THE DEPENDENCE OF FLUORESCENCE QUANTUM YIELD OF THE tRNA–ACRIFLAVINE COMPLEXES ON THE CONFORMATIONAL CHANGES IN tRNA

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1. Introduction

The "clover leaf" seems at present to be the most probable model for the tRNA secondary structure. A number of physical and chemical findings show that the clover leaf is folded into a compact three-dimensional structure. Many dyes interact only with double helical parts of tRNA at low dye to tRNA ratios [1–4]. The use of such dyes allows to get information about the role of the double helical regions in the formation of tertiary structure.

It was shown on the basis of binding isotherms of tRNA–acriflavine complexes that the conformational change in tRNA produced by the increase of ionic strength results in the inaccessibility for the dyes of some double helical regions [3].

However, the study of the binding isotherms cannot indicate which helical regions become inaccessible for dyes. In order to study this problem we examined the fluorescence characteristics of the tRNA–acriflavine complexes (tRNA–AF). It was shown earlier that acriflavine (AF), adsorbed on the double helical regions of DNA or double stranded synthetic polynucleotides exhibits fluorescence if the dye molecules interact with AT–AT, AU–AU or IC–IC base pairs [5–7]. The GC base pairs are shown to quench fluorescence of AF.

The conformational changes in tRNA resulting in the inaccessibility for dyes of several double helical regions in tRNA may influence considerably the fluorescence of the tRNA–AF complexes.

2. Results and discussion

As shown in fig. 1, absorption and fluorescence spectra of AF complexed with the total yeast tRNA coincide with those for double stranded poly A–poly U dye complexes.

The value of the fluorescence quantum yield (ρ) for the tRNA–AF complexes proves to be considerably less than that for the free AF ($\rho_I = 0.6$ [5]). It is shown that $\rho/\rho_I = 0.30 \pm 0.03$, therefore ρ is equal to 0.18 ± 0.02 for tRNA–AF. The fluorescence lifetime for tRNA–AF (τ) exceeds by $\cong 10\%$ the corresponding value τ_I for free dye and is equal to 5×10^{-9} sec.

The pronounced decrease of ρ for the tRNA–dye complexes in comparison with ρ_I while τ practically does not change, indicates that the fluorescence of some fraction of adsorbed dye molecules is decreased drastically.

The interaction of AF with double helical regions of tRNA appears to be similar to the sorption of this dye on native DNA or poly A–poly U [1,3]. It is reasonable to suggest on the basis of data obtained in [5,6], that AF adsorbed on tRNA exhibits fluorescence if it is interacting with AU–AU base pairs only, while the GC–GC or GC–AU base pairs quench the fluorescence.

The coincidence of fluorescence spectrum for tRNA–AF with fluorescence spectrum for poly A–poly U-dye complexes confirms this suggestion (fig. 1).

The effect of ionic strength (μ) on the ρ value of the complexes of AF with purified yeast $tRNA^{Val}$,

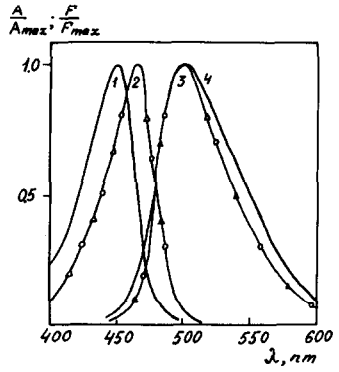

Fig. 1. The absorption and fluorescence spectra of AF complexes with total yeast tRNA and double stranded poly A–poly U. 1) the absorption and 4) the fluorescence spectra of free dye, 2) the absorption and 3) the fluorescence spectra of AF complexes with tRNA (○-○-○) and poly A–poly U (△-△-△). The molar ratio of tRNA phosphate to dye was P/D = 300; for poly A–poly U, P/D = 100. The concentration of AF was 3×10^{-6} M. Solutions contain 15 mM NaCl and 1.5 mM Na-citrate; pH = 6.8; Temp. = 22°. The absorption spectra were measured with ESP-2 "Hitachi" spectrophotometer. The fluorescence spectra were measured with ISL-51 spectrophotofluorometer and Photoelectric attachment FZL-1 [5], excitation λ was 436 nm.

