Structural investigation of nuclear RNP particles containing pre-mRNA by different fluorescence techniques

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ABSTRACT

Ethidium bromide (EB) adsorption isotherms on 30S nuclear RNP particles isolated from liver nuclei has revealed 6% of double-stranded regions in pre-mRNA (dsRNA).

It has been established by measurements of the EB fluorescence polarization that the bulk of dsRNA regions in RNP is rigidly attached to RNP. They are longer than 45 Å. The increase of NaCl concentration from 0.1 up to 0.4 M causes a significant loosening of dsRNA-protein bonds. As a result the dsRNA segments become more flexible.

Measurements of energy transfer from fluorescamine (covalently bound to the protein) to EB (adsorbed on dsRNA) have yielded information about dsRNA location. The fact that absorption of exciting light by fluorescamine causes pronounced increase of EB fluorescence is consistent with the idea that helical regions of RNA are located outside the RNP particles.

INTRODUCTION

It is assumed that processing of pre-mRNA and its transport in the cell nucleus take place in nuclear RNP complexes¹⁻⁴. These complexes consist of chains of repeating units and can be easily cleaved into monomers with mild RNAase treatment (30S particles)^{1,2}. Detailed knowledge of structural organization of the pre-mRNA and the protein (informoer) in these particles is important for understanding of the processes mentioned above.

Some experimental data show that a pre-mRNA chain (about 600 nucleotides in length) is located mainly on the surface of the 30S particle¹⁻⁵. Biochemical studies have revealed double-stranded regions in pre-mRNA of 30S particles (dsRNA), which are resistant to treatment with the mixture of pancreatic and T₁-RNAases⁶. Similar results have been obtained by Calvet and

Pederson, who in addition digested double-stranded (ds) segments by RNAase III ⁷.

In this paper further investigation of the structural organization of pre-mRNA in RNP particles has been carried out using a combined set of fluorescence methods. Their major advantage is the ability to gain information about the pre-mRNA structure in RNP particles without destroying the latter.

Some fluorescent dyes can be specifically bound either to RNA or to proteins. It is known that the ethidium bromide (EB) adsorption on double-stranded nucleic acids causes profound increase of its fluorescence yield ⁸. The nature of EB interaction with nucleic acids has been studied by many investigators ⁸⁻¹⁵.

Fluorescamine (FL) is now widely used as a highly sensitive reagent for the fluorimetric assay of amines, amino acids and proteins ¹⁶⁻²².

The EB fluorescence has been used in this work for quantitative determination of dsRNA in nuclear 30S particles. Measurements of fluorescence polarization of EB complexed with RNA have been used as a source of information about the size and the flexibility of ds sequences in 30S particles.

The relative measurements of energy transfer from fluorescamine to EB attached to different components of the same RNP particle may provide some information about dsRNA location.

MATERIALS AND METHODS

Material. (a) 30S RNP particles were isolated from rat liver nuclei as is described previously ^{2,5,6}. Fractions of 30S peak from the sucrose gradient were collected and dialyzed against a buffer containing either 0.1 or 0.36 M NaCl, 0.001 M MgCl₂, 0.01 M TEA-HCl; pH = 7 (STM-buffer). A portion of dialyzed material was treated with DNase (Worthington). This treatment did not change the fluorescence characteristics of bound dyes.

Pre-mRNA concentration in 30S particles was calculated using optical density measurements at 260 nm ²². Pre-mRNA and protein from the nuclear 30S particles and DNA from E.coli were isolated as in ²².

Lysine (Calbiochem) at a concentration $2 \cdot 10^{-4}$ M was dissolved in 0.005 M cacodylate buffer with 0.01 M NaCl, pH = 7.5.

(b) Covalent binding of fluorescamine with 30S RNP particles.

The microvolumes of FL solution in dry acetone (3 mg/ml) were added to dialysed RNP particles. The final dye concentration never exceeded $5 \cdot 10^{-5}$ M. The unbound FL was completely hydrolyzed in water solution¹⁸⁻²⁰.

