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ENERGY MIGRATION IN THE POLY(rA–rU)–ETHIDIUM COMPLEX. DETERMINATION OF THE UNWINDING ANGLE OF THE POLYRIBONUCLEIC HELIX *

Ph. WAHL, D. GENEST and J.L. TICHADOU **
Centre de Biophysique Moléculaire, 45045-Orléans Cedex, France

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The polarized fluorescence of the ethidium bromide (EB)–poly(rA–rU) complex has been studied by pulse fluorometry. As expected for a polynucleotide showing one single kind of intercalation site, the decay of the whole emission is a single exponential (time constant 27 ns). The anisotropy decay is analysed as follows: (1) A brownian contribution having two correlation times, one of which characterizes local motions and the other a macromolecular motion. (2) A contribution due to transfers between EB molecules fixed to the same polynucleotide molecule, is analysed by a method analogous to the method used in previous work on EB–DNA complexes. That method consists in choosing a molecular model of the complex depending on geometrical parameters, and in simulating the energy migration on that model with a Monte Carlo calculation. Poly(rA–rU) is assumed here to adopt the structure A of RNA. Intercalated EB molecules modify the angle between two consecutive base pairs by δ. The angular position of the EB transition moment is defined by an angle ϕ. One finds that the angle ϕ is situated between 0° and 30°, which corresponds to a whole intercalation of the chromophore as opposed to the semi-intercalation which has been proposed for certain dyes. The angle δ is negative; therefore there is an unwinding of the polyribonucleotide helix. Its absolute value is about 38°, sensibly greater than the value previously found for EB–DNA complexes.

1. Introduction

In our previous work, we studied the complexes of EB with calf thymus DNA and with poly d(A–T), using a pulse fluorometry method. The anisotropy decay for small values of D/P (number of dyes per nucleotide) was interpreted as being a result of a local brownian motion [1–3].

As the ratio D/P increased, the anisotropy decay was accelerated. This observation was assigned to energy migration between dyes bound to the same DNA molecule [2,4,5]. In order to interprete quantitatively these experiments, we performed a Monte Carlo calculation on a model of the complex in which the DNA molecule was assumed to be in the B form and in which the dye induced an angular deformation [4,5]. A good fit of the computed curve with the experimental results was obtained by assuming that the dye unwinds the DNA helix by a value of the order of 16° [3–5]. This is in fair agreement with the unwinding angle that Bauer and Vinograd [6] have determined in their analysis of buoyant density experiments of circular DNA, and which Füller and Waring [7] have predicted by model studies.

We thought it interesting to extend our measurements to a complex of EB with a ribonucleic acid in double helix, since the structure of these molecules is quite different from the structure of the DNA molecules [8]. In the present work, we present a study of the EB–poly(rA–rU) complex.

2. Theory: Energy transfers and model of the complex

For the rate of an elementary transfer between two chromophores A and B, we use here the formula of Förster [9], valid in the case of "very weak interactions"

$$V_T = (C/n^4)(J/\tau_0)K^2/R^6.$$

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** Present address: Centre de Recherches de Biochimie et de Génétique Cellulaires, 118, route de Narbonne, 31400 Toulouse, France.

C is an universal constant; J is the overlap integral of the absorption and fluorescence spectra; τ_0 the natural lifetime of the excited state; R the distance separating the chromophores A and B, K^2 is defined by:

$$K^2 = (\cos\theta - 3\cos\theta_A \cos\theta_B)^2.$$

If $\boldsymbol{\mu}_A$ and $\boldsymbol{\mu}_B$ are unit vectors in the direction of the emission transition moments of the molecules A and B, and $\boldsymbol{u}$ the unit vector along the line joining A and B, one has (see appendix and fig. 10):

$$\cos\theta = \boldsymbol{\mu}_A \cdot \boldsymbol{\mu}_B,$$

$$\cos\theta_A = \boldsymbol{\mu}_A \cdot \boldsymbol{u}, \qquad \cos\theta_B = \boldsymbol{\mu}_B \cdot \boldsymbol{u}.$$

The geometrical factors R and K^2 are determined by the structure of the EB–polynucleotide complex. In our previous work, the polynucleotides were polydeoxyribonucleotides, and the calculations were based on the B type structure. We assume that poly(rA–rU) molecules in solution have the A-type structure in double helix, the geometry of which has been determined by X-ray diffraction studies [8]. There are 11 base pairs per helix turn; consequently the angle between two neighbouring base pairs is $\alpha = 33°$. The projection, on the helix axis of the segment joining two equivalent points of two consecutive base pairs is $b = 2.81$ Å. The distance between the base centers and the helix axis is: $D = 4.32$ Å. The plane of the base is tilted by 14°. In our calculation, we neglect the angle of twist which is only 2.2° [8].

