis information is obtained about the molecular mechanism by which a conformation change is induced.

II a) MATERIALS

Poly A was purchased from Boehringer Mannheim, GmbH. It was subjected to a phase separation procedure according to Eisenberg and Felsenfeld.⁵ The high molecular weight fraction precipitating at 15.4° C in 1.2 M NaCl was dialysed against 10⁻⁴ M EDTA, 10⁻⁴ M Tris, pH 8.0, then against 10⁻⁴ M Tris, pH 8.0 and finally for a short period against quartz distilled water. The pH of the dialysed solution was adjusted to pH 8 by addition of NaOH and the solution was stored at -20° C. The sedimentation coefficient of this poly A sample was 10.3 Svedberg units in 0.1 M NaCl, 0.05 Na-cacodylate, pH 6.9. When a poly A sample was kept at room temperature without addition of salt (at neutral pH), the sample was slowly degraded as judged by a decrease in the absorbance change upon salt titration (cf. results, cf. ref. 6). The oligoadenylate of chain length 14 was isolated as described in reference 7.

b) METHODS

Absorbances were measured in a Zeiss PM QIII spectrophotometer supplied with a thermostated cuvette holder. CD spectra were obtained by a Cary 60 spectropolarimeter equipped with a CD accessory. The electric field experiments were performed with an apparatus developed by Grünhagen.⁸ The samples were

analysed in a 0.5 ccm cell equipped with platinum electrodes at an electrode distance of 5 mm and an optical pathlength of 10 mm.

c) DATA EVALUATION

The field jump measurements and the data evaluation were based on the fact that absorbance signal changes due to orientation vanish when the polarization plane of the light used for detection is oriented at an angle of 54.8° with respect to the vector of the electric field.^{9,10} Thus, measurements performed at 54.8° orientation of the polarizer yield amplitudes due to chemical processes ΔA_{ch} without interference by orientation effects. When the polarizer is oriented parallel (||) or perpendicular (⊥) with respect to the electric field, however, the absorbance change may be due to both orientation and chemical processes

$$\begin{aligned}\Delta A_{||} &= \Delta A_{||}^0 + \Delta A_{ch} \\ \Delta A_{\perp} &= \Delta A_{\perp}^0 + \Delta A_{ch}\end{aligned}\tag{1}$$

where $\Delta A_{||}$ (and $\Delta A_{\perp}$) is the absorbance change measured directly with parallel (and perpendicular) orientation of the polarizer and $\Delta A_{||}^0$ (and $\Delta A_{\perp}^0$) is the corresponding absorbance change caused by orientation exclusively. Using the equations (1) $\Delta A_{||}^0$ and $\Delta A_{\perp}^0$ may be evaluated most conveniently by an independent determination of ΔA_{ch} as described above. The resulting $\Delta A_{||}^0$ and $\Delta A_{\perp}^0$ should then agree with

$$\Delta A_{||}^0 = -2\Delta A_{\perp}^0\tag{2}$$

III UV-ABSORBANCE AND CD-SPECTRA OF POLY A AT LOW IONIC STRENGTHS

Addition of Tris buffer to desalted poly A at pH 8.1 leads to small yet clearly detectable changes in the poly A absorbance. Difference spectra obtained by titration of high molecular weight poly A with Tris buffer are given in Fig. 1. The addition of salt does not affect the position of the maximum absorbance, which is found at 256.7 nm at all salt concentrations up to 0.2 M at pH 8. However, the absorbance of poly A at the maximum decreases by about 8.3%, when the Tris-concentration is

Figure 1: Difference spectrum $E_c - E_o$ of poly A at various Tris concentrations (E_c absorbance at Tris concentration c and E_o absorbance without added electrolyte, pH 8, 20° C, --- 0.27 mM, ---- 0.54 mM, — 0.81 mM, - - - 1.08 mM, ... 1.35 mM, .. 2.01 mM, $E_o = 1.00$ at 257 nm).

