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THERMAL DENATURATION OF DISTAMYCIN A – DNA COMPLEXES AS FOLLOWED BY HYPERCHROMIC SPECTRA

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Interaction of distamycin A with calf spleen DNA is investigated by the method of hyperchromic spectra. Hyperchromic spectra of complexes are partitioned into the components corresponding to the denaturation A·T and G·C base pairs and dissociation of the ligand, fractions of respective components are found as a function of temperature. A scheme of melting of successive regions of DNA with different G+C content together with the scheme of distamycin A redistribution in the course of thermal denaturation is presented.

1. Introduction

Distamycin A (DstA) is an oligopeptide antibiotic which has specific affinity to A·T clusters of double helical DNA [1–16]. The crystal structure of DstA has been determined recently [17]. DstA as well as a similar antibiotic netropsin (Nt) binds to the B conformation of DNA [18]. The ligand molecules are located in the small groove [19], covering approximately 5 base pairs [7,18,20]. DstA and Nt inhibit DNA and RNA synthesis [2,3,9,21,22]. Nt has been applied to CsCl density gradients to increase the resolving power of gradients [3,23–27] and used as A·T specific probe or modulating agent in chromosome and chromatin analysis [28–30].

In the present investigation the melting of the eukaryotic DNA in the presence of this A·T specific ligand is reported. Hyperchromic spectra of complexes are partitioned into components corresponding to the denaturation of A·T and G·C base pairs [30,31] and the dissociation of DstA; fractions of respective components are found as a function of temperature.

The method outlined here allows one to determine the specificity of the ligand in respect to A·T or G·C base pairs making use of only a single eukaryotic DNA sample. Also, it becomes possible to follow the redistribution of the ligand in the course of thermal denaturation.

2. Materials and methods

Calf spleen DNA was obtained from Olaine works (Latvian SSR). Distamycin A was synthesized at the Institute of Molecular Biology (Moscow) by Dr. A.L. Zhuze and S.L. Grokhovsky. Concentrations of stock solutions were determined spectrophotometrically using molar extinction coefficients $\epsilon_{260} = 6480\ \mathrm{l \cdot mole^{-1}\ cm^{-1}}$ for DNA (P^-) and $\epsilon_{303} = 30000\ \mathrm{l \cdot mole^{-1}\ cm^{-1}}$ for distamycin A. 2.5×10^{-4} M EDTA adjusted to pH 7.0 was used as buffer.

The melting of A·T and G·C base pairs and the dissociation of the ligand were calculated by the formula (1) which was used previously for two-component analysis of spectra [31].

$$f_{kj} = (\beta_{ki} \cdot \beta_{ik})^{-1} \cdot (\beta_{ki} \cdot \Delta A_{ij}). \qquad (1)$$

j, i and k refer to the temperature, wavelength and spectral component, respectively; f_{kj} is a $3 \times j$ matrix of fractions of melted A·T and G·C base pairs and fraction of dissociated ligand at temperature j (f_{AT}, f_{GC}, f_L); ΔA_{ij} is a $i \times j$ matrix of hyperchromic spectra; β_{ki} is $3 \times i$ matrix of spectral coefficients.

$\beta_{AT,i}$ and $\beta_{GC,i}$ are calculated from respective molar coefficients [32] using mean nucleotide composition and concentration of DNA [33], $\beta_{L,i}$ is the differential spectrum of the interaction of DNA and DstA measured at room temperature at the concentration equal to that used in the experiment.

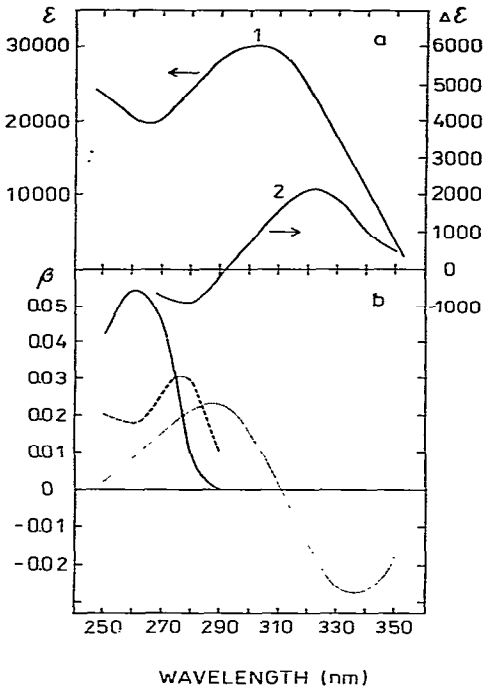

Fig. 1. (a). 1 – Ultraviolet spectrum of DstA, 2 – dependence of DstA absorbance upon temperature, $\Delta\epsilon = \epsilon_{25°} - \epsilon_{95°}$; (b) partial spectra ($\beta_{AT}$, β_{GC}, β_L) used for calculation of f_{AT}, f_{GC} and f_L according to formula (1); concentration of DNA 2.87×10^{-5} M (P^-), $r = 0.10$. —— β_{AT}, – – – β_{GC}, · · · β_L.

