BINDING ISOTHERMS OF tRNA–ACRIFLAVINE COMPLEXES

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1. Introduction

One of the approaches to the investigation of nucleic acid structure is the study of complexes of dyes with nucleic acids. It is known that at high nucleotide to dye ratios the acridine dyes interact presumably with double helical regions of tRNA [1–3] and therefore a smaller number of strong binding sites should be expected for tRNA in comparison with double stranded poly A–poly U or native DNA. Study of the binding isotherms of tRNA–dye complexes as compared with those for the double standed polymers may provide the information about the number of nucleotide base pairs accessible for dyes in tRNA. It may be expected that the number of the double helical regions accessible for dyes in tRNA is affected by the folding of tRNA into a compact tertiary structure.

In this study a quantitative interpretation of the binding isotherms has been obtained for complexes of acriflavine (AF) with total yeast tRNA and individual yeast $tRNA^{Val}$, $tRNA^{Ser}$ and $tRNA^{Phe}$. The binding isotherms were studied in their dependence on the ionic strength.

2. Theory

It is known that the binding isotherms clearly indicate the existence of a stronger (I) and weaker (II) mode of binding of the acridine dyes on nucleic acids. In this work we investigated only process I for complexes of AF with tRNA, DNA and poly A–poly U.

The binding isotherms are usually plotted as a dependence of r/m on r, where r is the number of binding dye molecules per nucleotide pair, and m is the concentration of free dye molecules in the solution. If the binding sites of polymer are independent, for a single class of binding sites a plot of r/m against r will be linear with an intercept of the r axis at $r = n$,

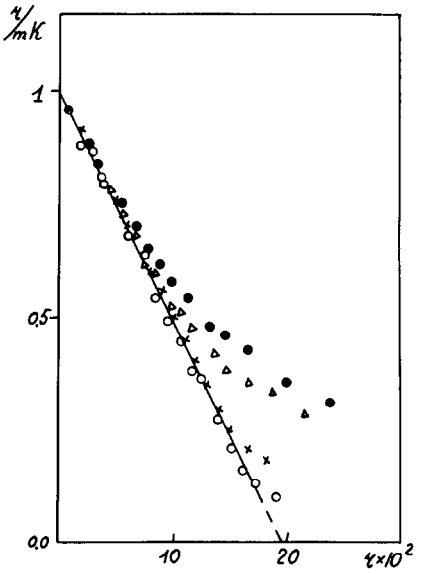

Fig. 1. The dependence of r/mK on r for native DNA–AF complexes: (×–×–×) at $\mu = 0.01$; (△–△–△) at $\mu = 0.4$; and for double stranded poly A–poly U–AF complexes: (○–○–○) at $\mu = 0.01$; (●–●–●) at $\mu = 0.4$. The characteristics of samples were described in [2]. The optical density for calculation of values r and r/m was measured with a Cary-16 spectrophotometer at two different wave lengths (440 nm and 470 nm) in a cuvette of 1 cm light path. The samples were dissolved in citrate buffer (ionic strength 0.01) with 1.0 mM EDTA, temp. = 20°. The ionic strength was changed by the addition of the concentrated NaCl solution. The concentrations of DNA and poly A–poly U were $(1–3) \times 10^{-4}$ M nucleotides. The concentrations of AF were changed from 5×10^{-7} M to 3×10^{-5} M.

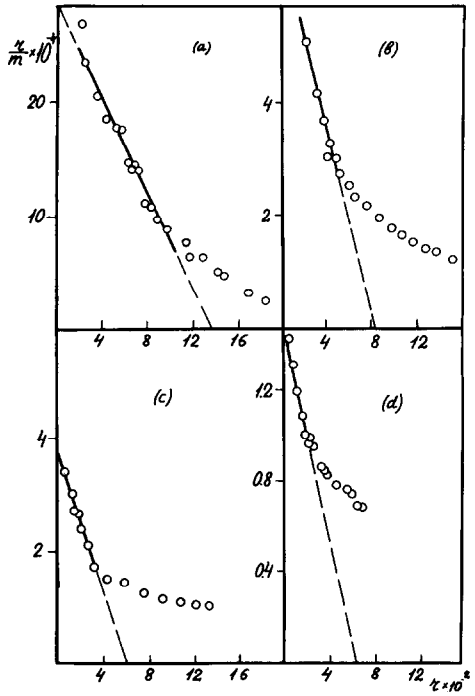

Fig. 2. The dependence of r/m on r for $tRNA^{Val}$–AF complexes: a) $\mu = 0.01$; b) $\mu = 0.1$; c) $\mu = 0.2$; d) $\mu = 0.4$. The concentration of $tRNA^{Val}$ was 1–2 μM. Solvents used and conditions of measurements are the same as in fig.1.

