Proc. Nat. Acad. Sci. USA
Vol. 69, No. 4, pp. 993–997, April 1972

Long-Lived Conformation Changes Induced by Electric Impulses in Biopolymers

(memory recording/metastability/biopolyelectrolytes/poly(A)/poly(U))

EBERHARD NEUMANN* AND AHARON KATCHALSKY

Polymer Department, The Weizmann Institute of Science, Rehovot, Israel

Contributed by Aharon Katchalsky, January 26, 1972

ABSTRACT Electric impulses are capable of inducing long-lived conformational changes in (metastable) biopolymers. Results of experiments with poly(A)·2 poly(U) and ribosomal RNA, which are known to develop metastabilities, are reported. A polarization mechanism is proposed to explain the structural transitions observed in the biopolymers exposed to the impulses. In accordance with this idea, the applied electric field (of about 20 kV/cm and decaying exponentially, with a decay time of about 10 μsec) induces large dipole moments by shifting the ionic atmosphere of multistranded polynucleotide helices. This shift, in turn, causes strand repulsion and partial unwinding. The fields used in our experiments are of the same order of magnitude as those in nerve impulses. The significance of the impulse experiments with regard to the question of biological memory recording is briefly discussed.

The initial step in the recording of biological memory is probably a physical, and not a biochemical, process (1, 2). A plausible mechanism for physical memory imprinting in living organisms is based on conformational transitions of metastable macromolecules or of macromolecular organizations, such as membranes (2, 3). Since metastabilities can lead to hysteresis, and because hysteresis loops were observed in several biopolymers (2, 4–6), these polymers could be possible matrices for a memory imprint. Such matrices should be able to record the electric impulses that are the information signals of the nervous system. Thus, we determined whether electric impulses can cause long-lived conformational changes in biopolymers. On physicochemical grounds, such long-lived changes are expected in domain structures capable of developing metastable states. Here, we report that electric impulses of about 20 kV/cm, and of a duration of a few μsec, do indeed induce long-lived conformational transitions in macromolecular complexes of polynucleotides (7), analyze this impulse effect, and suggest an explanation in terms of the physical chemistry of polyelectrolytes.

The polynucleotides investigated consist of polyriboadenylate, poly(A), and polyribouridylate, poly(U), mixed in the molar ratio 1:2 in aqueous NaCl solution. At sufficiently high salt concentration and neutral pH, these polymers form a three-stranded helical complex, poly(A)·2 poly(U). The base-pairing scheme of this complex (8, 9) is depicted in Fig. 1. The complex polyA·2 poly(U) can develop metastability, which underlies the time-independent hysteresis loops observed in potentiometric and spectrophotometric acid–base titrations (10). Such a hysteresis cycle is shown in Fig. 2. The structural changes leading to hysteresis are the transformations between poly(A)·2 poly(U) at neutral pH, and the protonated double-helix, poly(A)·poly(A) plus single-stranded poly(U) at acidic pH values:

$$2\,[\mathrm{poly(A)\cdot 2\,poly(U)}] \underset{\text{acid}}{\overset{\text{base}}{\rightleftharpoons}} \mathrm{poly(A)\cdot poly(A)} + 4\,\mathrm{poly(U)} \quad (1)$$

During titration from pH 7 to lower pH values, the three-stranded complex poly(A)·2 poly(U) is protonated and passes through a series of metastable states. The metastable configurations of the partially protonated complex are stabilized by an energy barrier that prevents the nucleation of the double helix poly(A)·poly(A). This barrier, which is mainly electrostatic, is strengthened by the mutual repulsion of the poly(A)·2 poly(U) triple helixes arising from the high density of negatively charged phosphates along these multistranded molecules (10). When the pH is lowered, the extent of protonation is increased. This further loosens the triple helix, lowering the nucleation barrier. Therefore, around pH'_m (see Fig. 2) the "transcrystallization" of poly(A)·2 poly(U) to poly(A)·poly(A) can occur. But even at pH values higher than pH'_m a suitable perturbation of the metastable complex, leading to a destabilization of the (U·A·U) base pairs, may induce the release of metastability (Fig. 2, *arrow A* to *B*). Such a perturbation could be effected through shifting the ionic atmosphere of the polynucleotide complex by an electric impulse.