The intercalation of EB increases the distance between the neighbouring base pairs by a distance $c = 3.4$ Å. We designate by ϕ the angle of the dye transition moment with the plane tangent to a cylinder of axis H and radius D.

According to Giacomoni and Le Bret [10], the transition moment of EB lies in the phenantridinium plane and makes an angle of 30° with its longitudinal axis. Therefore, if ϕ is of the order of 0° or 30°, this axis will be close to the direction of the line joining two paired bases. On the other hand, if ϕ is about 90°, the dye molecule has its longitudinal axis perpendicular to the base line. The first case is in agreement with a complete intercalation [7,11] while the second case corresponds to the semi-intercalation postulated by Pritchard et al. [12] for certain acridine dyes.
We assume that the intercalation of EB molecules does not change the diameter D of the helix and the angle of tilt, but the angle α between two adjacent base pairs is increased by a value δ. To superpose the chromophores A and B, we have to perform a translation h along the helix axis and a rotation γ in a plane perpendicular to this axis.

Under these conditions, the following relations can be established (see appendix I):

$$\begin{aligned} R^2 &= h^2 + (2D\sin\tfrac{1}{2}\gamma)^2, \\ \cos\theta &= (\sin^2\omega\cos\gamma + \cos^2\omega)\cos^2\phi + \sin^2\phi\cos\gamma, \\ \cos\theta_A &= (D/R)[(\cos\gamma - 1)\sin\phi + \sin\gamma\sin\omega\cos\phi] \\ &\quad - (h/R)\cos\omega\cos\phi, \\ \cos\theta_B &= (D/R)[(1 - \cos\gamma)\sin\phi + \sin\gamma\sin\omega\cos\phi] \\ &\quad - (h/R)\cos\omega\cos\phi, \end{aligned} \tag{1}$$

where ω is the complementary angle of the angle of tilt ($\omega = 76°$). When ϕ is replaced by $-\phi$ or $\pi - \phi$ in these relations, the expressions of $\cos\theta$ and of the product $\cos\theta_A \cos\theta_B$ are not changed.

$$\begin{aligned} h &= (n + i + 1)b + (i + 1)(C/\sin\omega - b)(\alpha - \delta)/2\alpha, \\ \gamma &= n\alpha + (i + 1)\delta. \end{aligned} \tag{2}$$

The anisotropy decay calculus has been performed by a Monte Carlo method which has been described in detail in a previous work [4,5]. A first simulation calculus is used to determine a chromophore distribution on the basis of an equal probability of all the sites with exclusion of two neighbouring sites [13, 14]. The energy migration is simulated step by step. At each step, the probability of the excitation stay is given by an exponential law depending on the total rate of energy transfer. This is the sum of the elementary rates of transfers towards the four dyes which are the nearest neighbours of the excited dye. The specification of the dye which effectively received the excitation is determined by a third simulation [14]. Each time, the anisotropy decay due to energy migration is given by:

$$r_T(t) = \tfrac{3}{2}\langle\cos^2\Phi(t)\rangle - \tfrac{1}{2}$$

where $\Phi(t)$ is the angle of the transition moment of the primary excited dye and the dye excited at time t. The angular brackets mean an average taken on a great number of simulations.

3. Material and methods

3.1. Biochemicals

The polyribonucleotides poly rA and poly rU come from Miles Laboratories. They have been purified by phenol extraction, dialysed against Tris-HCl buffer (NaCl 0.01 M, EDTA 10^{-4} M, pH 7.6) and against Tris-HCl 0.01 M, NaCl 0.01 M, in order to eliminate cations other than sodium. A last dialysis against bidistilled water has been followed by a lyophylisation.

Two preparations of poly rA and poly rU have been done.

The respective sedimentation coefficients $S_{20,w}$ of poly rA and poly rU, determined at 20°C in Tris-HCl buffer (0.01 M, NaCl 0.15 M, pH 7.5) were equal to 7.3 and 3.46 for sample I and to 4.76 and 3.92 for sample II.

The poly (rA–rU) solutions were obtained by mixing equimolar solutions of poly rA and poly rU. Concentrations of poly rA and poly rU were determined by spectrophotometry, using the following values for extinction coefficients [15]:

$\epsilon = 10^4$ M^{-1} cm^{-1} for poly rA at 257 nm,
$\epsilon = 0.943$ 10^4 M^{-1} cm^{-1} for poly rU at 260 nm.