where n is the number of binding sites per nucleotide pair. In fact a plot of r/m against r is curved for acridine dyes even with a single class of binding sites. This curvature may result from the existence of anticooperative interactions between neighbouring sites. Crothers [4] has suggested a model that can reproduce the observed binding isotherms. It is assumed that the monomer unity of polymer is a potential binding site, but binding of a dye molecule at one site prevents binding at the adjacent potential sites. It has been shown [5] that for a double helical polymer of infinite length the linear extrapolation of the initial part of the curve r/m against r intercepts the r axis at

$$r = n_{ap} = \frac{1}{2l-1},$$

where l is the 'length' of the dye molecule, i.e. the number of nucleotide pairs which become excluded for the next dye molecule when one dye molecule is adsorbed. It has been shown [5] that for a polymer

Table 1
Theoretical values of n_{ap} calculated for $tRNA^{Val}$–AF and $tRNA^{Ser}$–AF complexes in their dependence on the number of accessible for dyes Watson-Crick base pairs (N).

$tRNA^{Val}$		$tRNA^{Ser}$	
N	n_{ap}	N	n_{ap}
20	0.12	24	0.132
15	0.09	19	0.104
12	0.068	15	0.08

of finite length n_{ap} does not depend on the number of nucleotide base pairs (N) which the polymer contains, provided that $N \geqslant 2l$, and approximately is equal to $\frac{1}{2l-1}$ like in the case of a polymer of infinite length.

If a polymer contains parts of polynucleotide chains which do not adsorb dyes, then

$$n_{ap} = \alpha \frac{1}{2l-1},$$

where α is the share of accessible for dyes binding sites. In the case of tRNA at low r value dye molecules practically do not interact with single stranded parts of tRNA and α corresponds to the ratio of the number of nucleotides which form Watson–Crick base pairs to the total number of nucleotides. Thus the α value may be determined from the ratio of n_{ap} for tRNA–AF complexes to n_{ap} for poly A–poly U–AF complexes. However, as it will be shown further l is equal to 3 nucleotide base pairs when AF is adsorbed on nucleic acids at low r value. According to the cloverleaf model, tRNA has short double helical regions, containing from 3 to 7 nucleotide pairs so that there are both regions with $N > 2l$ and those with $N < 2l$. A more detailed analysis of binding isotherms which therefore is necessary for tRNA–AF complexes may be found elsewhere [6].

The values of n_{ap} resulting from the theoretical calculation [6] for tRNA–AF complexes, taking into account the anticooperative model of interactions between neighbouring sites, are shown in table 1. The calculations were performed for two different situations: i) when all double helical regions in tRNA are accessible for dyes and ii) when a part of them becomes inaccessible probably due to the tertiary folding of the cloverleaf.

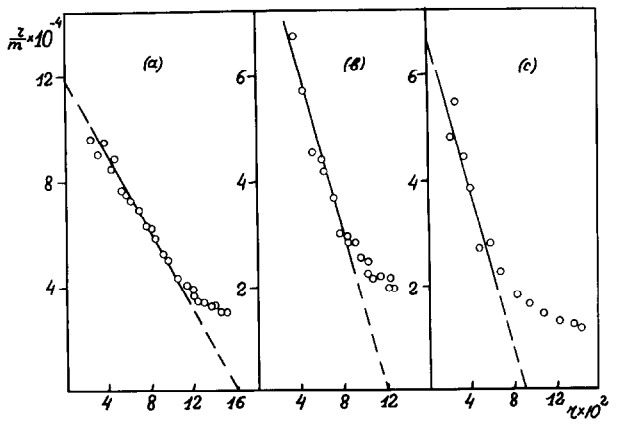

Fig. 3. The dependence of r/m on r for $tRNA^{Ser}$–AF complexes: a) $\mu = 0.01$; b) $\mu = 0.1$; c) $\mu = 0.2$. The concentration of $tRNA^{Ser}$ was 1 μM. Solvents used and conditions of measurements are the same as in fig.1.

3. Results and discussion

3.1. *DNA and poly A–poly U*

The dependence of r/mK on r for DNA–AF and poly A–poly U–AF complexes at different values of the ionic strength (μ) are shown in fig.1 (K is the binding constant). It is seen that the value of n_{ap} is equal to 0.2 ± 0.02, therefore, the dye 'length' is equal to 3 nucleotide base pairs. The value of n_{ap} coincides for DNA and poly A–poly U. Within the experimental accuracy in the range $0.01 \leqslant \mu \leqslant 0.4$ n_{ap} can be regarded as independent on the ionic strength for DNA–AF and poly A–poly U–AF complexes.