Multistranded polynucleotides in dilute solution can persist only when the interchain repulsion is sufficiently screened either by poly-cations, such as the histones, or by an atmosphere of low molecular weight counterions. As is known from the study of polyelectrolyte solutions (11), alkali metal counterions form a dense, but mobile, atmosphere surrounding the polyion. The average ion concentration of the atmosphere depends on the linear charge density of the polyelectrolyte

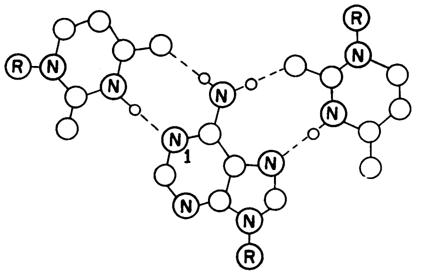

FIG. 1. Schematic representation of the (U·A·U) base pairings of an (A·2U) segment (ref. 9).

---

* On leave from the Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany.

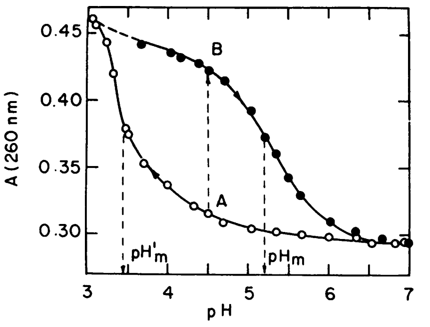

FIG. 2. Spectrophotometric acid-base titration of the system poly(A)-poly(U), molar ratio 1:2. ○, Titration of poly(A)·2 poly(U) with 1 N HCl; ●, titration of poly(A)·poly(A) and poly(U) with 1 N NaOH. Polymer residue concentration at the start of the acid titration at pH 7 is $c = 1.6 \times 10^{-5}$ M(U·A·U). $\mathrm{pH'}_m$, midpoint of the acid titration; pH_m midpoint of the base titration. $A \rightarrow B$, direction of the absorbance change in the impulse experiments at pH 4.5, 17°, and 0.105 M Na^+. The change in absorbance at 260 nm, is an approximate measure of the variation of the fraction of (U) residues incorporated in the base stacking and H-bonding interactions of poly(A)·2 poly(U) (ref. 10).

chain. The polyionic complex poly(A)·2 poly(U) has a higher linear charge density ($\simeq$1 charge/Å) than the single-stranded helical poly(A) ($\simeq$1 charge/3 Å) and poly(U) ($\simeq$1 charge/7 Å). The helix–coil transition of poly(A)·2 poly(U) is therefore accompanied by a release of counterions from the atmosphere of the polyionic complex. Thus,

$$(\mathrm{U\cdot A\cdot U}) = (\mathrm{A}) + 2(\mathrm{U}) + \Delta n, \qquad (2)$$

where Δn is the number of counterions released per phosphate charge. The magnitude of Δn is about 0.1 (see, e.g., ref. 12). Since the helix–coil transition liberates counterions, we reasoned that we might induce a transformation of the helix by an external force that can remove at least Δn counterions from the ionic atmosphere of poly(A)·2 poly(U). The partial removal of atmospheric ions from the polyionic complex by electric impulses of the proper intensity and duration does appear to cause an increase of the repulsion between the opposite strands, resulting in base-pair separation and unwinding of the triple helix.

MATERIALS AND METHODS

The Impulse Method. The electric impulse experiments were performed with an advanced model T-jump apparatus equipped with a highly sensitive optical detection unit (developed by C. R. Rabl, Göttingen). This device is used conventionally in the study of fast chemical relaxation processes (13). We used the apparatus to apply electric fields to biopolyelectrolyte solutions. The solution to be studied was placed between the two flat, platinum-covered electrodes of a thermostated Teflon cell. The change in the light transmission of the solution, after an impulse, was observed through the quartz windows of the cell.