In order to check that only the double helix form poly(rA–rU) and not the triple helix form poly-(rA–2rU) was present in solution , we determined the melting curves by measuring the temperature dependence of the optical densities at 280 and 283.5 nm [16].

3.2. Binding curve

The binding curve of EB to poly(rA–rU) was determined by absorption measurements as described by Waring [17,18]. The molar extinction coefficient if free EB (ϵ_f) was taken equal to 5600 cm^{-1} $mole^{-1}$ at 480 nm [18]. Absorbancy measurements were performed at 460 nm and 480 nm, keeping the EB concentration constant, and varying the polymer concentration. The apparent value of the molar extinction coefficient (ϵ_{app}) reaches a plateau at high polymer concentrations, which provides the molar extinction coefficient of the bound chromophore ϵ_b.

The fraction C_b of EB bound to poly(rA–rU) was determined by using the formula:

$$C_b = [(\epsilon_{app} - \epsilon_f)/(\epsilon_b - \epsilon_f)]\, C_0,$$

where C_0 is the total dye concentration. The binding isotherm was plotted according to the method of Scatchard [19].

3.3. Fluorescence measurements

The principle of the method is only outlined here. Full details have been given in previous work [2,5, 20]. In order to determine the overlap integral J, the absolute fluorescence spectrum of EB–poly(rA–rU) was determined with a Cary special product no. 50903 spectrofluorometer.

The fluorescence decay in polarized light $s(t)$ and $d(t)$ was obtained with a pulse fluorometer.

The "true decay" of the fluorescence, $S(t)$, was determined by deconvolution of $s(t)$, with the apparatus response $g(t)$. This function has been determined by using a scattering solution in the case of sample I, and a reference compound as described before [3] in the case of sample II. The curve $d(t)$ was compared with curves $d'(t)$ calculated according to the following formula [4,5]:

$$d'(t) = g(t) \times [S(t) r_0 r_B(t) r_T(t)].$$

r_0 is the anisotropy at time 0, $r_B(t)$ the anisotropy due to the brownian motion. $r_0 r_B(t)$ is measured with a solution of the complex containing a small amount of dye, so that energy transfers are negligible and $r_T = 1$. r_T, the anisotropy due to energy migration, is calculated as described above.

The values of $d(t)$ and $d'(t)$ are known in n points. For each computed curve, one determines the mean weighted residue [21]:

$$F = n^{-1} \sum_{i=1}^{n} (d_i - d_i')^2 / d_i.$$

The computed curve which corresponds to the best geometrical model gives the smalles value of F.

4. Results

4.1. Binding curve

The binding isotherms of EB to poly(rA–rU) was determined with sample II in a Tris-HCl 0.05 M

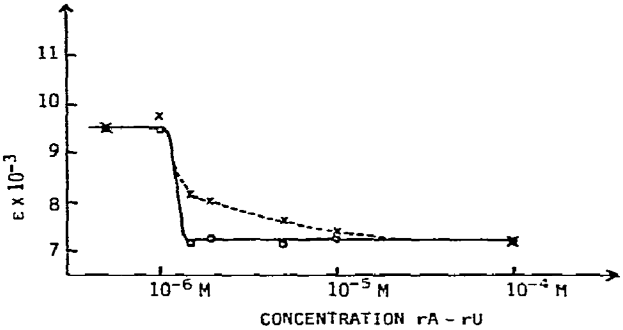

Fig. 1. Variation of the molar absorbancy of poly(rA–rU) with concentration (o: measurements made immediately after dilution, ×: measurements made 48 h after dilution).

NaCl 0.15 M buffer (pH 7.6), as described in section 3.

In a first step we verified the stability of poly(rA–rU) at low concentrations by measuring the absorbancy of successive dilutions, at 260 nm. The molar absorptivity of poly(rA–rU) was taken to be equal to 7.14×10^3 M^{-1} cm^{-1} at high concentrations [15]. For each solution, measurements have been done with the freshly prepared solution (time smaller than $\frac{1}{2}$h) and after a delay of 48 h. The results are shown in fig. 1. It can be seen that for concentrations above 10^{-5} M, poly(rA–rU) is stable. Consequently we used in our measurements poly(rA–rU) concentrations greater than 3×10^{-5} M.

Fig. 2 shows the apparent molar absorptivity ϵ_{app} of EB at 460 nm and 480 nm, for different polymer concentrations. As stated in section 3, ϵ_b was determined by the plateau value of ϵ_{app}.