raised from 0 to 0.1 M (at 20° C, pH 8.1). Salt titrations at different temperatures (cf. Fig. 2) show that the absorbance change is maximal in the temperature range 10 to 20° C and decreases both at higher and lower temperatures. Most of the experiments described in this investigation were performed with Tris-buffer. It was shown, however, that corresponding results are obtained, when e.g. NaCl is added to poly A. The titration curves also did not depend upon pH (within the small pH range accessible for measurements without interference by double helix formation). All the results described above were obtained with a high molecular weight fraction of poly A ($S = 10.3$). Poly A samples of lower molecular weight showed smaller absorbance changes and also attained constant absorbance levels at lower

Figure 2: Poly A absorbance at 260 nm as a function of the Tris concentration at different temperatures (pH 8).

ionic strengths. No absorbance change was observed upon salt titration using an oligomer containing 14 adenylate residues.

Most of the conformation changes observed in nucleic acids are reflected by changes in ORD and CD spectra.¹¹ Thus it was tested whether the CD spectrum of poly A is affected by variations of the salt concentration. However, for changes in salt concentrations resulting in substantial changes in the UV absorbance, the CD-spectrum of poly A showed only relatively small changes. For instance, addition of Tris buffer from zero concentration up to 0.1 M at 20°C yielded a 1.8% increase in the CD-spectrum at the peak at 264 nm, whereas the absorbance changed by about 8.3% at the maximum of absorbance.

IV ELECTRIC FIELD EFFECTS

a) Amplitudes

1) Chemical Effect

An example for a relaxation effect induced in poly A by an electric field is given in Fig. 3. A few μsec after the electric field is applied to the poly A sample, the absorbance of the sample is increased to a new constant value corresponding to the new equilibrium state in the presence of the electric field. When the electric field is switched off, the original absorbance is restored within a few μsec . The absorbance change relative to the total absorbance is independent of the nucleotide concentration, when compared at identical conditions of ionic strength and temperature. Application of electric fields

Figure 3: Field jump experiment with $1.9 \cdot 10^{-4}$ M poly A in $5 \cdot 10^{-4}$ M Tris, pH 8.0, 20°C , $\lambda=240\text{ nm}$. Plane of the polarized light at 55° with respect to the direction of the electric field. Field strength 74 kV/cm.

Figure 4: Field jump amplitudes as a function of the field strength at different Tris concentrations: + 0.5 mM, o 1.0 mM, x 3.0 mM, □ 6.0 mM, Δ 10.0 mM (poly A concentration $9.5 \cdot 10^{-5}$ M, pH 8, 20°C).

leads to an increase in the absorbance in the wavelength range from 240 nm to 275 nm, whereas at wavelengths around 285 nm a decrease in the absorbance is observed. These absorbance changes suggest that the effect of electric fields upon poly A conformation is similar to that induced by a decrease in the ion concentration (cf. Fig. 1).

The absorbance changes increase with increasing field strength (Fig. 4). Within experimental accuracy the absorbance change is a linear function of the electric field strength up to relatively high values, where the field effect apparently approaches saturation. Addition of salt to the poly A solution leads to a shift of the linear part of the electric field-absorbance function to higher field strengths. The measurements at different ionic strengths suggest the existence of apparent

threshold values of the field strength producing chemical effects (cf. Fig. 4). These threshold values increase with increasing ionic strength. In addition, a decrease in the slope of the linear part of the electric field-absorbance function is observed with increasing ionic strength.

Most of the field jump experiments reported in the present investigation were performed at 20° C. Some experiments performed at 30° and 40° C showed a decrease in the field jump amplitudes with increasing temperature. This result is consistent with the decrease in the absorbance change upon salt titration of poly A at increased temperatures (cf. Fig. 2).

2) Electrodichroism

The dichroism due to the orientation of the polymer molecules was measured for the same conditions as the chemical field effect. Since the absorbance changes due to orientation cannot be measured without interference due to chemical effects, all the orientation data had to be corrected for the chemical field effect. One example for the superposition of orientation and chemical field effects is given in Fig. 5. The chemical field effect first leads to a decrease in transmission. This decrease is then overcompensated by an orientation effect, characterized by a slightly lower rate. When the field is switched off, the effects are reversed. However, due to the influence of the electric field on the rate constants (cf. following section), the signals are not symmetrical. When the signal changes produced by the electric field are corrected for the chemical field effect, the change observed for light and field vector being parallel are twice as high as obtained for the perpendicular orientation of the light and field vectors. The reduced dichroism (definition cf. ref. 1) evaluated from these measurements approaches values around -0.3 at high electric field strengths.