The standard deviations Δf_{kj} were calculated from the respective matrix equations [34]. The values of Δf_{kj} were found to be in the range from 0.5% to 4.0%, they are not shown in the figure for the sake of clarity of representation.

The values of f_{kj} found at the highest temperature were ordinarily in the range 0.9—1.1 and were normalized to 1.00.

Spectrophotometer "Specord UV-Vis" (VEB Carl Zeiss, Jena, GDR) was used with digital output. Absorbance differences ΔA_{ij} at 14 wavelengths in the regions 263–286 nm and 322–351 nm were recorded as a function of temperature. The numerical data were corrected for water expansion.

The ratio $r = D/P^-$ (the number of the dye molecules per phosphate group of DNA) was in the range from 0 to 0.10; the saturation of DNA is achieved at much higher concentrations of the ligand [2].

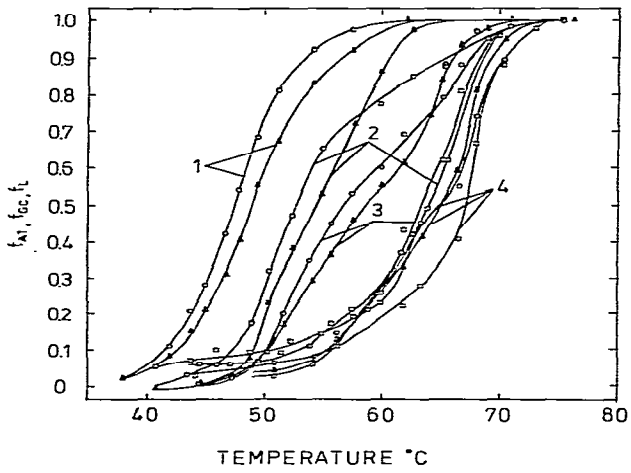

Fig. 2. Thermal denaturation of DNA complexes with DstA in 2.5×10^{-4} M EDTA pH 7.0. 1 – DNA, 2 – DNA+DstA, $r = 0.03$, 3 – DNA+DstA, $r = 0.05$, 4 – DNA+DstA, $r = 0.10$. ○ – f_{AT}, △ – f_{GC}, □ – f_L.

The method used in present investigation is described in detail by Räim et al. [35].

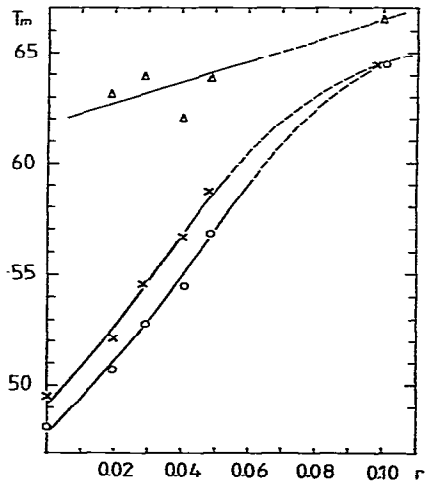

Fig. 3. Dependence of T_m upon the ratio of the bound DstA per phosphate of DNA (r); melting temperature of A-T (○) and G-C (×) base pairs and temperature corresponding to 50% dissociation of DstA (△).

3. Results

DstA has an ultraviolet absorption band with maximum at 303 nm (fig. 1a, curve 1); the intensity of this band decreases with temperature (curve 2). When calculations were carried out, the hyperchromic spectra were corrected for absorbance decrease caused by the change in the absorbance of the free ligand.

Upon interaction of DstA and DNA a red shift was observed in the DstA absorption band with an isosbestic point at 311 nm. This difference spectrum was regarded as a third partial spectrum (β_L), it is depicted in fig. 1b together with other partial spectra (β_{AT} and β_{GC}). To enhance the resolution of the melting and dissociation curves the spectra were recorded both in the 263–286 nm and 322–351 nm region. It must be mentioned that the red shift of the absorption spectrum of DstA is not specific for DNA complexes only, a similar difference spectrum is observed in the case of netropsin interaction with inorganic polyphosphate [36].

The results are represented in fig. 2. The denaturation of A·T and G·C base pairs always precedes the dissociation of the ligand regardless of the initial value of r. The temperature corresponding to $f_L = 0.50$ depends only a little upon r and increases from 63°C to 67°C when the ratio of r is raised from 0.03 to 0.10 (fig. 3). In the case of DNA the fraction of melted A·T base pairs is always higher in comparison with melted G·C base pairs throughout the melting region. When the complexes of DNA and DstA are investigated, the inversion of stability of A·T and G·C takes place at the terminal part of the melting curves.