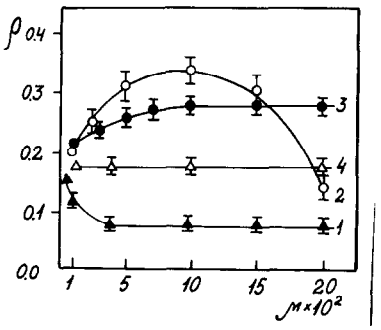

Fig. 2. Fluorescence quantum yield ρ of AF complexes with purified yeast $tRNA^{Ser}$ (curve 1), $tRNA^{Phe}$ (curve 2), $tRNA^{Val}$ (curve 3) and total yeast tRNA (curve 4) as the function of ionic strength μ. The fluorescence quantum yield measurements were described in [5]. $tRNA^{Ser}$ and $tRNA^{Phe}$ were obtained by the courtesy of Prof. H.G. Zachau. $tRNA^{Val}$ and total tRNA were obtained by the courtesy of Dr. A.D. Mirsabekov. Measurements were made in citrate buffer (ionic strength 0.005) with 1.0 mM EDTA; Temp. = 22°. The ionic strength was changed by the addition of the concentrated NaCl solution. In order to eliminate the energy transfer between dyes absorbed on tRNA [2,5,6], the measurements of the fluorescence quantum yields were made at P/D ratios $\geq 10^3$. The concentration of free dye at high ionic strength was calculated from the fluorescence depolarisation of tRNA–AF system as described in [8]. The concentrations of tRNA's were ≈ 2 μM and of the AF 0.1 μM.

$tRNA^{Ser}$ and $tRNA^{Phe}$ is shown in fig. 2. The ρ is nearly equal to 0.2 for all three tRNA's investigated as well as for the total tRNA in the region $0.005 \leqslant \mu \leqslant 0.01$. However, each particular tRNA is characterized by its own $\rho(\mu)$ dependence in the region of $\mu > 0.01$. In the case of $tRNA^{Ser}$ ρ decreases twofold as μ increases and then remains constant in the range of μ values $0.05 \leqslant \mu \leqslant 0.2$. For $tRNA^{Val}$ and $tRNA^{Phe}$ ρ increases till $\mu = 0.1$. Then for $\mu > 0.1$ it becomes constant for $tRNA^{Val}$ and is sharply decreased for $tRNA^{Phe}$. However, for the total tRNA the ρ value practically does not depend on μ and is approx. equal to 0.18 ± 0.02.

The experiments show that ρ value of AF complexes with poly A–poly U and DNA does not depend on μ. Thus the observed dependence $\rho(\mu)$ for AF complexes with individual tRNA is probably due to a conformational change in tRNA.

One can calculate theoretically the ρ value for individual tRNA–AF for different conformations of tRNA using the following suggestions.

i) The clover leaf model is valid for the secondary structure of tRNA.

ii) The dye molecules when bound are distributed randomly on the double helical regions of tRNA. (It is known that the adsorption binding constant of AF does not depend on the nucleotide sequence of DNA [5,9]).

iii) The adsorbed dye molecules fluorescence being bound by the AU–AU nucleotide pairs only* [5,6].

According to the assumptions mentioned above one can write the following formula for the quantum yield of tRNA–AF complexes.

$$\rho = \rho_{AU} \frac{n_{AU}}{n} \qquad (1)$$

* There are no experimental data on the influence of the GU pairs on the AF fluorescence. Therefore two cases should be considered for $tRNA^{Val}$ and $tRNA^{Phe}$ containing GU pairs in their acceptor parts: a) GU pairs quench or b) do not quench the dye fluorescence.

Table 1

The influence of the tRNA tertiary structure on the fluorescence quantum yield ρ of the acriflavine bound to some individual tRNA's.

