

POLARIZED FLUORESCENCE OF ACRIDINE ORANGE-TRANSFER RNA COMPLEXES

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1. Introduction

Previously we attempted to analyse the molecular morphology of mixed tRNAs from the rotational relaxation time (ρ_h) measured from fluorescence polarization of acridine orange (AO) adsorbed on the tRNA molecules [1]. The same approach was also used by Churchich [2] and by Millar and Steiner [3] using a dye covalently attached to the acceptor end of the mixed tRNA molecules. The ρ_h values they found were, however, considerably lower than when the dye was adsorbed on the double-helical regions of tRNA [1]. This difference is probably attributable to the local mobility of the dye coupled with the relatively flexible ACC end in Mg^{2+} -free medium.

The aim of this work was to carry out similar investigations on two individual tRNA fractions. Moreover, calculations were made taking into account various orientations of the dye molecule with respect to the long axis of the tRNA particle in solution and also various molecular volumes of the tRNA depending on the degree of its hydration.

2. Results and discussion

The temperature dependence of the polarization of fluorescence of the adsorbed AO molecules is shown in fig. 1. From the linear left-hand parts of these curves the rotational relaxation time ρ_h could be calculated according to the equation [4]

$$\rho_h = \frac{3\eta\tau(\frac{1}{P_0} - \frac{1}{3})}{\beta T}, \text{ where}$$

τ - lifetime of the excited state of AO adsorbed on the tRNA,

P_0 - limiting polarization when $T/\eta \rightarrow 0$,

β - a regression coefficient in the plot of $1/P$ against T/η ,

T - absolute temperature,

η - viscosity of the solvent.

The lifetime of the excited state, $\tau = 5 \pm 0.2$ nsec, was determined directly with a phase fluorometer [5].

For the tRNA^{Val} ρ_h varies between 36 and 40 nsec depending on the tRNA preparation; average value 38 nsec at 20°C.

A characteristic break in the slope of the curve between 22 and 25°C was noticed which is also pronounced but less sharp with mixed tRNA preparations (fig. 1C). For the tRNA^{Val} an initial slope $\beta_1 = (2.5 \pm 0.15) \times 10^{-5}$ whereas after the break $\beta_2 = (5.7 \pm 0.2) \times 10^{-5}$. Thus the β coefficient changes more than 2 fold, while τ is constant in this temperature interval [1].

A priori this change could be caused (1) by desorption of the dye at increasing temperatures, (2) by structural transition from more asymmetric to less asymmetric conformation, and (3) by loss of rigidity of the macromolecule.

We have analysed all these possibilities.

(1) It was shown for the mixed tRNAs [1] that appearance of the free dye in solution began at 40°C. With tRNA^{Val} we followed the process of desorption by measuring the intensity of fluorescence of the AO adsorbed on the macromolecule, since it is known [6] that the intensity of fluorescence of the free dye is 3 times weaker than that of the AO fixed on the tRNA. It is seen from fig. 1A (curve 2) that changes in the fluorescence intensity and hence desorption of the dye

