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Physics

CALCULATION OF CENTRAL MOMENTS OF DIFFERENTIAL MELTING CURVE OF DNA MOLECULE

R. S. GHAZARYAN[1] *, S. N. HAKOBYAN [2], M. A. SHAHINYAN [3]**

[1] *Chair of Molecular Physics YSU, Armenia*
[2] *Department of Mechanical Engineering SEUA, Armenia*
[3] *Chair of Biophysics YSU, Armenia*

Central moments of function of the differential melting curve (DMC) of DNA have been calculated through expansion of block DNA DMC to Gaussian components. Calculations showed that the first moment of DNA DMC characterizes the melting temperature, the second is DNA melting interval. Central moments of the third and fourth orders characterize distribution of nucleotides along DNA chain. It was shown that the distribution of nucleotides along DNA chain differs from quasi-arbitrary distribution.

***Keywords*:** differential melting curve, central moments of DMC of DNA.

Introduction. It is known that DNA of most of organisms is composed of blocks extended up to 10^5–10^6 bases of pairs [1] and their sequence for each block is quasi-arbitrary, but blocks differ from each other by GC-content [1, 2]. It was shown earlier that by the expansion of the differential melting curves (DMC) of DNA block structure to Gaussian components one can determine an average GC-content of block DNA [3]. Problem of the expansion is circumstantially observed in other works [3,4], where it is shown that DNA DMC of the given organism may be expanded by several modes. Moreover, calculations show that some integral characteristics of DNA (average GC-content and dispersion) are stable to choice of the expansion mode [3].

In the present work it is suggested a mode of determination of several values characterizing distribution of nucleotides along DNA chain via expansion of DMC of DNA having block heterogeneity on components given by Gaussian function.

Materials and Methods. DNA from *E. coli* and Calf Thymus (“Sigma”, USA), which have a block structure, were used in experiments. The melting curves were obtained through spectrophotometer Cary 219 (USA). Thermostating was carried out using thermostat Haake-F3 (Italy). Experiments were carried out in water solution containing 0.015 *M* NaCl, 15 *mM* Na-citrate (pH 7.3). Measurements were

* E-mail: ruzkhazaryan@ysu.am **E-mail: mshahinyan@ysu.am

carried out in thermostat cells using 10 *mm* quartz cuvettes with hermetically closing caps. The melting curves (absorbance at 260 *nm* versus temperature) were measured by continuous heating of DNA solutions at a rate of 0.25 *grad/min* over the range of temperature 293–363 *K*. Precision of temperature determination was equal to ± 0.05 *K* and optic density was equal to 10^{-4} optic units.

DMC was obtained by numeric differentiating of normalized melting curves. To exclude random errors the obtained DMC was fitted several times (up to 5 times) using polynomials of the second and third orders, presented via 13, 9 and 7 points correspondingly by the least square method.

Results and Discussion. Form of DNA DMC in constant external conditions depends on DNA average GC-content and distribution of nucleotides along DNA chain [5]. It is very difficult and even impossible to write an analytic form of DNA melting curve precisely. That is why in this work it is suggested to calculate the central moments of DNA DMC function and through these values to judge about distribution character of GC-pairs along DNA chain. For this aim DMC of Calf Thymus and *E. coli* DNA was obtained by numeric differentiation method. DMC was expanded to Gaussian components according to the method described in [3, 6] in details. For DNA DMC approximation it is convenient to use superposition of Gaussian functions, since DMC of DNA with quasi-arbitrary distribution of bases of pairs is described with high precision by Gaussian function [5, 6].

$$\vartheta'_T(T) = \sum_{l=1}^{n} \frac{S_l}{\sqrt{2\pi}\sigma_l} \exp\left[-\frac{(T-T_l)^2}{2\sigma_l^2}\right], \tag{1}$$

where S_l, σ_l and T_l are surface, average-square width and position on temperature scale l respectively, and $\sum_{l=1}^{n} S_l = 1$. According to definition the first central moment of the function $\vartheta'_T(T)$ is expressed as

$$M_1 = \int_{T_{AT}}^{T_{GC}} \left[-\vartheta'_T(T)\right] T dT, \tag{2}$$

where T_{AT} and T_{GC} are the melting temperatures of polynucleotides poly(dA)·poly(dT) and poly(dG)·poly(dC) respectively. Expression for $\vartheta'_T(T)$ from (1) replacing in (2) and widening boundaries of integrating from $-\infty$ to $+\infty$ (widening of the integrating boundaries does not affect on the integrating result, since integral expression out of boundaries T_{AT} and T_{GC} is equal to zero) it will be obtained:

$$M_1 = \sum_{l=1}^{n} S_l T_l. \tag{3}$$

As it follows from (3), M_1 is immediately connected to the melting temperatures each of its components represents a simple average melting temperature. For DNA with quasi-arbitrary sequence of bases of pairs M_1 coincides with T_m determined from $\vartheta(T_m) = 0.5$ condition.

