DIFFERENCES IN STABILIZATION OF ADENINE–THYMINE AND GUANINE–CYTOSINE PAIRS IN DEOXYRIBONUCLEIC ACIDS BY DIAMINOACRIDINE DYES*

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It is well established by now that at least two different types of binding sites can be identified[1–4] in complexes of acridine dyes with DNA. At low ratio of bound dye molecules to nucleotide residues of DNA (r), the stronger type of binding took place (the resulting complex being usually denotet as complex *I*). At higher r values (above 0·2) the weaker binding occurred forming complex *II*. Further investigations[5–7] of acridine dye–DNA interaction were in agreement with this idea and enabled the construction of models for both types of interaction. According to Lerman's model[8] the dye molecules in complex *I* intercalate between two neighboring base pairs in the DNA double helix. In the modification of the latter model by Pritchard, Blake and Peacocke[9] the intercalated dye molecules are situated so that they interact only with two adjoining bases of one strand of the DNA double helix. The dye bound in complex *II* forms stacks of parallel oriented and mutually interacting molecules on the surface of the DNA helix[1,4].

In our studies of DNA stabilization by diaminoacridine dyes it was shown that DNAs with higher content of adenine–thymine pairs were more stabilized against thermal denaturation than DNAs rich in guanine–cytosine pairs[10,11]. It was also observed that the stabilization effect was due predominantly to the complex *I* formation, while further binding of dye molecules as complex *II* influenced the melting temperature only slightly[10]. These results are in good agreement with the calculations of interaction energies of proflavine with individual base pairs for both types of complexes[12]. Thus, within each of the above described structural types of complexes, there exist further heterogenities depending on the strength of the dye–base (or dye–base pair) interaction. They are exhibited pronouncedly in complex *I*, where the dye molecules are in such position that they can interact with different types of adjacent bases (the dye–dye interaction being very small in complex *I*) and are negligible in complex *II*, where predominantly the dye–dye interactions take place.

In the present paper an attempt is made to evaluate the relative contributions to the stabilization effect exerted by the two principal types of DNA base pairs, *i.e.* adenine–thymine and guanine–cytosine pairs.

EXPERIMENTAL

The values of melting temperatures for DNAs isolated from *Micrococcus lysodeikticus* (72·2% GC) *Escherichia coli* (52·2% GC), and *Bacillus cereus* (34·0% GC) and their complexes with proflavine and acridine orange were taken from our preceding communication[10], where all experimental details are given. Here it should be noted that the complexes were prepared by spectrophotometric titration in the medium of 10^{-3}M sodium acetate. The composition of the complexes is expressed

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as the ratio, r, of the number of binding sites occupied by dye molecules to the total number of binding sites.

RESULTS AND DISCUSSION

In the helix–coil equilibrium in DNA the more stable conformation is characterized by lower value of free energy. If the free energy change is $\Delta G = G_{coil} - G_{helix}$, then $\Delta G = 0$ at the temperature of melting and for $\Delta G > 0$ and $\Delta G < 0$ the helix and the coiled conformation is more stable, respectively. For pure DNA it can be written

$$T_m = \frac{\Delta H}{\Delta S}, \quad (1)$$

where ΔH is the change of enthalpy and ΔS the change of entropy in the course of helix–coil transition.

It was pointed out by Gersch and Jordan[12] that the increase of DNA melting temperature upon formation a complex with a dye was a consequence of an increase of stability of the complex native DNA–dye and not of a decrease of stability of the system denaturated DNA–dye. Thus, the decrease of free energy of native DNA upon complexing with dye should be higher than the difference between free energies of denaturated DNA and its complex with dye, respectively.

For the simple evaluation of free energy change, which corresponds to the shift of melting temperature in the complexes of DNA with dyes, the semiquantitative model of Schildkraut and Lifson[13] was used[14]. It was shown, however, that the stabilization effect of the bound dye is only partially due to the electrostatic interaction between dye cations and negatively charged phosphate groups[10] and the free energy change includes also the contribution from the weak dye–DNA base interactions to the stabilization.

Thus, the melting temperature of a DNA–dye complex ($T_{m,c}$) is given by

$$T_{m,c} = T_m + \frac{\Delta G_s}{\Delta S}. \quad (2)$$

The free energy change ΔG_s corresponding to the stabilization of helical conformation by the bound dye can be directly related to the increase of denaturation temperature ΔT_m, which is the difference between $T_{m,c}$ and the melting temperature of pure DNA T_m

$$\Delta T_m = \frac{\Delta G_s}{\Delta S}. \quad (3)$$

The values of ΔG_s listed for complexes of different DNAs in Table I were calculated using the value[14] $\Delta S = 22$ cal./grad/(mol of base pairs) for all DNAs, because it was shown that the change of entropy in the course of helix–coil transition was practically independent on base composition[15].