The cell is part of a high-voltage discharge circuit, characterized by the cell resistance R, and a high-voltage "storage capacitor" C. At time zero this capacitor is discharged, and thereby the voltage V_0 is applied to the electrodes of the cell. The finite resistance of the solution causes a decay of the initial field strength $E_0 = V_0/d$, where d is the distance between the electrodes. Due to the low inductance of the discharge circuit, the time dependence of the field decay is given by

$$E(t) = E_0\cdot\exp(-t/RC). \qquad (3)$$

The resistance of the filled cell was $R = 250\ \Omega$ at 17°; $C = 5 \times 10^{-8}$F. Since V_0 was 30 kV and $d = 1.35$ cm, $E_0 = 22.2$ kV/cm. The rise-time of the field, after the voltage V_0 is applied, is about 10^{-10} sec. The dissipation of energy during the decay of the field occurs within psec(decay time $\tau_E = RC = 12.5\ \mu$sec). Thus no local overheating lasting more than 10^{-12} sec could occur, not even in the region of high ion concentration in the ionic atmosphere of a polyelectrolyte. The total change of temperature due to Joule heating is $\Delta T = C\cdot V_0^2\cdot(8.36\, c\rho q d)^{-1}$, where c is the specific heat of the solution [$\simeq$1 cal/(g-deg)] and qd is the cell volume [$\simeq$0.65 cm^3]; the density of the solution, ρ, is taken to be $\simeq$1 g/cm^3. In our experiments ΔT was about 8.5°.

Materials. Poly(A) and poly(U) (Miles Lab., Elkhart, Indiana) were fractionated in concentrated NaCl solutions (14, 15). The polymer fractions with mean sedimentation coefficients of $s_{20} \simeq 5$ were selected. Poly(A) and poly(U), dissolved in 0.1 M NaCl–5 mM Na-phosphate buffer (pH 7.1), were mixed in the molar ratio 1:2 and equilibrated for 4–6 days at 17° to permit complete complex formation. The complex poly(A)·2 poly(U) was titrated with 1 N HCl to pH 4.5 and equilibrated until no further change in A_{260} was observed. The concentrations of the individual polymers were determined after alkaline hydrolysis with the following extinction coefficients, ϵ; for AMP, ϵ (259 nm) = 15,400 (M-cm)$^{-1}$ and for UMP, ϵ (262 nm) = 10,000 (M-cm)$^{-1}$, at pH 7.1 and 20°. All measurements were performed under sterile conditions (10).

RESULTS

Effect of a Single Impulse. After a single impulse was applied to a solution of metastable poly(A)·2 poly(U), at pH 4.5 and 17°, the time course of the transmission at 260 nm was followed on an oscilloscope. The transmission first decayed rapidly, then leveled off slowly towards a constant value, which was used in the calculation of the corresponding change in absorbance, ΔA. The observed increase in the absorbance is not a temperature effect. The rise in temperature from 17 to 25.5° caused by the impulse cannot induce the conformational transition (Eq. 1) thermally, since at pH 4.5 the cooperative "melting" of the metastable complex occurs only in a temperature range around 47° (10).

Effect of a Series of Impulses. In order to penetrate further into the hysteresis loop (see Fig. 2) we applied a series of impulses. If A_n is the absorbance measured after the n'th impulse, the change in absorbance, ΔA_n, resulting from the n'th impulse, is given by $\Delta A_n = A_n - A_{n-1}$. The data from such an impulse series are plotted in Fig. 3a, from which we see that ΔA_n decreases with increasing n.

This finding can be explained by considering the anisotropic polarizability of the ionic atmosphere of rod-like polyelectrolytes (16) (see *Discussion*). In an external electric field only those poly(A)·2 poly(U) rods will be affected and converted to poly(A)·poly(A) that have an orientation sufficiently close to the direction of the field vector E. Because of the short duration and the relatively low intensity of the applied impulse we may neglect any possible rotary orientation of the polyions in the electric field (16). The field intensity along the axis of a rod that forms an angle θ with the field vector, E, is $E \cdot \cos \theta$ (Fig. 4). We can assume that there is a limiting minimum value of the field intensity, E_l, that is sufficient to induce a conformational change. Corresponding to this value there exists a maximum value for θ, θ_m, which is given by

$$E_l = E_0 \cdot \cos \theta_m \tag{4}$$

Thus all the poly(A)·2 poly(U) rods lying between $\theta = 0$ and $\theta = \theta_m$ will undergo the conformational transition, since they will be exposed to an *initial* field intensity $\geq E_l$. These rods represent the fraction $(1 - \cos \theta_m)$ of the total number of complexes (Fig. 4). The fraction ϕ of the poly(A)·2 poly(U) rods that are converted per pulse to poly(A)·poly(A) and poly(U) is $(1 - \cos \theta_m)$. Thus, with Eq. **4**,

$$\phi = 1 - E_l/E_0 \tag{5}$$

Furthermore, we assume that the absorbance change is a measure of the variation of the degree of conversion (10), and we denote the relative absorbance change, caused by the n'th impulse, by

$$\alpha_n = \Delta A_n / \Delta A_{\text{tot}} \tag{6}$$

where $\Delta A_{\text{tot}} = \sum_{n=1}^{\infty} \Delta A_n$ is the distance between the points A and B in Fig. 2.