For the second central moment of the function $\vartheta'_T(T)$ it is obtained:

$$M_2 = \int_{T_{AT}}^{T_{GC}} (T-M_1)^2 \left[-\vartheta'_T(T)\right] T dT = \sum_{l=1}^{n} S_l T_l^2 - M_1^2. \tag{4}$$

The average square deviation for DNA DMC is calculated through M_2, which is immediately connected to the width of the transition interval (ΔT):

$$\sigma = \sqrt{M_2}. \tag{5}$$

The third central moment is calculated analogously:

$$\begin{aligned} M_3 = & \int_{T_{AT}}^{T_{GC}} (T - M_1)^3 \left[-\vartheta_T'(T)\right] T dT = \\ = & \ 3\sum_{l=1}^{n} S_l T_l^3 - 3M_1 \sum_{l=1}^{n} S_l \sigma_l^2 - 3M_1^2 \sum_{l=1}^{n} S_l T_l^2 + 2M_1^3. \end{aligned} \tag{6}$$

Asymmetry of the function $\vartheta_T'(T)$, which depends on distribution of DNA nucleotides, is characterized by a parameter

$$A_C = \frac{M_3}{\sigma^3}. \tag{7}$$

If DNA melting temperature is shifted to high temperatures compared to T_m, determining from $\vartheta(T_m) = 0.5$ condition, $A_C > 0$, in the contrary case $A_C < 0$.

For the fourth central moment we have:

$$\begin{aligned} M_4 = \int_{T_{AT}}^{T_{GC}} (T - M_1)^4 \left[-\vartheta_T'(T)\right] T dT = 3\sum_{l=1}^{n} S_l \sigma_l^4 + 6\sum_{l=1}^{n} T_l^2 S_l \sigma_l^2 + \sum_{l=1}^{n} T_l^4 S_l - \\ -12M_1 \sum_{l=1}^{n} T_l S_l \sigma_l^2 - 4M_1 \sum_{l=1}^{n} T_l^3 S_l + 6M_1^2 \sum_{l=1}^{n} S_l \sigma_l^2 + 6M_1^2 \sum_{l=1}^{n} S_l T_l^2 - 3M_1^4. \end{aligned} \tag{8}$$

How DMC, which is given by the function $-\vartheta_T'(T)$ and is determined by distribution of nucleotide pairs along DNA chain, differs from Gaussian distribution by its sharpness is determined by E_K parameter:

$$E_K = \frac{M_4}{\sigma^4} - 3. \tag{9}$$

If $E_K > 0$, the distribution is sharper than the Gaussian, and it means that blocks in DNA differ little by GC-content. Otherwise, the distribution is comparatively less sharp than the Gaussian, consequently, the blocks in DNA strongly differ by GC-content.

The above mentioned parameters were calculated for Calf Thymus and *E. coli* DNA DMC.

Parameters were calculated for several modes of the expansion. In the Tab. 1 the expansion parameters of DNA DMC from Calf Thymus are presented. Four modes of the expansion modes of DMC to Gaussian components are given in Figure.

Calculations show, that DMC of DNA from $E.\ coli$ can be expanded to three Gaussian components. For four expansions presented in Figure and for expansion of DMC of DNA from $E.\ coli$ values of M_1, σ, A_C and E_K are calculated by (3), (5), (7) and (9) formulas respectively. Data are presented in Tab. 2. As it follows from Tab. 2, the above mentioned parameters for different modes of the expansion of the same DMC of DNA in error frames of experiment coincide: they are stable to expansion mode and are comparative to the average values of calculated parameters, which characterize DNA thermostability and distribution of nucleotide pairs along DNA chain of Calf Thymus and $E.\ coli$ (see Tab. 2).