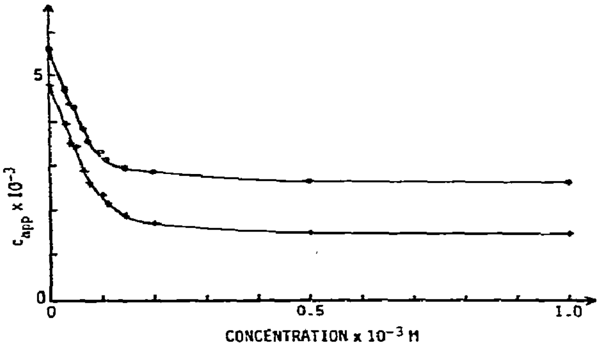

Fig. 2. Variation of ϵ_{app} of EB with polymer concentration. The EB concentration is equal to 1.77×10^{-5} M (o: absorption at 480 nm; +: absorption at 460 nm).

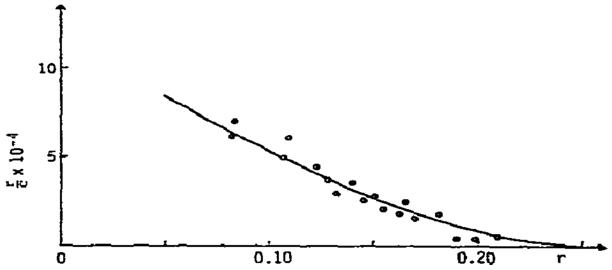

Fig. 3. Scatchard plot for the binding of EB to poly(rA–rU). The EB concentration was equal to 1.77×10^{-5} M (o: experimental data; ——: theoretical curve computed with the excluded site model).

In fig. 3 the binding of EB to poly(rA–rU) is plotted using the Scatchard representation. On the same figure is shown the curve computed according to the excluded site model with an association constant of 2.36×10^5 M^{-1}. It can be seen that the theoretical curve fits the experimental data.

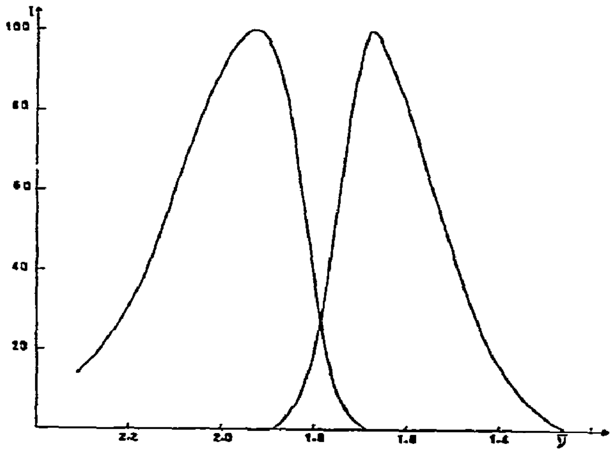

Fig. 4. Absorption and fluorescence spectra of EB–poly(rA–rU) complex. The fluorescence intensity I is expressed in arbitrary units and the wavenumber ν in $m^{-1} \times 10^{-6}$.

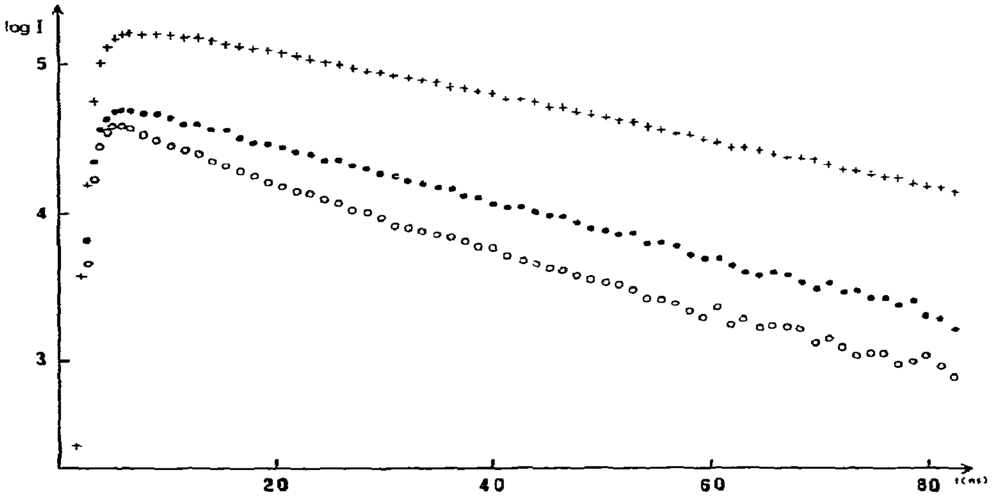

Fig. 5. Experimental curves $s(t)$ (+) and $d(t)$ for P/D = 160 (•) and P/D = 7.35 (○).