3.2. *tRNA*

The dependence of r/m on r for $tRNA^{Val}$–AF and $tRNA^{Ser}$–AF complexes at different μ is shown in fig.2 and fig.3. Similar plots were obtained for $tRNA^{Phe}$–AF and total tRNA–AF complexes. The experimental values of n_{ap} for investigated tRNA's are given in table 2. The n_{ap} values which are proportional to the number of sites accessible for dye binding are approximately similar for different tRNA's. For $tRNA^{Ser}$ n_{ap} is slightly larger, than that for $tRNA^{Val}$. This may be due to the existence of an extra arm consisting of four nucleotide base pairs. While the n_{ap} value for DNA and poly A–poly U does not vary with μ increasing, the n_{ap} decreases with the increase of μ for all tRNA's studied in this work. The decrease of the number of binding sites in tRNA which follows from this fact may be associated with shielding of a part of double helical regions of tRNA due to a conformational change of its tertiary structure. This suggestion is consistent for example with Connor's model of the tertiary structure of tRNA [7]. That model assumes that the TVC loop occupies a narrow groove of the anticodon double helical stem. This probably results in the inaccessibility of that stem for dyes [6,8].

Table 2
Experimental values of n_{ap} for complexes of AF with different individual tRNA and total tRNA in their dependence on μ.

Samples (μ)	*Total tRNA	*$tRNA^{Val}$	*$tRNA^{Ser}$	*$tRNA^{Phe}$
0.01	0.14 ± 0.02	0.14 ± 0.02	0.16 ± 0.02	0.14 ± 0.02
0.1	0.09 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.1 ± 0.01
0.2	0.08 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.09 ± 0.01

*$tRNA^{Ser}$ and $tRNA^{Phe}$ were obtained by the courtesy of Professor H.G.Zachau, total tRNA and $tRNA^{Val}$ were obtained by the courtesy of Dr. A.D.Mirzabekov.

From the comparison of the experimental n_{ap} values with those calculated theoretically, one can conclude that at $\mu = 0.01$ all double helical regions in tRNA appear to be accessible for dyes (20 nucleotide base pairs for $tRNA^{Val}$ and 24 for $tRNA^{Ser}$). In the range of the μ value 0.1 to 0.2 the number of the nucleotide base pairs accessible for dyes diminishes to 12–15.

Tao et al. [9] found only one strong type binding site per $tRNA^{Phe}$ molecule in the presence or absence of magnesium at $\mu = 0.01$ and using ethidium bromide (EB). This result has been explained by the existence of a tertiary structure favoring the binding to only those helices which protrude from the bulk of the molecule. But it should be noted that a single strong binding site was observed at $\mu = 0.01$ without magnesium as well, when the tRNA probably has no rigid tertiary structure [10,11].

In another our work [6] in the range of low r values we have also revealed a complex corresponding approximately to a single binding site per tRNA molecule for proflavine adsorbed on total tRNA.

However a similar complex was revealed on tRNA fragments (5′-halves), which have a structure differing greatly from that of the whole tRNA molecule. Thus the explanation of this complex relating to a specific tertiary structure of tRNA, seems rather doubtful. The analysis of experimental errors of spectrophotometric measurements in this low r range has shown that the evidence on this complex can not be considered as quite reliable.

The results of the present work imply that at low values all helical regions existing in the cloverleaf model of tRNA are exposed for binding of the dyes. This conclusion is confirmed by the data of Dourlent and Hélène [3] concerning the proflavine–$tRNA^{Phe}$ complex at $\mu = 0.02$. At higher μ the folding of tRNA in a rigid tertiary structure probably results in the inaccessibility of 20% to 40% of the helical regions for the dyes.

Acknowledgements

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References

[1] H.Grosjean, J.Werenne and H.Chantrenne, Biochim. Biophys. Acta 166 (1968) 616.
[2] S.Trubitsyn, A.Surovaya and O.Borissova, Molekul. Biol.SSSR 5 (1971) 419.
[3] M.Dourlent and C.Hélène, European J.Biochem.23 (1971) 86.
[4] D.M.Crothers, Biopolymers 6 (1968) 575.
[5] A.Zasedartelev, G.Gursky and M.Volkenstein, Molekul. Biol.5 (1971) 245.
[6] A.Surovaya and S.Trubitsyn, Molekul. Biol.SSSR (1972) in press.
[7] P.Connors, M.Labanauskas and W.Beeman, Science 166 (1969) 1528.
[8] G.Gursky, Biofizika 11 (1966) 737.
[9] T.Tao, J.H.Nelson and C.R.Cantor, Biochemistry 9 (1970) 3514.
[10] D.B.Millar and R.F.Steiner, Biochemistry 5 (1966) 2289.
[11] A.Rosenfeld, C.L.Stevens and P.Printz, Biochemistry 9 (1970) 4971.