The first impulse caused the fraction ϕ to react, while the fraction $(1 - \phi)$ remained unreacted. Thus, $\phi = \alpha_1$ and therefore ϕ can be calculated from the absorbance change, ΔA_1, resulting from the first impulse. The second impulse transforms the fraction ϕ of the unreacted portion $(1 - \phi)$, hence $\alpha_2 = \phi(1 - \phi)$. The remaining fraction is now $(1 - \phi) - \phi(1 - \phi) = (1 - \phi)^2$. The relative change obtained after a third pulse is $\alpha_3 = \phi(1 - \phi)^2$, and therefore in general

$$\alpha_n = \phi(1 - \phi)^{n-1} \tag{7}$$

Combining Eqs. **6** and **7** we find that

$$\log \Delta A_n = (n - 1) \log(1 - \phi) + \log(\phi \Delta A_{\text{tot}}) \tag{8}$$

The data of Fig. 3*a* are replotted in Fig. 3*b* calculated according to Eq. **8**. Within the experimental error, the dependence of $\log \Delta A_n$ on $(n - 1)$ is linear, and the slope yields $\phi = 0.083$ (± 0.005). The result is in good agreement with the value of ϕ obtained from the relation $\alpha_1 = \phi$. Since, per cm path-length, ΔA_{tot} is 0.106 (± 0.004) and $\Delta A_1 = 0.0083$ (± 0.0005), we obtain $\alpha_1 = 0.0783$ (± 0.008).

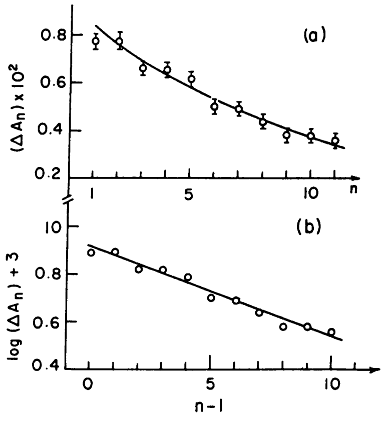

FIG. 3. Change in absorbance, ΔA_n at 260 nm as a function of the impulse number n (see Fig. 2).

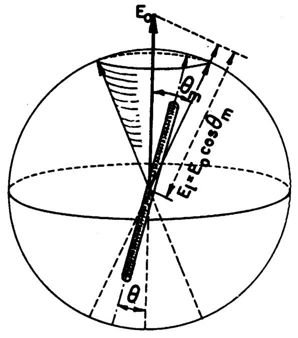

FIG. 4. Schematic representation of the rod vectors pointing to the spherical surface of the spherical sector having the apical angle $2\theta_m$. The fraction of these rods is $(1 - \cos \theta_m)$.

Using $\phi = 0.08$, we may state that under our experimental conditions each impulse caused 8% of the intact poly(A)·2 poly(U) molecules to undergo the conformational transition described by Eq. 1. From Eq. 5 we obtain $E_l = 18.6$ kV/cm as a limiting value of the initial field strength sufficient to permit the nucleation of (A·A) base pairs, and by Eq. 4, $\theta_m = 23°$. The total fraction of poly(A)·2 poly(U) molecules converted after the n'th impulse is

$$\sum_{n=1}^{n} \alpha_n = 1 - (1 - \phi)^n \tag{9}$$

Thus, after only 10 impulses, ~56% of all the complexes are transcrystallized. It should be mentioned that, on energetic grounds, even a series of impulses of the field strength and duration applied here cannot cause any damage to the primary structure of polynucleotides. A back titration of the pulsed solution to pH 7 and a subsequent titration to pH 4.5 resulted in the original absorbance curve as measured before the pulsing.

DISCUSSION

We cannot present a detailed quantitative analysis of long-lived conformational changes in biopolymers, induced by strong electric impulses. However, we can outline semiquantitatively the main factors involved in the processes and estimate their magnitudes and contributions.