4.2. Fluorescence measurements

The overlap integral was found to be:

$J = 1.07 \times 10^{-15}$ cm^6 mole^{-1}.

As refractive index of poly(rA–rU) we take n = 1.6 [5] and as the natural lifetime of the excited state τ_0 = 73 ns [5].

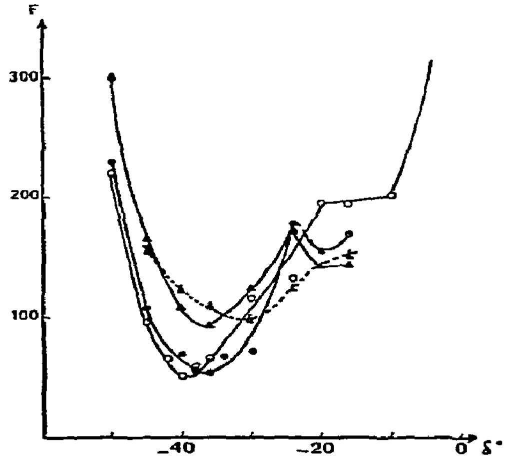

Fig. 6. Average weighted residues versus δ for different conformations of EB–poly(rA–rU) complex (sample I): (○ A form of RNA with ϕ = 0°; • A form of RNA with ϕ = 30°; △ A form of RNA with ϕ = 90°; ▲ B form of DNA).

Fig. 4 shows the absorption and fluorescence spectra of EB bound to poly(rA–rU).

We determined the transient fluorescence of EB on the following conditions: (1) poly(rA–rU) sample I was used in a Tris-HCl 0.005 M, NaCl 0.015 M buffer

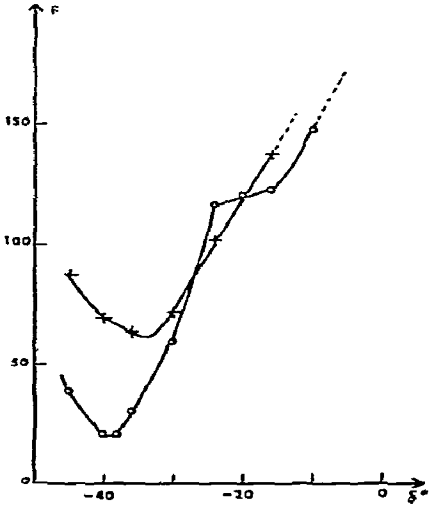

Fig. 7. Average weighted residues versus δ for different conformations of EB–poly(rA–rU) complex (sample II) (○ A form of RNA with ϕ = 0°; + A form of RNA with ϕ = 90°).

(pH 6.6) with P/D equal to 160 and 7.35.

(2) Poly(rA–rU) sample II was used in a Tris-HCl 0.05 M, NaCl 0.15 M buffer (pH 7.6) with P/D equal to 115 and 7.35. In all cases, the $S(t)$ decay is described by a single exponential:

$$S(t) = e^{-t/\tau}$$

with $\tau = 27$ ns.

The $d(t)$ curves obtained with poly(rA–rU) sample I are shown in fig. 5.

We determined $r_0 r_b(t)$ for P/D = 160 and we find:

$$r_0 = 0.345,$$
$$r_B(t) = 0.436e^{-t/\rho_1} + 0.564e^{-t/\rho_2},$$

with $\rho_1 = 22$ ns and $\rho_2 = 270$ ns.
With poly(rA–rU) sample II and with P/D = 115 we obtained:

$$r_0 = 0.345,$$
$$r_B(t) = 0.29e^{-t/\rho'_1} + 0.71e^{-t/\rho'_2};$$

with $\rho'_1 = 8.6$ ns and $\rho'_2 = 120$ ns.

We computed the $r_T(t)$ functions assuming P/D = 7.35 for 3 values of ϕ: 0°, 30° and 90°, and for δ values varying from −50° to −16°. In the case of ϕ = 0°, we added the value −10°, 0° and +10°.

Figs. 6 and 7 show the average weighted residue F versus δ for the different cases. One can see on these figures that the best fits are obtained with $\phi = 0°$ and $\delta = -40°$, $\phi = 30°$ and $\delta = -36°$, with both poly(rA–rU) samples. It can also be seen on these figures that the curves computed with $\phi = 90°$ disagree with the experimental ones.

In fig. 8, the experimental $d(t)$ curve relative to sample I is compared with computed curves for three values of δ taking $\phi = 0$.