4. Discussion

The dissociation of the bulk of DstA from DNA proceeds at temperatures up to 8°C higher compared with denaturation of DNA base pairs in the conditions of present experiment. If ligand molecules had bound evenly along the double helix of DNA, the number of molecules released into solution is to be proportional to the number of broken base pairs. In the case of A+T specific binding the proportionality in respect to broken A·T pairs is to be expected. However, it may be seen (fig. 2) that far fewer ligand molecules are released in comparison with the number of broken base pairs. Therefore, the ligand molecules which were initially bound to double helical DNA either must remain bound to single stranded DNA in the course of thermal denaturation or migrate to these regions of DNA which have maintained their double helical structure at this particular temperature. It was shown by Zimmer et al. [1] that the melting of DstA complex with denatured DNA proceeds cooperatively but the respective T_m is lower when compared with the T_m of the complex with native DNA.

It follows that the ligand molecules are released from single stranded regions of DNA and migrate to the double helical regions of DNA. Since the fraction of double stranded DNA decreases gradually, the ratio of the ligand bound per phosphate of DNA double helix increases continuously and reaches the maximum at 65–68°C irrespective of the initial r. The migration of the ligand is described in greater detail in the case of DNA complexes with phenosafranine [37].

It must be recalled that the stabilizing effect of a single DstA molecule extends over neighbouring regions including G·C base pairs [38]; the cooperativity of melting is even much more higher. Therefore, the melting curves must not be described in terms of individual base pairs but in terms of DNA block heterogeneity [24,39].

Our results confirm the suggestions made by Aktipis et al. [40] on the basis of CD and absorbance measurements of DNA complexes with ethidium bromide. The following description of the melting of DstA complex with heterogeneous DNA may be proposed (fig. 4). The initial distribution (at temperature T_1) of DstA on DNA molecules is uneven because of higher affinity of DstA in respect to A·T base pairs. However, in the range of the ligand concentrations used in this investigation, the additional stabilization of A+T rich regions caused by a greater amount of bound DstA molecules is insufficient to inverse the stability of A+T and of G+C rich regions. As a result, the melting begins from the A+T rich regions ($f_{AT} > f_{GC}$) like in the case of free DNA. Most of the ligand molecules which are released from the single stranded regions migrate to the double stranded regions of DNA. The concentration of the ligand on the remaining sections of double helical DNA increases until the slope of A·T curves begin gradually to decrease (temperature T_2); at the same time the slope of G·C curves increases. It follows that the stability of the A+T rich

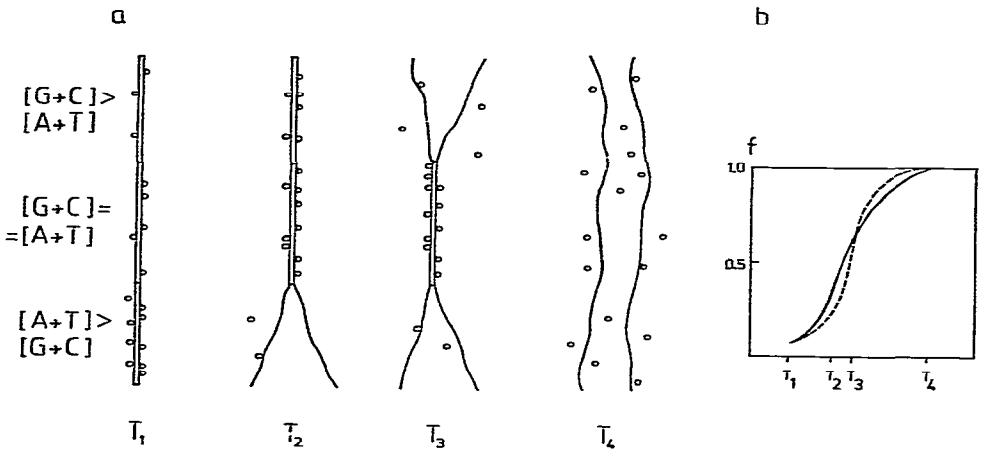

Fig. 4. (a). Denaturation of the complex of DNA with DstA $r \leq 0.10$. Eukaryotic DNA is shown to consist of blocks of different base composition as indicated on the left of the figure. (b) Schematic representation of denaturation, —— f_{AT}, – – – f_{GC}; the state of the system at temperatures $T_1 \ldots T_4$ is depicted in part (a). ▭ – double helical DNA, —— –single stranded DNA; DstA molecules are shown by circles.

regions exceeds that of the G+C rich regions at this temperature. G+C rich regions begin to denature; at temperature T_3 the fraction of melted G+C base pairs becomes equal to that of A+T base pairs ($f_{AT} = f_{GC}$). The ligand is concentrated now to the double helical DNA, which has a nucleotide composition moderately rich in A+T; these regions of DNA melt last (temperature T_4).

When the initial ratio r is higher, the saturation of the A+T rich regions and, hence, the inversion of A·T and G·C stability proceeds at a lower temperature in the relative scale. At $r = 0.10$ all the three curves are placed in close proximity.

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