Samples	ρ_I Calculated (a)	(b)	$\rho_\mu = 0.01$ Experimental	ρ_I/ρ_{II} Calculated (a)	(b)	$\rho_\mu = 0.1/\rho_\mu = 0.01$ Experimental
$tRNA^{Ser}$	0.15	0.15	0.13 ± 0.01	0.4	0.4	0.6 ± 0.15
$tRNA^{Phe}$	0.11	0.16	0.20 ± 0.02	1.3	1.3	1.6 ± 0.3
$tRNA^{Val}$	0.11	0.17	0.22 ± 0.02	1.4	1.3	1.3 ± 0.2
tRNA Total			0.18 ± 0.02			1.0 ± 0.2

ρ_I is calculated from equation (1) for the clover leaf model when all double helical regions in tRNA are accessible for AF provided that a) GU pairs quench fluorescence of AF, or b) GU pairs do not quench fluorescence of AF.

ρ_{II} is calculated for clover leaf model when anticodon stem become inaccessible for AF.

where n is the total number of dye binding sites in the double helical tRNA parts; n_{AU} is the number of binding sites for fluorescing dye molecules (the quantity of the AU–AU pairs); ρ_{AU} is the fluorescence quantum yield of the dye molecules interacting with AU–AU pairs which is equal to 0.9 as in the case of poly A–poly U–AF complexes [5].

The AF fluorescing sites (there are two of them in the case a) and three in the case b), see the footnote) are located in the acceptor double helical arm of $tRNA^{Val}$ and $tRNA^{Phe}$. The $tRNA^{Ser}$ has only one fluorescing site in the acceptor stem and two sites in the anticodon double helical region.

The increase in ionic strength causes the formation of a rigid compact tertiary structure of tRNA. In this case 20–40% binding sites may become inaccessible for AF [3]. The ρ value may be decreased if the AU–AU base pairs become inaccessible for dye or increased if only GC base pairs are shielded. The decrease in ρ for $tRNA^{Ser}$–AF and increase in ρ for $tRNA^{Val}$–AF and $tRNA^{Phe}$–AF complexes when μ increases, permits to suppose that the anticodon helical stem becomes inaccessible for the dye. These data confirm the suggestion of Connors et al. [10], that TψC-loop is inserted into the narrow groove of anticodon double helical region which may become inaccessible for dye. The ρ values calculated for the clover leaf model, as well as for the case when anticodon stem is shielded for dye, are shown in table 1 for $tRNA^{Ser}$, $tRNA^{Phe}$, and $tRNA^{Val}$. In addition the experimental ρ values are given at $\mu = 0.01$ and $\mu = 0.1$. It may be seen that the relative changes of ρ calculated for tRNA structure where the anticodon stem is shielded, correspond to the experimental $\rho(\mu)$ dependence in the region $0.01 \leqslant \mu \leqslant 0.1$.

One additional fact is worth noting. In this work ρ value for $tRNA^{Phe}$–AF was observed to decrease sharply in the range $0.15 \leqslant \mu \leqslant 0.20$. It is known that the 8th and 13th nucleotides are located in close proximity [11]. In the particular case of $tRNA^{Phe}$ the AU–AU pairs are located near the 8th nucleotide and therefore GC pair of diHU-stem may quench the fluorescence of AF molecules bound to those sites. On the contrary there are no fluorescing sites near the 8th nucleotide in $tRNA^{Val}$ and $tRNA^{Ser}$ and hence the interaction with the GC pair of diHU-stem may not influence ρ. Our experiments confirm this suggestion.

Thus our experimental data show a considerable variation of the ρ value for the individual tRNA–AF. Each individual tRNA is characterized by its own specific ρ (μ) dependence.

The variations of ρ may reflect the interactions between different regions of clover leaf while compact rigid tertiary structure is formed. We believe that the variations of ρ (μ) provide a sensitive instrument for the study of the tRNA tertiary structure. This method may be also useful for the study of the role of the double helical regions in the tRNA–enzyme interactions.

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