In order to determine the influence of the polyribonucleotide structure on the curve $r_T(t)$, we used the curve computed in our previous works for the DNA-B structure [4,5]. Fig. 9 shows that the $d'(t)$ curves thus obtained do not fit $d(t)$ even with the best value of $\delta = -36°$.

In order to study how the chromophore distribution along the polymer influences our results, we computed the anisotropy corresponding to a "two excluded sites model", in which the four sites adjacent to an occupied one cannot bind a chromophore. The corresponding results are shown in table 1 where ϕ is taken equal to zero. Similar results were obtained

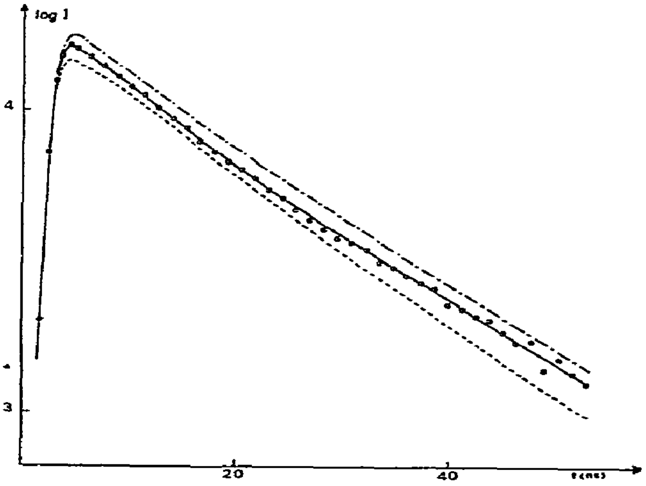

Fig. 8. Comparison between the experimental curve $d(t)$ (o) for P/D = 7.35 and the computed $d'(t)$ curves assuming the A structure of RNA with $\phi = 0°$ for different values of δ: (— . — . $\delta = -50°$; —— $\delta = -40°$; — — — $\delta = -16°$).

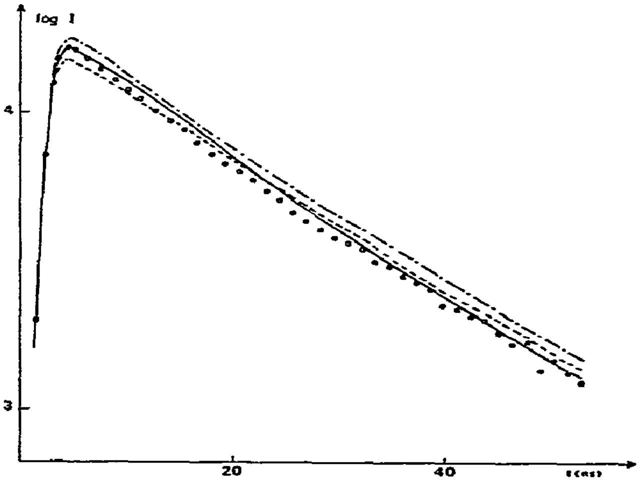

Fig. 9. Comparison between experimental curve $d(t)$ (o) for P/D = 7.35 and computed curves $d'(t)$ assuming the B structure of DNA for different values of δ: (— . — . $\delta = -50°$; —— $\delta = -36°$; — — — $\delta = -16°$).

Table 1
Variation of F with δ, assuming a two excluded sites model of EB–poly(rA–rU) complex, with $\phi = 0$

δ (deg)	F
–45	600
–40	580
–36	610
–30	595
–26	585
–20	635
–16	815
–10	2060

with $\phi = 90°$. It can be seen that the F values remain very high whatever the δ value is.

5. Discussion

We first notice that the fluorescence decay of the EB–poly(rA–rU) complex is a single exponential. This result corresponds to a great homogeneity of the intercalation sites.

The brownian anisotropy comprises two exponential terms. The exponential time constants are the molecular correlation times. The shortest correlation time has a value which is of the same magnitude to the one found with the EB–DNA complex [3,5]; By analogy with that case, we attributed it to a torsional motion of the double helix polynucleotide. The greatest relaxation time must involve much longer sections of molecular helix and perhaps a rotation of the whole molecule. One must envisage indeed that in this synthetic polynucleotide, the helix sections are interrupted by single strand sections which are relatively flexible. In addition, the molecular weight of the polymers used here is relatively small and some contribution of the rotation of the whole molecule may also exist.

On the contrary, in the case of EB–DNA, the second correlation time was infinite. This was well explained since the molecular weight was very high and no helix interruption was expected with these molecules.