It was shown²² that FL adsorption did not change the sedimentation coefficient of 30S particles. The FL concentration measurements in 30S particles were described earlier²². The number of FL molecules per a 30S particle in our experiments varied from 10 to 60.

Methods. (a) Measurements of absorption and fluorescence spectra. Absorption spectra were recorded with spectrophotometer Cary-118 C. Fluorescence and excitation spectra were recorded with spectrofluorimeter Hitachi MPF-2A. The fluorescence decay kinetics was studied with nanosecond fluorescence spectrometer Ortec 9200. Fluorescence polarization (P) was measured with spectrofluorimeter Aminco SPF 1000. The fluorescence polarization coefficient of EB adsorbed on 30S particles (P_2) was calculated from equation:

$$P = I_1 P_1 / (I_1 + I_2) + I_2 P_2 / (I_1 + I_2) \quad (1),$$

where $I_i = I_{\parallel i} + I_{\perp i}$ are fluorescence intensities of free ($i = 1$) and bound ($i = 2$) EB; $I_{\parallel}$ and $I_{\perp}$ - the fluorescent light parallel and perpendicular, respectively, to the direction of polarization of the exciting light²³.

Emission was measured at 610 nm with 520 nm excitation.

(b) Absorption isotherms. Absorption isotherms of EB on 30S particles expressed as r/c_1 versus r (r is the ratio of the amount of the dye adsorbed to the number of nucleotides) were obtained by means of fluorescence intensity measurements at 610 nm (excitation at 520 nm). Concentration of adsorbed dye C_2 was calculated from equation, advanced in⁸:

$$C_2/C_0 = (I - I_1)/(I_2 - I_1) \quad (2).$$

(c) Energy transfer. From weak dipole-dipole coupling the rate of energy transfer W from the donor (D) to the acceptor (A)

may be expressed by the Förster equation ²⁴:

$$W = \tau_D^{-1} R_0^6 / R^6 \quad (3),$$

where R - distance between the D - A dipoles, τ_D - decay time of D emission in the absence of A molecules; R_0 - critical distance ²⁴.

To study the energy transfer from FL to EB in 30S particles the excitation was achieved at 395 nm, where the molar extinction coefficient of EB did not exceed 10% of its value for FL ²². The fluorescence intensity of the acceptor was measured at 610 nm.

RESULTS AND DISCUSSION

1) Investigation of the secondary structure of pre-mRNA in RNP particles by EB fluorescence. When EB is adsorbed on 30S particles, its fluorescence yield is increased. Similar changes occur when EB associates with free RNA's ²⁵⁻²⁷. The fluorescence lifetime (τ) of EB is equal to 22.5 ± 0.5 nsec and 23.5 ± 0.5 nsec for its complexes with RNP and free pre-mRNA, respectively. Note that lifetime of EB in water solution is only 1.8 ± 0.2 nsec, and the fluorescence yield is correspondingly lower. The coincidence of absorption and fluorescence spectra of EB on RNP and free pre-mRNA and nearly equal values of its lifetimes seem to prove that this dye associates with double-stranded regions of pre-mRNA in both the cases.

To evaluate the portion of double-stranded regions in pre-mRNA available for dye binding in 30S particles, the adsorption isotherms of EB on 30S particles, free pre-mRNA and DNA have been compared (Fig. 1). The isotherms in Fig. 1 are plotted as r/c_1 versus r (see Methods). The binding isotherm for RNP (Fig. 1, curve 1) in the range $0.002 \leq r \leq 0.02$ is essentially nonlinear and can be represented as the sum of two rectilinear parts. The first one ($0.002 \leq r \leq 0.008$) is characterized by a steep slope which corresponds to a high intrinsic association constant $K_1^{\text{RNP}} = 1.6 \cdot 10^6 \text{ M}^{-1}$ approximately equal to that of EB association with free pre-mRNA ($K_1^{\text{RNA}} = 1.4 \cdot 10^6 \text{ M}^{-1}$) (Fig. 1, curve 2). The association energy of