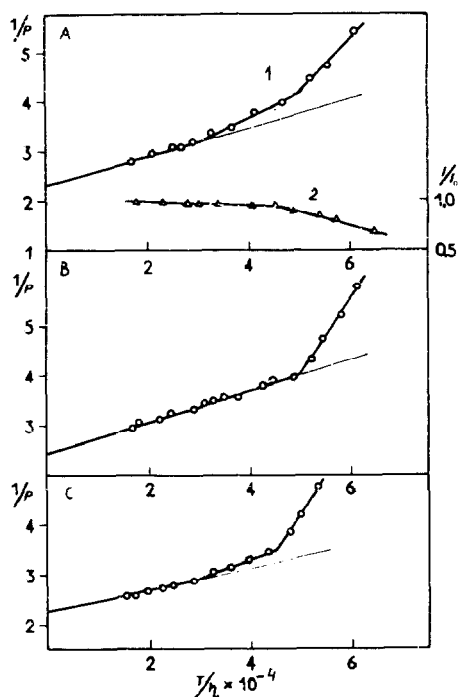


Fig. 1. Dependence of $1/P$ on T/η for tRNA^{Val} (A), tRNA^{Lys} (B) and mixed tRNA (C). Purification of tRNA^{Val} from yeast and tRNA^{Lys} from *E. coli* was achieved chromatographically as described elsewhere [23, 24]. The purity of these fractions was $\geq 80\%$. All physical measurements were made as described earlier [5, 25] in solutions containing 50 mM NaCl and 5 mM Na-citrate, pH 6.8. The concentration of the tRNA was 2–8 μ M and of the AO 0.1–0.5 μ M. In this concentration range energy migration between the AO molecule adsorbed on the tRNA is practically absent. In fig. 1A (curve 2) the variation with temperature of the relative intensity of the fluorescence is plotted for AO adsorbed on the tRNA^{Val} (I_0 is the intensity of the fluorescence at 4°C).

in solution begins only at 38–40°C in accordance with our previous results [1]. Therefore it is clear that the transition revealed at 22–25°C has nothing to do with desorption of the dye from the macromolecule.

(2) Henley et al. [7] discovered that mixed tRNA from yeast undergoes a conformational transition between 20 and 40°C; its molecular asymmetry being reduced with a slight change in molecular volume. Fig. 2 shows the theoretical changes of the relative relaxation time ρ_h/ρ_0 with the degree of asymmetry of the macromolecule (ρ_0 is a relaxation time of a sphere with a volume equal to that of the equivalent ellipsoid

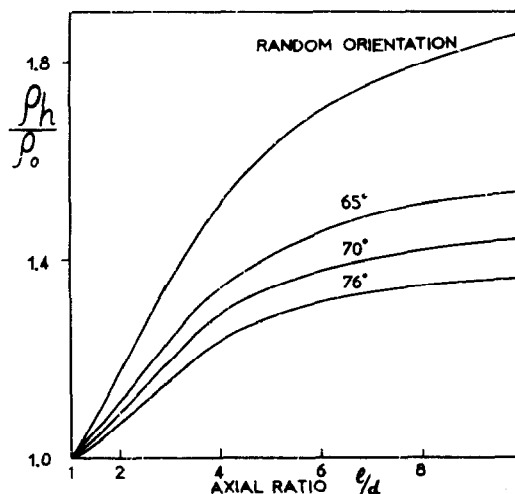


Fig. 2. Theoretical dependence of ρ_h/ρ_0 on the axial ratio (l/d) of an ellipsoid of revolution. The curves are plotted for various orientation angles between the dye emission oscillator and the long axis of the ellipsoid.

of revolution). Fig. 2 also shows that ρ_h/ρ_0 changes no more than 1.5-fold with a change of l/d from 4 to 1.

Thus if the molecular volume is constant even a large change in the asymmetry of the macromolecule is not enough to explain more than a 2-fold change of β .

(3) With the exclusion of the first two possibilities it follows that the loss of rigid conformation is the main source of the observed transition. It is likely that melting of some part of the tertiary structure of the tRNA^{Val} produces a rotational freedom between helical parts of the molecule, since the secondary structure remains practically intact at this temperature [8].

In addition, flexibility of the tRNA induced by heating could also diminish the asymmetry of the particle and therefore contribute to the transition.

From fig. 1B, $\rho_h = 36$ nsec at 20°C was calculated for tRNA^{Lys} which is practically equal to that of tRNA^{Val}. However, no break was found at 22–25°C. A change in the slope of the curve only appears at 40°C which is mainly attributable to the desorption of the dye. Possibly the melting of the tRNA^{Lys} tertiary structure occurs at higher temperatures and coincides with the melting of its secondary structure and therefore cannot be revealed by this method.