Since we are working with very dilute polyelectrolyte solutions, each macromolecular complex may be treated as an isolated polyion surrounded by its ionic atmosphere. The residue concentration used in this study was 1.6×10^{-5} M (U·A·U), which for an average degree of polymerization $P \simeq 400$—estimated from the mean s-values of the single polymers (14, 15)—is equivalent to a mean macromolecular concentration of $\sim 4 \times 10^{-8}$ M, or to $\sim 2.4 \times 10^{13}$ polyionic complexes per cm^3. The electrostatic repulsions within the polyions, combined with the stiffness due to base stacking, allows us to represent the macromolecules as rather stiff rods of mean length l. Since the approximate length of an (U·A·U) base triplet along the axis of a poly(A)·2 poly(U) rod is 3 Å, we estimate $l \simeq 1.2 \times 10^{-5}$ cm. If we assume that each bipolymer rod is the center of a cylindrical cell of solution (11), the average distance between the polyionic molecules is several times the length of the triple helix. This may justify the treatment of the impulse effect as an interaction of electric fields with isolated polyionic systems.

A polyelectrolyte and its ionic atmosphere form a highly polarizable electrical system (11, 16). In the presence of an electric field of sufficiently high intensity, polarization of the polyelectrolyte will occur even at higher ionic strengths. In rod-like polyelectrolytes this polarizability is anisotropic. Because of the powerful electrostatic field, the movement of the counterions is restricted in directions *radial* to the polyion cylinder. The counterion "mobility" along equipotential surfaces *parallel* to the cylindrical polyion surface can be assumed to be similar to the mobility of these ions in the bulk solution. Since the ion density of the ionic atmosphere is appreciably higher than that of the surroundings, a strong electric field can provoke a displacement of the ionic atmosphere that will not be fully compensated for by an influx of ions from the medium towards the polyion. The shift thus induced of the counterions results in a large dipole moment.

For the three-stranded helix, the negative pole of the induced macro-dipole will be transiently in a nonequilibrium state with respect to the paired bases. Due to the displaced ionic atmosphere, the increased repulsive forces between the phosphate residues will cause dissociation of (U·A·U) base pairs and strand separation, starting at the dipole end and proceeding along the triple helix. At acidic pH values, the A residues decoupled from the base pairing with U residues are readily protonated. When the degree of protonation is sufficiently large (corresponding to a $\mathrm{pH} < \mathrm{pH}'_m$ in the titration experiment, Fig. 2), no reassociation to (U·A·U) sequences, and hence no reestablishment of the metastable state, can occur. Instead, the protonated poly(A)·poly(A) double helix is formed spontaneously. The pH difference between pH 4.5 and pH'_m corresponds to about 38% change in the absorbance (Fig. 2). Since the triple helix has ~400 base triplets, at least ~150 (U·A·U) base pairs must dissociate before (A·A) base-pair formation can start.

We can estimate a value for the time interval, Δt, required to decouple ~150 (U·A·U) residues from base pairing. Cohen and Crothers (17) have shown that the rate of unwinding small DNA (~10^5 daltons) is controlled by the dissociation time of one base pair. At the thermodynamically unstable negative pole of the polarized triple helix, the relative stability constant of the base pairings is $\ll 1$. Hence, dissociation of an (A·U) pair can occur within less than 10^{-7} sec (18), and the opening of n (A·U) base pairs requires about $n \times 10^{-7}$ sec. To a first approximation, we may (formally) consider the field-induced triple helix-coil transition as two subsequent double helix-coil transitions, (U·A·U) → (U·A) + (U) and (U·A) → (A) + (U). Thus, $n \simeq 2 \times 150$, and we obtain $\Delta t \simeq 30$ μsec.

During this time of "effective polarization" the field intensity decays. We may now define a lower limiting field intensity, E_m, required to shift the ionic atmosphere to such an extent that still one (U·A·U) triplet can dissociate. For $E < E_m$, no further base-pair separation occurs. To estimate the order of magnitude of E_m, we may write Eq. 3 as:

$$E_m = E_l \exp\{-(\Delta t - \bar{\tau})/\tau_E\} \tag{10}$$

where the relaxation time of the field decay is $\tau_E = 12.5$ μsec. We may use the approximation $\Delta t - \bar{\tau} \simeq 30$ μsec, provided the response time $\bar{\tau}$ of the ionic atmosphere to the electric field is small compared to Δt. We show below that this assumption is justified. With $E_l = 18.6$ kV/cm, we obtain $E_m \simeq 1.7$ kV/cm.