Fig. 1. EB adsorption isotherms on RNP (1), pre-mRNA (2) and DNA *E. coli* (3) as r/c_1 on r dependence obtained by fluorescence measurements. RNP and RNA are dissolved in STM-buffer containing either 0.1 M NaCl (ooo) or (●●●) 0.36 M NaCl; $pH=7$; $T = 20^\circ C$. The concentration of pre-mRNA in RNP was $6 \cdot 10^{-5}$ M nucleotides; free pre-mRNA - $3.3 \cdot 10^{-5}$ M; DNA - $3 \cdot 10^{-5}$ M. EB concentration varied from 10^{-7} to $5 \cdot 10^{-6}$ M.

such binding is equal to $\sim 7 \text{ kcal} \cdot \text{M}^{-1}$ (strong complex) which is similar to the energy of EB association with double-stranded RNA and DNA ²⁶. The number of EB binding sites (obtained at the crossing of corresponding isotherm with the abscissa) is much less for RNP as compared to free pre-mRNA and especially to DNA (Fig. 1, curves 1,2,3). This may be due not only to a really low level of the secondary structure of pre-mRNA in particles but also to inaccessibility to the dye of some ds regions caused either by the tertiary structure of pre-mRNA or by their screening with protein in the particles ^{28,29}.

In order to evaluate the portion of double-stranded regions

in RNP accessible to EB, one should compare the EB binding isotherms with RNP and with double-stranded DNA which is completely accessible to EB. The DNA-dye isotherm theory is described in 30,31.

The number of binding sites for DNA and RNP (n_1) are equal to 0.18 and to 0.01 respectively (Fig. 1, curve 1,3). Consequently, $n_1(\text{RNP})/n_1(\text{DNA}) = 0.06$. This means that pre-mRNA in RNP contains only 6% of ds sequences.

The ratio of free pre-mRNA binding sites to those of DNA is equal to 0.22 (Fig. 1). In other words, only 22% of free pre-mRNA is double-stranded and accessible to the dye. At the same time it is known that in solution approximately 55-65% of free pre-mRNA is double-stranded 32,33. Therefore a considerable portion of these sites is inaccessible to EB.

The isotherms (Fig. 1, curve 1,2) with a relatively small slope ($K_2^{\text{RNP}} = 6 \cdot 10^4 \text{ M}^{-1}$) are similar to those of pre-mRNA ($K_2^{\text{RNA}} = 8.5 \cdot 10^4 \text{ M}^{-1}$). Previously, it has been established that the single-stranded regions of pre-mRNA (65%) in RNP particles are also accessible to the EB binding under appropriate conditions ($r \geq 0.01$) 34.

It is known that the increase of NaCl concentration leads to dissociation of pre-mRNA and protein in 30S particles. In the 2 M NaCl solution 30S particles are completely destroyed. However, these particles can resist the NaCl concentration up to 0.3-0.4 M 35-37.

To get information about the changes in the secondary structure of pre-mRNA induced by the increase of the NaCl concentration (from 0.1 up to 0.4 M), we have studied the isotherms of EB sorption on 30S particles in 0.36 M NaCl. Fig. 1 (curve 1) shows EB adsorption isotherms on RNP in 0.1 M and 0.36 M NaCl to be practically the same. This implies that the increase of NaCl concentration up to 0.36 M has no effect on a number of double-stranded regions of pre-mRNA in RNP accessible to the strong complex formation.

However, the analysis of sorption isotherms does not provide precise information about the length of the double-stranded regions in RNP. It is established that at least two nucleotide pairs in DNA or RNA are needed for strong binding

to EB ³⁰. To evaluate the average length of dsRNA in RNP particles the fluorescence polarization method has been introduced.

2) Fluorescence polarization of EB as a tool for estimation of dsRNA flexibility in 30S particles. Fluorescence polarization may decrease because of several reasons: 1) intrinsic mobility of the dye molecules adsorbed; 2) the flexibility of whole double-stranded regions of pre-mRNA; 3) energy transfer between the dye molecules adsorbed (concentration depolarization), or; 4) the presence of a fraction of free dye molecules in solution. All these mechanisms are reviewed in a number of works ³⁸⁻⁴². The contribution of the free dye fraction into the measured polarization coefficient has been calculated according to formula (1). The concentration depolarization may be neglected in experiments conducted at $r \rightarrow 0$. Consequently, the remaining depolarization effects should be attributed to the mobility either of dsRNA or EB molecules adsorbed.