The dichroic signal observed at a given field strength decreases with increasing salt concentration. This observation supports the results of temperature jump measurements reported previously.¹² These measurements showed the absence of orientation effects in poly A solutions at high ionic strength

Figure 5: Field jump experiment with $1.9 \cdot 10^{-4}$ M poly A in $5 \cdot 10^{-4}$ Tris pH 8.0, 20°C , $\lambda=240\text{ nm}$. Plane of the polarized light in the direction of the electric field. Field strength 74 kV/cm.

under the conditions of fast temperature jump experiments.

b) Relaxation Time Constants

The time constants associated with the conformation change in poly A may be evaluated both from the relaxation process observed when the electric field is switched on, and from the relaxation observed when the field is switched off. For small perturbations it is expected that both processes are characterized by the same time constant. Actually the time constants observed for the "on"- and "off"-process are identical, when relatively low electric fields are applied. The relaxation curves both of the "on"- and of the "off"-process can be represented within experimental accuracy by single exponentials. For high electric fields, the time constant observed for the

Figure 6: Relaxation time as a function of the reciprocal field strengths. (poly A concentration $6.25 \cdot 10^{-5}$ M, pH 9, no added salt, 20° C).

"on"-process decreases with increasing field strength. A graphical representation of this dependence is given in Fig. 6. The data show a linear dependence of the relaxation time upon the reciprocal field strength.

The results described below were obtained at zero field strength, i.e. derived from the "off"-process. At constant ionic strength, the relaxation time associated with this process is independent of the nucleotide concentration. Measurements at different salt concentrations revealed a distinct dependence of the relaxation time upon the ionic strength. The relaxation time constants decrease with increasing ionic strength. In Fig. 7 the data obtained from field jump experiments are plotted together with data obtained previously from temperature

Figure 7: Reciprocal relaxation time as a function of the square root of the ionic strength (measured at 240 nm, points indicated by an + are taken from reference 10).

jump measurements.¹² Apparently the relaxation time constants attain a constant level at high ionic strengths, where the electrostatic repulsion between adenylate residues is shielded.

Most of the field jump data were recorded using the absorbance change in the wavelength range around 240 to 250 nm. For comparison some relaxation data were measured in the wavelength range around 285 nm. For identical conditions ($3 \cdot 10^{-3}$ M Tris, $9.5 \cdot 10^{-5}$ M poly A, 20°C) the time constant observed at 285 nm ($\tau_{285} = 1.08 \pm 0.1 \text{ } \mu\text{sec}$) was slightly higher than that observed at 240 nm ($\tau_{240} = 0.87 \pm 0.1 \text{ } \mu\text{sec}$). Apparently the difference of the time constants τ_{285} and τ_{240} observed previously by temperature jump experiments at higher ionic strengths decreases with decreasing ionic strength (cf. ref. 12).

The time constants associated with the orientation of poly A derived from measurements of the dichroism were in general lower than those associated with the conformation change. Moreover, the orientation of poly A is characterized by a spectrum of time constants. The orientation decay of the poly A fraction used in the present investigation showed a major contribution in the time range of 10 μ sec with small contributions up to the time range of a few msec (cf. Fig. 5).

V DISCUSSION

a) The Conformation of Poly A at Low Ionic Strength

Generally the conformation of single stranded poly A has been considered to be almost independent of the salt concentration.¹³⁻¹⁵ Melting curves of single stranded poly A, obtained at different salt concentrations, show only rather small effects of the ionic strength upon the stability of the single strand structure.^{14,15} This result can be explained by the fact that the distance between adjacent phosphate charges is hardly changed upon stacking. However, if base stacking in poly A leads to a single stranded helix with helix parameters similar to those of the Watson Crick double helix, electrostatic repulsion is expected between subsequent helical turns, at least at low ionic strengths. Since helix turns are separated by about 35 Å, an influence of the ionic strength upon the poly A conformation is expected when the Debye radius is of that order of magnitude, i.e. at ionic strengths below 10 mM. The results of the poly A salt titrations obtained from UV absorbance measurements are in accord with this estimation.