Well-known theories provide simple relations for the mean relaxation time of the ionic atmosphere and the polarizability of rod-like polyelectrolytes in weak electric fields and low ionic strengths (19–21). In the relatively strong fields used in our experiments, the linear dependence of the induced dipole moment on field strength does not hold any more and saturation phenomena are expected. Moreover, at the higher ionic strengths (close to the physiological range) required to stabilize the triple helixes, the polarizability of polyelectrolytes is much lower than in salt-free solutions. The theoretical treatment of Mandel accounts for saturation (20), while Hornick and Weill (22) have shown that the theoretical treatment of Oosawa (21) can be extended to describe the effect of the ionic strength on polarization. By Mandel's approach, the mean time $\bar{\tau}$ required to establish an average displacement $\overline{\Delta\xi}$ of the ionic atmosphere is given by

$$\bar{\tau} = \overline{\Delta\xi}/\bar{u}\cdot E, \tag{11}$$

where $\bar{u}$ is the mean mobility of the counterions in the atmosphere. As mentioned, we assume that $\bar{u} \simeq u(\mathrm{Na}^+, 17°, 0.1\ \mathrm{M\ NaCl}) = 4.2 \times 10^{-4}$ (cm/sec)/(V/cm). The displacement $\overline{\Delta\xi}$ corresponds to an induced dipole moment m, which, for monovalent counterions, can be expressed as:

$$\overline{\Delta\xi} = m/\{e_0\nu(1 - 2\phi_p)\} \tag{12}$$

where the elementary charge $e_0 = 4.8 \times 10^{-10}$ stat C; $\nu(1 - 2\phi_p)$ is the number of the condensed, but mobile, counterions (11, 23); $\nu = 3P \simeq 1.2 \times 10^3$ is the number of fixed charges on the triple helix; and ϕ_p is the osmotic coefficient. For our triple helix, we estimate that $\phi_p \simeq 0.15$ (see ref. 12). On the basis of Mandel's model we obtain the approximate equation for the dependence of the induced dipole moment on the field strength:

$$m \simeq {}^1/_2(1 - 2\phi_p)e_0\cdot l\,\{\nu \coth\,(e_0\cdot \mathrm{l}\cdot E/2kT) - \coth\,(e_0\cdot l\cdot E/2\nu kT)\}\cdot f \tag{13}$$

This expression contains a correction factor, f, which depends on ionic strength,

$$f \simeq [1 + 2(1 - 2\phi_p)\lambda_0\, ln(R/\bar{a})]^{-1} \tag{14}$$

where the charging parameter $\lambda_0 = e_0^2\nu/(\epsilon kTl)$. Since the value of the dielectric constant ϵ close to the polyions is not known, we assume $\epsilon = \epsilon(\mathrm{H_2O}, 17°) = 81$. The radius of the cylindrical cell volume of a triple helix is $R \simeq 3.8 \times 10^{-5}$ cm. The mean radius $\bar{a}$ of the counterion layer is the sum of the radius of the triple helix ($r \simeq 10$ Å) and the radial thickness of the ionic atmosphere which, for a 0.1 M NaCl solution at 17° is ~10 Å, so that $\bar{a} \simeq 20$ Å. Due to the great length of the helix, $l \gg r$, the intensity of the polarizing field in the polyelectrolyte is, to a good approximation, equal to the external field strength. With $E = E_l = 18.6 \times 10^3/300$ stat V/cm, we calculate $m \simeq 3.5 \times 10^4$ debye (which is close to the saturation value of ~4×10^4 debye). Inserting $m \simeq 3.5 \times 10^{-14}$ stat C·cm into Eq. 12, we obtain $\overline{\Delta\xi} \simeq 8.7$ Å, and with Eq. 11, the mean response time of the ionic atmosphere is $\bar{\tau} \simeq 0.01$ μsec, which is appreciably smaller than Δt.

Thus, in accordance with our rough estimates, the trigger effect of the electric impulse consists of a transient polarization of the triple helix followed by base-pair dissociation and strand separation to a critical extent, after that the new structure develops.