In Fig. 2 the fluorescence polarization coefficient of EB

Fig. 2. Dependence of EB fluorescence polarization P_2 on its concentration in RNP particles. Pre-mRNA concentration in RNP was 15 $\mu\text{g/ml}$. EB concentration was changed from 10^{-7} to $5 \cdot 10^{-6}$ M. RNP was dissolved in STM-buffer containing 0.1 M NaCl (ooo) and 30% sucrose (xxx).

adsorbed on ds regions of pre-mRNA in particles is shown along with the increase of r in conditions of "strong" complex formation. One can see that fluorescence polarization of this dye drops greatly as r grows and is hardly influenced by solution viscosity increase. Consequently, the fluorescence depolarization observed is only due to energy transfer between EB molecules. However, in the limitation ($r \rightarrow 0$) conditions the polarization coefficient tends to reach 42%. This value corresponds to the EB maximal polarization coefficient ⁴¹.

Thus, we can conclude that EB is rigidly attached to the ds sequences which themselves are rigidly bound to RNP particles. On the other hand, the fluorescence polarization coefficient of EB complexes with free pre-mRNA is considerably lower than 42%. Even in the r limitation conditions ($r \rightarrow 0$), it never exceeds 28%. This means that ds regions of free RNA are flexible in solution ³⁸.

The increase of NaCl concentration from 0.1 up to 0.4 M leads to an appreciable change in the polarization curve of EB complexed with RNP (Fig. 3). It is seen that in the course of the NaCl concentration increase from 0.1 to 0.4 M the polarization declines and then remains constant. The coefficient of fluorescence polarization reaches only 36% in the limita-

Fig. 3. Dependence of EB fluorescence polarization P_2 in RNP particles on NaCl concentration. RNP was dissolved in STM-buffer; $T = 20^\circ\text{C}$. EB concentration in RNP $r = 8 \cdot 10^{-3}$; $4 \cdot 10^{-3}$ and $7 \cdot 10^{-4}$ (curves 1, 2 and 3, respectively).

tion conditions ($r \rightarrow 0$). At the same time, as it is shown above, there are no indications of any changes in the dye binding to RNP in 0.4 M NaCl (the same adsorption isotherm curve). This decrease in polarization observed in 0.4 M NaCl seems to be due to partial breaking of bonds between the protein moiety of RNP and double-stranded parts of pre-mRNA which acquire some flexibility.

3) The length of dsRNA in 30S particles as studied by means of energy transfer between EB molecules. The average length of double-stranded regions of pre-mRNA in RNP is estimated by measurements of energy transfer between EB molecules. The above described experiments show that in 30S particles about 6% of pre-mRNA capable to adsorb the dye molecules, are double-stranded. This means that about 38 nucleotides in a 30S particle can form either one ds chain ($50 \text{ \AA}$) or several short double-stranded structures. Evidently, the efficiency of energy transfer in long or short double-stranded regions must be different as is demonstrated by Gursky et al.⁴³. The theory of such phenomenon predicts⁴³ that the energy transfer should be weaker in the cases of helical length less than $2R_0$ (R_0 is the critical distance of the EB intermolecular energy transfer) as compared to the cases of rather long double-stranded chains ($> 2R_0$). The R_0 for EB is equal to $\sim 16 \text{ \AA}$ ⁴², therefore, the energy transfer between the dye molecules bound to different double-stranded regions looks improbable.