The results of Bradley and coworkers[1,4] indicate that the attachment of the dye molecules to DNA proceeds in a statistically random pattern and the dye distribution along the double helix is not significantly influenced by the distribution of individual bases. In such a case the relative values of ΔG_s corresponding to the stabilization of a GC pair ($\Delta G_{s(GC)}$) and an AT pair ($\Delta G_{s(AT)}$) can be estimated. The stabilization free energy change of given DNA was expressed by the equation

$$\Delta G_{s(DNA)} = a\,\Delta G_{s(GC)} + b\,\Delta G_{s(AT)}, \quad (4)$$

TABLE I

Calculated Values of the Changes of Free Energy Stabilization ΔG_s for DNAs with Different Base Composition and the Relative Contributions of Adenine–Thymine pairs (ΔG_s(AT)) and Guanine–Cytosine Pairs (ΔG_s(GC)) to the Stabilization for the DNA–Dye Complexes with r 0·05 and r 0·5

The isolation of bacterial DNAs, the determination of their GC content and the spectrophotometric determination of the denaturation temperatures of the complexes are described in our previous communication[10]. In the Table the differences ΔT_m between the denaturation temperatures of the complexes (characterized by the value r) and of corresponding pure DNAs are given.

Dye component of the complex	Content of GC/pairs in DNA %	r 0·05				r 0·5			
		ΔT_m °C	ΔG_s cal/(mol of base pair)	$\Delta G_{s(AT)}$ cal/(mol of base pair in complex I)	$\Delta G_{s(GC)}$ cal/(mol of base pair in complex I)	ΔT_m °C	ΔG_s cal/(mol of base pair)	$\Delta G_{s(AT)}$ cal/(mol of base pair in complex I and II)	$\Delta G_{s(GC)}$ cal/(mol of base pair in complex I and II)
Acridine orange	72·0	10·4	229			21·0	462		
Acridine orange	52·2	12·7	280	4 110 ± 470	1 520 ± 470	24·5	539	840 ± 60	280 ± 60
Acridine orange	34·0	14·0	308			30·5	671		
Proflavine	72·0	12·5	275			20·7	455		
Proflavine	52·2	14·5	319	5 000 ± 390	1 660 ± 380	25·2	554	840 ± 20	300 ± 20
Proflavine	34·0	18·2	400			30·0	660		

where a and b were the fraction of GC pairs and AT pairs in given DNA respectively. The equation (4) applied to n DNAs of various base composition formed a set of n equations, from which $\Delta G_{s(GC)}$ and $\Delta G_{s(AT)}$ were determined. These mean values of $\Delta G_{s(GC)}$ and $\Delta G_{s(AT)}$ represented the average values with respect to the dye content in the complex. By dividing by $2r$ the values which correspond to one base pair with the dye molecule attached were obtained.

The values of $\Delta G_{s(GC)}$ and $\Delta G_{s(AT)}$ calculated for proflavine and acridine orange complexes of r 0·05 and r 0·5 are given in Table I. There is only a small difference between the data for these diaminoacridine derivatives at r 0·05 and practically no difference at r 0·5.

Since at r 0·05 all dye molecules are bound in complex *I*, the corresponding values of stabilization free energy characterize the stabilization effect of the strongly bound dye molecules on respective base pairs. On the other hand, at r 0·5 the dye molecules are bound in both complex *I* and complex *II*. The dependence of ΔT_m on r ar well as the course of dye dissociation at elevated temperatures[10,16] indicate that the dye bound in complex *II* is partially split off from the complex at predenaturation temperatures and has none or negligible stabilization effect. Considering the fact that even at the high r values the stabilization free energy change is due only to the dye molecules bound in complex *I*, it could be estimated that the stronger binding process proceeds up to r 0·08–0·1 (*i.e.* one intercalated dye molecule per 5–6 base pairs) and then the dye molecules bind in the weak complex *II*. This value is lower than that obtained by Peacocke and Skerret[3], who found that proflavine was bound to DNA in complex *I* up to r 0·22, but it agrees reasonably with the estimation based on the analysis of the changes of ultraviolet spectra during heating the complexes DNA–acridine orange[16].

The calculation of interaction energy for proflavine binding to DNA made by Gersch and Jordan[12] are in agreement with the obtained results: while the interaction free energy between the dye molecules and DNA bases was very low and not dependent on the nature of neighboring bases in complex *II* (−1·2 kcal per repeating unit), it became much higher for the intercalated dye molecules in complex *I* strongly depending on the nature of adjacent bases (*e.g.* −12·6 and −59·2 kcal per repeating unit for the units CG : GC and AT : TA with intercalated proflavine, respectively).

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