As was shown by Weber [9, 10] the rotational relaxation time of a macromolecule computed from the fluorescence polarization is an average of the principal relaxation times of the associated hydrodynamic ellipsoid weighted according to the orientation of the transition moments in absorption and emission with respect to the ellipsoid's axes. If oscillators of absorption and emission are coincident (and in the low-frequency band of AO absorption this is a satisfactory approximation) then

$$\frac{\rho_h}{\rho_0} = \frac{1}{\frac{\cos^2\alpha}{n_1} + \frac{1-\cos^2\alpha}{n_2}}, \text{ where}$$

α is the angle that the emission oscillator makes with the axis of revolution of the ellipsoid, and n_1 and n_2 are the ratios of the two principal relaxation times of the ellipsoid to the relaxation time for the sphere of the same volume. Usually the orientation of the dye covalently fixed or adsorbed on the macromolecule is unknown and random orientation is assumed for calculations. For random orientation

$$\overline{\cos^2\alpha} = \frac{1}{3}; \quad \frac{\rho_h}{\rho_0} = \frac{1}{\frac{1}{3}(\frac{1}{n_1} + \frac{2}{n_2})}$$

In the case of AO adsorption on tRNA at high polymer/dye ratios, the dye interacts only with the double-helical regions forming a so-called strong

complex [11, 12] which is similar to the sorption of the dye on native DNA or poly(A + U).

Two models have been suggested for the interaction between the dye and the double helix. According to Lerman's model [13] the intercalated dye molecules form the same angle with the long axis of the particles as do the base pairs. According to Gursky's model [14] AO occupies the narrow groove of the double helix and the angle between the oscillator and the long axis of the particle is 65–70°. Both models suggest sufficiently rigid attachment of the molecule to the nucleic acid molecule.

Furthermore, it has been shown [15] that the rate constant of dissociation of the strong complex between native DNA and proflavine is about 10^4 – 10^2 sec⁻¹ and therefore, during the lifetime of excited state (5 nsec) desorption of the dye could not take place. This is also so for the AO-tRNA complex since the binding constant of this complex is of the same order of magnitude as for the DNA-proflavine complex [16].

It has been demonstrated by the flow birefringence technique that most of the base pairs in the tRNA are orientated normally to the long axis of the particle [17]. On the other hand the angle between the planes of the base pairs and the long axis of the particle is 70–76° for the double helical RNA segments [18] and 70° for the A-form of DNA.

Taking into account all these considerations it is reasonable to calculate the asymmetry of the tRNA molecule for various angles between the long axis of the particle and the dye oscillator.

Table 1
Asymmetry of tRNA^{Val} in solution.

Hydration, ml H ₂ O/g tRNA	Molecular volume of tRNA ^{Val} , Å ³	ρ_0 at 20°C, nsec	ρ_h/ρ_0	Axial ratio for equivalent ellipsoid of revolution (<i>l/d</i>)			
				Orientation of the dye			
				Random	65°	70°	76°
0	22000	16.1	2.37	∞	∞	∞	∞
0.35	37700	27.4	1.40	3.5	5.0	6.5	10
0.4	39000	28.6	1.33	3.0	4.0	4.5	7.0
0.5	44000	31.9	1.20	2.0	2.7	3.0	3.5

The second important parameter is the molecular volume of the tRNA. From the partial specific volume $\bar{V} = 0.504$ ml/g [19] and molecular weight (26500 daltons) of tRNA^{Val}, the molecular volume without hydration is about 22000 Å³. However, the effective hydrodynamic volume depends on the hydration which, unfortunately, has not been measured accurately for tRNA molecules. Hence we took three values which seemed to be probable on the basis of some indirect evidence: 0.35 ml/g, the measured hydration for DNA [cf. 20]; 0.4 ml/g, determined by calorimetric measurements for mixed tRNA; and 0.5 ml/g, from crystallographic data [19].

The relationships between the parameters discussed above and the axial ratio of the tRNA^{Val} molecule are shown in table 1.

It is clear from the table that, even with the high degree of hydration, the minimum ratio is not much less than 3 for both Lerman's and Gursky's models. For random orientation of the dye this ratio could be as low as 2 but this value seems to be very doubtful since, from physical data [17, cf. 21], the double helical segments of the tRNA are mainly parallel to the long axis of the macromolecule and therefore the orientation of the dye chromophore is non-random.

The degree of asymmetry found is in good agreement with electron microscopic observations on tRNA molecules [22].

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