In part 2 it is shown that EB molecules are tightly bound to dsRNA in 30S particles. Then the observed decrease of their fluorescence polarization P_2 should be entirely attributed to the energy transfer processes. The dependence of the P_2 value on efficiency of the energy transfer is expressed by equation^{23,42}:

$$\frac{1}{P_2} - \frac{1}{3} = \frac{2(P_0^{-1} - 1/3)}{(3 \cos^2 \theta - 1)} \quad (4),$$

where $\cos^2 \theta = \sum I_i \cos^2 \theta_i$, θ_i - angle between adsorbing and irradiating dipoles separated by i nucleotide pairs; I_i - normalized intensity of the i dipole fluorescence; P_0 - coefficient of fluorescence polarization in the absence of energy transfer⁴².

$\cos^2 \theta$ represents the function averaged over the ensemble

of interacting EB molecules. It depends on both the rate of energy transfer W and the EB mutual positions in double-stranded regions of nucleic acids^{39,42,43}. Parameters of mutual orientation of EB adsorbed on nucleic acids in B- and A-conformations are given in the works by Paoletti and Le Pecq⁴² and Wahl et al.³⁹. We have used these parameters for estimation of W and $\cos^2\theta$ as function of r .

The values of $\cos^2\theta$ as function of EB concentration r in helical regions are calculated theoretically for polymers in A-conformation having the number of nucleotide pairs from 4 to ∞ ^{39,42,43}. The data are represented in Fig. 4 versus the concentration of the dye molecules adsorbed on dsRNA (r_{ds}). In our experiments, the real value of r_{ds} for dsRNA in 30S particles is equal to $r/0.06$. It is seen from Fig. 4 that the experimental points of $\cos^2\theta$ for EB adsorbed on dsRNA in 30S particles coincide rather well with the theoretical curves for the helical polymers in A-conformation containing more than

Fig. 4. $\cos^2\theta$ as function of EB concentration on double-stranded polymers in A-conformation (—) containing 5, 7, 11, 17, 20 and ∞ nucleotide pairs. Curve (---) for ∞ long polymer in B-conformation. (o o o) experimental data of $\cos^2\theta$ for RNP and DNA ($\triangle \triangle$), calculated from equation (4).

17-20 nucleotide pairs. The experimental curve for DNA coincides with the theoretical calculated for the infinite double-stranded polymer in B-conformation and is in good agreement with the data of Paoletti and Le Pecq⁴².

To this end, a conclusion can be drawn, that the bulk of helical regions of pre-mRNA are longer than 45 Å. Therefore, it is likely that a 30S particle has only one double-stranded region which includes 18 nucleotide pairs (≈ 50 Å), or more but in this case not every 30S particle contains it.

4) The localization of dsRNA in RNP as studied by means of energy transfer from FL to EB. It is important to know whether dsRNA is buried inside the protein moiety or sticks out of the RNP particle. To solve this problem, we have used the method of energy transfer from FL to EB adsorbed on the same RNP particle. The FL (donor) is covalently bound to the N-terminal and lysine- ϵ -amino groups of protein moiety of RNP particle^{17,21}. EB (acceptor) is adsorbed on the double-stranded regions of RNA. In order to calculate the critical distance for such heterogeneous energy transfer (R_0), some fluorescence characteristics of FL complexes with RNP have been studied.

The excitation and emission spectra of FL for 30S particles and the protein isolated from them are shown in Fig. 5. These spectra are very similar and coincide with those for FL covalently attached to lysine. The fluorescence lifetimes are also measured for the above-mentioned preparations. The emission decays for the FL complexes with RNP and protein are best fitted to the double-exponential decay with two distinct lifetimes: 3.6 nsec for short-lived and about 10 nsec for long-lived components. The fluorescence quantum yield for the short-lived (ρ_1) and the long-lived (ρ_2) components are calculated from the well-known equation: $\rho_i = \tau_i / \tau_o$, where τ_o - intrinsic radiative lifetime ($\tau_o \approx 30$ nsec). The average value of fluorescence quantum yield for the FL-RNP complexes is equal to 0.27. The increase of NaCl concentration from 0.1 to 0.4 M does not cause changes in the fluorescence quantum yield of FL complexed with RNP. However, the same increase of the NaCl concentration brings about a linear increase in FL

Fig. 5. Excitation (a) and fluorescence (b) spectra of FL complexes with lysine (curve 1), RNP (ooo) and protein (x x x).

fluorescence, as is seen from Fig. 6. It can be explained by the increasing number of free amino groups accessible to FL.