Table 1

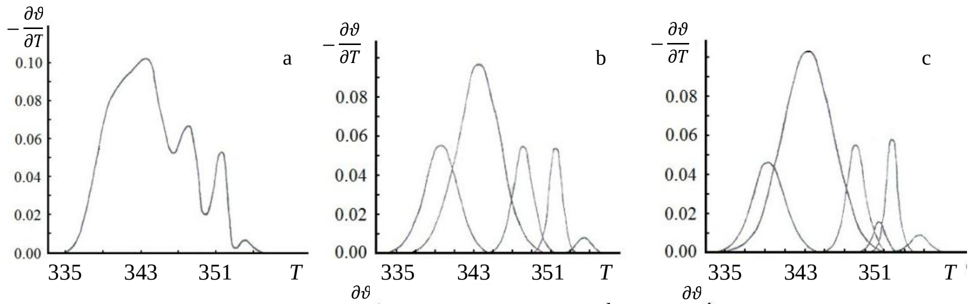
Different modes of expansion of the differential melting curve of DNA from Calf Thymus to Gaussian components

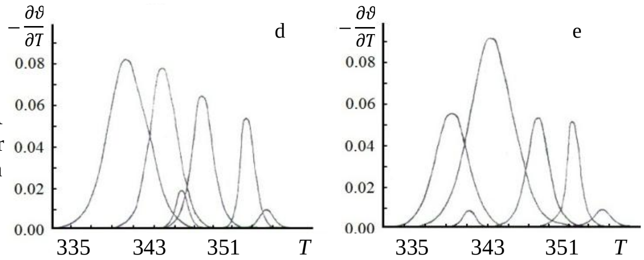
Variant	Parameter	1 comp.	2 comp.	3 comp.	4 comp.	5 comp.	6 comp.
1	T_l, K	339.73	343.62	348.24	351.70	354.70	
	S_l	0.230	0.528	0.141	0.863	0.014	
	σ_l	1.555	2.042	0.917	0.601	0.698	
2	T_l, K	339.53	343.44	348.26	350.54	351.79	354.63
	S_l	0.175	0.602	0.117	0.017	0.074	0.016
	σ_l	1.475	2.290	0.835	0.445	0.500	0.780
3	T_l, K	340.45	344.06	346.00	348.08	341.70	354.70
	S_l	0.418	0.277	0.026	0.180	0.085	0.014
	σ_l	1.883	1.316	0.510	1.035	0.600	0.700
4	T_l, K	339.69	341.42	343.65	348.21	351.71	354.70
	S_l	0.235	0.007	0.508	0.150	0.090	0.014
	σ_l	1.504	0.390	1.941	1.002	0.597	0.701

Table 2

Values of several parameters of DNA DMC from different sources, calculated for different modes of DNA DMC expansion to components

Expansion variants of DNA DMC	M_l	σ	A_C	E_K
DMC of DNA from Calf Thymus				
1	71.18	4.05	0.47	–0.405
2	71.08	4.05	0.47	–0.400
3	71.04	4.08	0.47	–0.410
4	71.07	4.05	0.47	–0.410
Average data for Calf Thymus	71.10	4.06	0.47	–0.410
DMC of DNA from $E.\ coli$				
1	74.51	2.69	–0.91	0.950
2	74.51	2.71	–0.90	0.960
Average data for $E.\ coli$	74.50	2.70	0.91	0.960





Differential melting curve of DNA from Calf Thymus (a) and four modes of its expansion to Gaussian components: (b, c, d, e).

Comparing values of parameters, presented in Tab. 2, the following conclusions can be done. DNA from $E.\ coli$ is less heterogeneous, than DNA from Calf Thymus. By distribution type the Calf Thymus DNA nucleotides (for which $A_C > 0$ and $E_K < 0$) differ from those of DNA from $E.\ coli$ (for which $A_C < 0$ and $E_K > 0$). Consequently, blocks in DNA from $E.\ coli$ by their GC-content less differ from each other, while for DNA from Calf Thymus differ strongly. That is why at investigation of behavior of certain regions of block DNA under the effect of different biological active compounds it is useful to apply DNA from Calf Thymus, in which there are blocks with sufficiently strongly different GC-content, Gaussian components of which does not almost cover each other.

Conclusion. Generalizing above mentioned it can be concluded that the first moment of DNA DMC (M_1) characterizes DNA melting temperature and for DNA with quasi-arbitrary distribution of bases of pairs it coincides with the melting temperature (T_m) determined from $\vartheta(T_m) = 0.5$ condition. Parameter σ, which is determined through M_2, characterizes the melting interval (ΔT) of DNA. It should be noted that in works [3, 7] it is shown that if instead of T_m and M_1 is used, DNA average GC-content determined by chemical mode and T_m does not differ. Simultaneously, ΔT and σ are directly proportional and behave themselves in a similar way. Consequently, the melting parameters (ΔT and T_m) can be determined from the melting curves by (3) and (5) formulas. Parameters A_C and E_K can be determined by higher moments. These parameters characterize distribution of nucleotides along DNA chain and show how strong DMC of DNA differs from Gaussian distribution.

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