For each sample I and II we selected the parameters of the brownian anisotropy function, which corresponds to the minimum value of the residual F. However we found that F increases only slightly when the parameters are varied about the values corresponding to the minimum. So we will not speculate in this work about the different parameters values obtained with the two samples I and II.

In a first attempt to interpret the fluorescence depolarization due to energy transfers in the complex EB–poly(rA–rU), we simply used the calculations we made in our previous work [4,5]. This means that in the Monte Carlo simulation, the B structure of DNA was used. In this case the only adjustable parameter was the angle δ. By this means, it was not possible to achieve a good fit of the calculated curves with the experimental one.

We then decided to introduce in the calculation the A structure of RNA defined above. Due to this relatively complicated structure, energy migration among intercalated EB depends, in addition to δ, on two other parameters. In particular, we had to evaluate the projection $b + \Delta b$ of the distance between two base pairs separated by an EB molecule. The variation of this parameter must depend on the variation of δ. We assumed somewhat arbitrarily that this dependence is linear. In order to estimate the error involved by this approximation, calculations have been made for different δ values with fixed values of $b + \Delta b$ equal to 2.8 Å and 3.5 Å. In both cases, the best δ value was changed by less than 5°.

Another parameter involved in the calculation is the angle ϕ which defines the position of the EB transition moment in the site. As we have seen, ϕ can vary from 0° to 30° without sensibly altering the quality of the fit.

However, ϕ cannot have an arbitrary value; when ϕ equals 90°, the average residue is considerably increased. According to the calculation of Giacomoni and Le Bret [10]. the transition moment makes an angle of 30° with the longitudinal axis of the EB molecule.

We then arrive at the interesting result, namely that the position of the EB longitudinal axis is about parallel to the line joining two appaired bases.

It also emerges from our analysis that the depolarisation decays agree well with the "one excluded site" model, but not with a "two excluded site" one. This result is in good agreement wiht the binding curve of ethidium on poly(rA–rU).

As with the EB–DNA complex, the best value of δ

is negative, which implies that EB induces an unwinding of the polynucleotide helix. However, the precise value of the parameter depends on the chosen value of ϕ, δ being equal to $-40°$ with $\phi = 0°$ and δ equal to $-36°$ with $\phi = 30°$. In any case, δ has clearly an absolute value greater than in the case of EB–DNA complex where it was equal to $-16°$ [3–5].

It is interesting to compare out results with the structures proposed by Füller and Waring [7] and by Pigram et al. [22]. For EB–polynucleotide complexes, X-ray diffraction and model studies lead these authors to assume that a dye molecule is intercalated with its long axis along the line joining two appaired bases. In addition, they found that the value of the unwinding angle must be situated between 10° and 25° in the case of the B structure of DNA, and between 24° and 38° in the case of the A structure of DNA. We note that our results are in fair agreement with these proposals since the A structures are very similar in DNA and in RNA [8].

We must however stress that Pigram et al.'s work concerning the A structure of DNA was based only on model studies.

It then appears that our work gives the first experimental data concerning a complex of EB with a polynucleotide in structure A. We think that this fact illustrates well the possibilities of our method.

Acknowledgement

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Appendix I

We compute the components of the unit vectors μ_A and μ_B in the xyz axes system having its origin in A (fig. 10).

$$\mu_A = (\sin\phi,\ \cos\phi \sin\omega,\ -\cos\omega \cos\phi),$$

$$\mu_B = (\sin\phi \cos\gamma - \sin\omega \cos\phi \sin\gamma,\ \sin\phi \sin\gamma + \sin\omega \cos\phi \cos\gamma,\ -\cos\omega \cos\phi).$$

The A and B coordinates in the same system are:

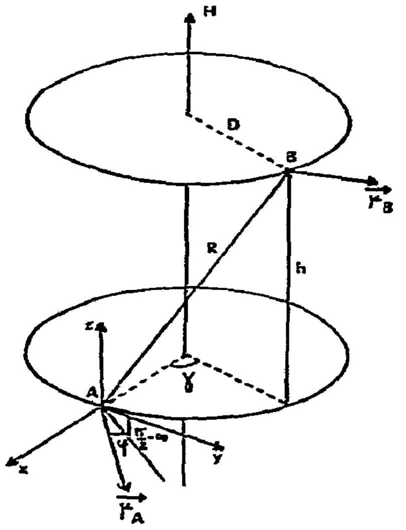

Fig. 10. Geometrical model of EB–poly(rA–rU) complex.