The fluorescence polarization coefficient for the FL-RNP is equal to 26% in the range of FL concentrations from 10 to 60 molecules per a 30S particle. It is lower than its limiting value ($P_0 = 34\%$)⁴⁴. The invariability of polarization degree observed proves that no energy transfer occurs under these conditions. The critical distance for FL energy transfer in RNP particles is equal to $11 \text{ \AA}$ ²². Judging from the above-mentioned data the intermolecular energy transfer between FL molecules is hardly possible.

The critical distance R_0 for the FL-EB pair is calculated according to the Förster theory^{24,45}. Using the mean value of the FL fluorescence yield $\bar{\rho} = 0.27$, we have calculated this parameter to be equal to $32 \pm 2 \text{ \AA}$. The value of the orientation coefficient used is $0.47 \leq K^2 \leq 0.67$ according to^{45,46} for a random orientation of the dipoles.

The efficiency of energy transfer (E) can be derived from

Fig. 6. Increase of FL fluorescence intensity, covalently attached to RNP as a function of NaCl concentration in solution. FL was added to RNP solutions at different concentration of NaCl. Pre-mRNA concentration in RNP was 20 $\mu\text{g/ml}$. FL concentration was $5 \cdot 10^{-5}$ M. STM-buffer containing different concentrations of NaCl.

the donor fluorescence yield or the acceptor fluorescence intensity. In our preparations EB has been adsorbed on a small portion of dsRNA (6%) and, therefore, it can quench only a limited number of donor molecules. It is established that the fluorescence yield and the lifetime of donor molecules are not changed with the increase of the acceptor concentration ($0.001 \leq r \leq 0.01$). Therefore it is more convenient to measure the fluorescence intensity of acceptor with excitation being adsorbed by donor molecules. The equation for acceptor fluorescence intensity can be written as:

$$(I - I_0)/I_0 = EA_D/A_A \quad (5),$$

where I_0 and I - fluorescence intensity of acceptor molecules in the absence and in the presence of donor molecules, respectively. A_D and A_A are absorption coefficients of donor and acceptor at the wavelength of exciting light (395 nm).

The magnitude of the increasing fluorescence intensity of acceptor as a function of donor concentration N_D is shown in Fig. 7.

For the acceptor content ranging from 2 to 5 molecules per

Fig. 7. Relative increase of EB fluorescence as a function of FL concentration N_D in RNP particles. (N_D is the average number of FL molecules per a 30S particle). EB fluorescence was measured at 610 nm with excitation 395 nm in the absence (I_0) and in the presence (I) of FL. RNP was dissolved in STM-buffer containing 0.1 (curve 1) and 0.36 M NaCl (curve 2).

a 30S particle all the curves (after being normalized as I/I_0) lie on one straight line. Lower curve (2) in Fig. 7 represents the same function but in 0.36 M NaCl. It is important that the efficiency of energy transfer is weaker in this case. The straight lines in Fig. 7 indicate that the average transfer efficiency E is independent of the donor concentration in RNP particles and depends linearly on the acceptor concentration. These facts apparently show that acceptor molecules are spaced so that their regions of strong quenching in an RNP particle do not overlap⁴⁷.

Let us consider two distinct models of FL distribution:

(a) the even incorporation of FL into RNP particle; (b) distribution of FL on the surface of RNP particles. **Assumption** (b) appears to be more probable as in this case we deal with compact 30S particles and the readily hydrolyzed FL^{18,48-51}.

Taking into consideration the above said we should analyse

the case with an acceptor and the N donor molecules in an RNP particle. In this case, the efficiency of energy transfer can be calculated according to equation introduced by Pearlstein⁴⁷:

$$(I - I_0)/I_0 = (A_D/A_A)N^{-1} \sum_{i=1}^N \left[1 + R_i^6/R_0^6 \right]^{-1} \quad (6)$$

This equation may be simplified by assumption that donor molecules are fully quenched at $R_i < R_0$ and are not quenched at all at $R_i > R_0$, where R_i is the distance between the acceptor and the donor molecules.