CONCLUDING REMARKS

By applying electrical impulses, it is possible to penetrate into the hysteresis loop of ribosomal RNA (4,5). Remarkably, the effect is detectable also at neutral pH—at which no hysteretic metastability was observed. Impulses applied to rRNA (at 0.1 M NaCl, pH 7, and 20°) produce an initial increase of A_{260}, followed by a gradual decrease to a level higher than the initial one. This behavior seems to reflect a transient opening of base pairs and the subsequent association of unfolded regions. During the phase of recombination, mismatching of nucleotide sequences may occur that will result in a spectrum of unmatched configurations. The extent of this reassociation to conformations different from the original one is determined by the intensity and the number of impulses applied. The mismatched macromolecules remain in these long-lived metastable states until slow annealing brings them back to the original stable state.

It is an intriguing question whether nerve impulses affect certain neuronal structures in a manner similar to the way in which electric impulses affect the biopolyelectrolytes in our experiments. During excitation of a nerve, the potential across the nerve membrane changes from about -70 mV to about $+50$ mV. Since the thickness of the membrane is about 100 Å, the potential change corresponds to a variation of the field intensity from -70 kV/cm to $+50$ kV/cm. We may assume that these electric fields act not only across the nerve membrane, but also in its close proximity and probably across the synaptic junction. Hence, any polyvalently charged system exposed to these powerful impulses could undergo far-reaching and long-lived conformational changes similar to those discussed above. We may therefore consider (metastable) conformational changes of biopolymers induced by electric impulses as a *model reaction* for the process of imprinting nerve impulses in the structures involved in the physical record of memory.

We thank I. Pecht for his help in using the T-jump apparatus, A. Revzin for preparation of rRNA, J. L. Jackson for critical comments, and the Stiftung Volkswagenwerk for a grant to E.N.

1. Roberts, R. B. & Flexner, L. B. (1969) *Quart. Rev. Biophys.* **2**, 135–173.
2. Katchalsky, A. & Neumann, E. (1972) *Intern. J. Neurosci.*, in press.
3. Katchalsky, A. & Oplatka, A. (1966) *Neurosci. Res. Symp. Sum.* **1**, 352–374; (1966) *Is. J. Med. Sci.* **2**, 4–13.
4. Cox, R. A., Jones, A. S., Marsh, G. E. & Peacocke, A. R. (1956) *Biochim. Biophys. Acta* **21**, 576–577.
5. Cox, R. A. & Katchalsky, A. (1971) *Biochem. J.*, in press.
6. Zimmer, Ch. & Triebel, H. (1969) *Biopolymers* **8**, 573–593.
7. Neumann, E. & Katchalsky, A. (1971) *Proc. 1st Eur. Biophys. Congr.* **6**, 91–95.
8. Felsenfeld, G. & Rich, A. (1957) *Biochim. Biophys. Acta* **26**, 457–468.
9. Miles, H. T. (1964) *Proc. Nat. Acad. Sci. USA* **51**, 1104–1109.
10. Neumann, E. & Katchalsky, A. (1970) *Ber. Bunsenges. Phys. Chem.* **74**, 868–879.
11. Katchalsky, A. (1971) *Pure Appl. Chem.* **26**, 327–373.
12. Auer, H. E. & Alexandrowicz, Z. (1969) *Biopolymers* **8**, 1–20.
13. Eigen, M. & De Maeyer, L. (1963) in *Techniques of Organic Chemistry*, eds. Friess, S. L., Lewis, E. S. & Weissberger, A. (Wiley, New York), Vol. 8, part 2, 895–1054.
14. Eisenberg, H. & Felsenfeld, G. (1967) *J. Mol. Biol.* **30**, 17–37.
15. Inners, L. D. & Felsenfeld, G. (1970) *J. Mol. Biol.* **50**, 373–389.
16. Eigen, M. & Schwarz, G. (1957) *J. Colloid Sci.* **12**, 181–194; (1962) in *Electrolytes*, ed. Pesce, B. (Pergamon Press, Elmsford, N.Y.), pp. 309–335.
17. Cohen, R. J. & Crothers, D. M. (1971) *J. Mol. Biol.* **61**, 525–542.
18. Pörschke, D. & Eigen, M. (1971) *J. Mol. Biol.* **62**, 361–381.
19. Schwarz, G. (1959) *Z. Phys. Chem. (Frankfurt am Main)*, **19**, 286–314.
20. Mandel, M. (1961) *Mol. Phys.* **4**, 489–496.
21. Oosawa, F. (1970) *Biopolymers*, **9**, 677–688.
22. Hornick, C. & Weill, G. (1971) *Biopolymers*, **10**, 2345–2358.
23. Manning, G. (1969) *J. Chem. Phys.* **51**, 934–938.