It remains unclear, however, why for a given change in the salt concentration the CD spectrum of poly A is only slightly changed, whereas the UV absorbance is changed substantially. The significance of this observation may be illustrated by a comparison with the change of optical parameters induced by a variation of the temperature. As can be seen from Fig. 2, the absorbance change due to a change in the salt concentration from 0.1 M to 0.1 mM at 20° C, is equivalent to that induced by a temperature change from 20° to about 33° C at 0.1 M. For the same variation in the temperature at 0.1 M

salt, the CD peak of poly A at 264 nm decreases by about 21%,¹⁶ whereas the CD spectrum remains almost constant when the salt concentration is changed from 0.1 M to 0.1 mM.

This difference in the optical parameters suggests that the change in the molecular structure induced by a change in the salt concentration is different from that induced by a change in the temperature. It may be that the conformation change induced by a variation in the salt concentration does not involve a great change in the number of stacked adenylate residues, but is mainly due to a change in the relative orientation of the single stranded helix segments. A possible explanation is a change in cooperativity of adenylate stacking. The analysis of oligo- and polyadenylate melting curves measured at high salt concentrations (0.15 M) revealed a slight cooperativity.^{17,18} It is conceivable that due to electrostatic repulsions at low ionic strength the adenylate stacking is a slightly anti-cooperative reaction. This would mean that a conformation with few long single-strand-helices is slightly favored at high salt concentrations, whereas a conformation with many short single-strand-helices is slightly favored at low salt concentration. The change from one conformation to the other may be reflected preferentially in the UV-absorbance and less clearly in the CD-spectrum. Finally it should be mentioned that recent relaxation measurements performed at high salt concentration revealed the existence of more than two conformations of adenylate chains.¹² Changes in the salt concentration may influence the equilibrium between these different stacking conformations.

b) The Field Effect and its Mechanism

The field jump measurements performed in the present investigation demonstrate that high electric fields induce conformation changes in single stranded poly A. The absorbance of poly A increases in the presence of electric fields, indicating an unstacking process of the bases in the single stranded structure. From this result it must be concluded that either the unstacking of adenine bases or a reaction coupled to the unstacking is sensitive to the electric field. According to the theory of chemical field effects, a field induced unstacking of

adenine bases could be due to an increase in the dipole moment upon unstacking.¹⁹ However, even if all the dipole moment of the adenine base (around 3 Debye²⁰) would be compensated in the stacked state, the increase in the dipole moment upon unstacking would not be sufficient to explain the high field sensitivity of the poly A conformation.

Thus it is more likely that a reaction coupled to the unstacking reaction is responsible for the field dependence. The salt titrations performed in the present investigation demonstrate that the adenine stacking reaction is influenced by the ion concentration. Furthermore it is well known that electric fields may cause perturbations of ion equilibria by a "dissociation field effect".¹⁹ According to the theoretical treatment of Onsager,²¹ the change in an ion dissociation constant K of an equilibrium

is linearly dependent upon the electric field strength E

$$\frac{\delta K}{K} = \frac{Z_a \cdot u_a + Z_b \cdot u_b}{u_a + u_b} \cdot Z_a \cdot Z_b \cdot \frac{e_o^3}{2\epsilon k^2 T^2} |E| \quad (3)$$

(Z valency, u mobility, e_o elementary charge, ϵ dielectric constant). The field effect increases with increasing valency of the ions and leads to appreciable changes of ion equilibria in polyelectrolytes. Large dissociation field effects were found by conductivity measurements in a variety of polyelectrolyte systems including DNA.²²⁻²⁵