Having all this in mind we should calculate the number of donor molecules within the sphere of radius R_0 with acceptor in the centre. The number of donor molecules involved in the quenching sphere is equal to: $n_D = N_D V_A / V_{RNP}$, where V_A is a volume obtained from the intersection of the quenching sphere with the RNP particle. The V_{RNP} is the volume of the 30S particle. A 30S particle is considered as a sphere with the radius equal to about 100 Å^{50,51}. Then, equation (6) will be:

$$I/I_0 - 1 = \epsilon_D N_D V_A / \epsilon_A V_{RNP} \quad (7)$$

For the model of FL being adsorbed on the RNP surface, this equation is:

$$I/I_0 - 1 = \epsilon_D N_D S_A / \epsilon_A S_{RNP} \quad (8),$$

where S_{RNP} - 30S particle surface; S_A - part of the 30S surface obtained by its intersection with the acceptor quenching sphere; ϵ_D and ϵ_A - molar extinction coefficients at 395 nm.

The calculations of dependence of the EB fluorescence increase on its distance from RNP particle (1) are carried out for both models (Fig. 8). It is seen that in the case of the first model the EB molecule should be fixed outside the RNP particles at a distance of 13 Å, while in the second model two alternative positions for EB on ds sequences are satisfactory: the EB molecule may be fixed either inside or outside the particle at a distance of about 27 Å from its surface.

In 0.36 M NaCl this distance is slightly increased (see Fig. 8) in accordance with our polarization data, which show that in these conditions dsRNA regions with adsorbed EB become flexible. This is hardly possible inside RNP particles.

Fig. 8. Theoretically calculated relative increase of acceptor fluorescence intensity as a function of its distance (l) from RNP surface. Curve 1 was calculated from equation (7), curve 2 - from equation (8). $N_D/N_A = 20$; $\epsilon_D/\epsilon_A = 10$ at excitation 395 nm. (oo) and ($\Delta\Delta$) are experimental data from Fig. 7.

Consequently, we come to a conclusion that these dsRNA regions are located outside RNP at a distance of $13 \text{ \AA} \leq l \leq 27 \text{ \AA}$.

CONCLUSION

The above described experimental data show that a significant part of pre-mRNA in nuclear 30S particles is available for EB. It is established that about 6% of pre-mRNA in RNP are involved in a strong complex formation with EB, which proves their double-stranded nature. These data are in good agreement with the results of biochemical experiments^{6,7} which show that 3-5% of pre-mRNA in RNP particles are double-stranded.

The average length of double-stranded regions of pre-mRNA in RNP is estimated by means of energy transfer between EB molecules adsorbed on them. According to our data this size can not be less than $45 \text{ \AA}$. It is consistent with the assumption that each 30S particle contains one ds stretch of about 18 nucleotide pairs long (6% of total pre-mRNA) or larger but in this case not in each 30S particle.

It is established that the EB polarization coefficient tends to reach 42% ($r \rightarrow o$) in RNP particles. This proves inevitably that dsRNA is tightly bound to the particle. However the increase of NaCl concentration from 0.1 to 0.4 M leads to a pronounced weakening of dsRNA bonds with the RNP particle. Further increase of NaCl concentration in solution ^{3,36,37} causes destroying of 30S particles and dissociation of their protein and RNA moieties.

The average efficiency of energy transfer from FL to EB in 30S particles has been calculated. It is shown that EB adsorbed on dsRNA is located outside the RNP particle at a distance of 13-27 Å from its surface. In 0.36 M NaCl this distance increases up to 19-30 Å. These data are in a good agreement with the results of work ⁵.

Thus elongated double-stranded regions of pre-mRNA on which EB is being adsorbed seem to stick out from the surface of RNP particle.

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FOOTNOTES AND REFERENCES

- ¹ Abbreviations used: EB, ethidium bromide; FL, fluorescamine; dsRNA, double-stranded sequences of pre-mRNA; ds, double-stranded regions.
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