$$A = (0, 0, 0), \qquad B = (D(\cos\gamma - 1), D \sin\gamma, h).$$

We can then write:

$$\begin{aligned} R^2 &= D^2(\cos\gamma - 1)^2 + D^2 \sin^2\gamma + h^2 \\ &= 2D^2(1 - \cos\gamma) + h^2 = 4D^2 \sin^2\tfrac{1}{2}\gamma + h^2 \\ &= (2D \sin\tfrac{1}{2}\gamma)^2 + h^2. \end{aligned}$$

On the other hand, we have:

$$\begin{aligned} \cos\theta = \mu_A \cdot \mu_B &= \sin^2\phi \cos\gamma - \sin\omega \sin\phi \cos\phi \sin\gamma \\ &\quad + \sin\phi \cos\phi \sin\omega \sin\gamma + \cos^2\phi \sin^2\omega \cos\gamma \\ &\quad + \cos^2\omega \cos^2\phi \\ &= \cos^2\phi(\cos^2\omega + \sin^2\omega \cos\gamma) + \sin^2\phi \cos\gamma. \end{aligned}$$

Let u be the unit vector on AB. Its components are:

$$u = (D/R)(\cos\gamma - 1), ((D/R)\sin\gamma, h/R).$$

The scalar products of μ_A and μ_B with u give the two last equations of eq. (1) in the text.

Appendix II

We designate by $2x$ the projection on the helix axis of the distance between two consecutive base pairs separated by an EB molecule. Furthermore, we assume that this projection varies linearly with $\beta = \alpha + \delta$.

For $\beta = 0, \delta = -\alpha$ one has $x = b' = c/\sin\omega$.
For $\beta = 2\alpha, \delta = \alpha$ one has $x = b$.

This immediately leads to the formula:

$$x = b + \Delta b,$$

with

$$\Delta b = [(\alpha - \delta)/2\alpha](c/\sin\omega - b).$$

For the projection between any chromophores A and B one obtains:

$$\begin{aligned} h &= nb + (i+1)x = (n+i+1)b + (i+1)\Delta b \\ &= (n+1+i)b + (i+1)(c/\sin\omega - b)(\alpha - \delta/2\alpha). \end{aligned}$$

References

[1] Ph. Wahl, J. Paoletti and J.B. Le Pecq, Proc. Natl. Acad. Sci. US 65 (1970) 417.

[2] D. Genest and Ph. Wahl, Biochim. Biophys. Acta 259 (1972) 175.

[3] J.L. Tichadou, D. Genest, Ph. Wahl and G. Aubel-Sadron, Biophys. Chem. 3 (1975) 142.

[4] D. Genest and Ph. Wahl, in: Dynamic aspects of conformation changes in biological macromolecules, ed. Ch. Sadron (Reidel, Dorderecht, 1973) pp. 367–379.

[5] D. Genest, Ph. Wahl and J.C. Auchet, Biophys. Chem. 1 (1974) 266.

[6] W. Bauer and J. Vinograd, J. Mol. Biol. 33 (1968) 141.

[7] W. Fuller and M.J. Waring, Ber. Bunsenges. Physik. Chem. 68 (1964) 805.

[8] S. Arnott, in Progress in Biophysics and Molecular Biology, Vol. 21, eds. Butler and Noble (Pergamon, Oxford, 1970) pp. 267–319.

[9] Th. Förster, Ann. Physik. 2 (1948) 55; in Modern quantum chemistry, Vol. 3 ed. O. Sinanoğlu (Academic Press, New York, 1965) pp. 93–137.

[10] P.U. Giacomoni and M. Le Bret, FEBS Letters 29 (1973) 227.

[11] L.S. Lerman, J. Mol. Biol. 3 (1961) 18.

[12] N.J. Pritchard, A. Blake and A.R. Peacoke, Nature 212 (1966) 1360.

[13] W. Bauer and J. Vinograd, J. Mol. Biol. 47 (1970) 419.

[14] J. Paoletti and J.B. Le Pecq, J. Mol. Biol. 59 (1971) 43.

[15] D.E.V. Schmechel and D.M. Crothers, Biopolymers 10 (1971) 465.

[16] R.D. Blake, J. Massoulić and J. Fersco, J. Mol. Biol. 30 (1967) 291.

[17] M.J. Waring, Biochim. Biophys. Acta 114 (1966) 234.

[18] M.J. Waring, J. Mol. Biol. 13 (1965) 269.

[19] G. Scatchard, Ann. NY Acad. Sci. 51 (1949) 660.

[20] Ph. Wahl, Biochim. Biophys. Acta 175 (1969) 55.

[21] A.E.W. Knight and B.K. Selinger, Spectrochim. Acta 27A (1971) 1223.

[22] W.J. Pigram, W. Fuller and L.D. Hamilton, Nature New Biol. 253 (1972) 17.