In the present investigation, the effect of the electric field upon poly A was observed by measuring the absorbance of the adenine chromophores attached to the polyelectrolyte. This method supplies information about conformation changes of the polymer but not directly about its ion equilibria. However, the results obtained from salt titrations allow at least a qualitative correlation of the field jump amplitudes to the extent of counterion condensation. The field jump amplitudes are consistent with a decrease in the ion concentration in the

environment of the polyelectrolyte induced by the electric field, and thus are consistent with a dissociation field effect. According to equation 3, a linear dependence of the dissociation constant upon the field strength is expected. The observed linear dependence of the absorbance change upon the field strength is also in agreement with the theoretical equation. These results suggest that the conformation change in poly A is induced by a dissociation field effect. A quantitative comparison of the experimental results to a theory of the dissociation field effect, however, is not possible at present, since the theory required for polyelectrolyte systems is not yet available.

Neumann and Katchalsky³ explained a field induced conformation change in a multistranded polynucleotide complex by ion polarization along the helix axis. The electric field induces large dipole moments by shifting the ion atmosphere of the polyelectrolyte. The ion depletion of the helix then induces a conformation change. Such ion polarization is also found in poly A. The induction of large dipole moments by ion polarization causes the orientation of poly A molecules observed by dichroism. The polarization of the ion atmosphere might also be a reason for the conformation change in poly A. However, if the ion polarization actually would be responsible for the conformation change in poly A, a quadratic dependence of the equilibrium shift upon the field strength would be expected. Although the conversion of the absorbance into a quantitative measure of the equilibrium shift has not been carried out directly, the measured linear dependence of the absorbance change upon the field strength certainly favors the dissociation field effect as a cause of the poly A conformation change.

One major advantage of the present model system is the possibility to measure the field effect at a variety of different ionic strengths, up to rather high ion concentrations. These measurements revealed the existence of threshold values of the electric field strength required for the induction of conformation changes at high ionic strengths. Apparently these threshold values are a measure of the strength of the ion atmosphere

around the polyelectrolyte. At high ionic strengths the ion atmosphere has to be disrupted, at least partially, before great changes in the polymer conformation can be achieved. This result can be explained qualitatively in terms of Onsager's theory of the dissociation field effect. At high ion concentrations most of the charges of the polyelectrolyte can be considered to be neutralized by counterions. Thus, the effective charge of the polyelectrolyte is relatively low, resulting in low dissociation field effects according to equation 3. When counterions are removed from the polymer, its effective charge increases resulting in much higher dissociation field effects (cf. eq. 3). Apparently there is a critical charge density of the polyelectrolyte. Below this critical charge density the dissociation field effect remains rather small. A more detailed discussion of the measured field effects would require a quantitative theory of the dissociation field effect in polyelectrolytes with consideration of the ion atmosphere at varying ionic strength.

The time constants associated with the field effect in poly A at low ionic strength are quite similar to some time constants published previously for dissociation field effects in synthetic polyelectrolytes under similar conditions.²²⁻²⁵ This correspondence suggests that the rate determining step in all these systems is influenced by the same process of ion equilibration. Thus the ion binding may be the rate limiting step in the poly A conformation change at low ionic strength. However, the measured relaxation time constants may also be associated directly with the poly A conformation change. The decrease in the rate at low ionic strength may then be explained by an increase in the electrostatic repulsion between the phosphate groups. At high ionic strength however, it is obvious that the rate is determined by the rearrangement of the nucleotides into the stacked structure.¹² This may be concluded from the relatively low rate of the poly A conformation change observed at high ionic strengths (cf. Fig. 7), which is lower than the rate of ion binding expected for high ion concentrations.

c) Conclusions

The present investigation provides several types of new information. First of all evidence is given for an ionic strength dependence of the conformation of a single stranded polynucleotide. Then it is shown that this ionic strength dependence leads to the induction of conformation changes in the presence of an electric field. According to the author's knowledge, the present investigation gives the first experimental evidence for a conformation change induced by a dissociation field effect. Further analysis of these effects may yield important information about the ion atmosphere of the polyelectrolyte. Finally, the present findings may have general implications on field induced conformation changes in biological macromolecules. Many conformation changes in nucleic acids, proteins and membranes are accompanied by a change in the charge density and thus may be induced in electric fields by a dissociation field effect.

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