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Influence of strongly stabilized sites on DNA melting: A comparison of theory with experiment

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Abstract – Strong local stabilization of the double helix can be an alternative to the interstrand crosslinks formed in DNA by some antitumor drugs. Therefore, we have carried out a computer modeling of the thermodynamic properties of DNA that contains strongly stabilized sites (SSSs). Melting of DNA locally fastened by SSSs was compared with DNA that includes interstrand crosslinks. Using experimental data from the literature and the results of our calculations, we have shown that SSSs really exist: some irreversibly bound protein molecules and chemical modifications caused by some ruthenium and antitumor platinum compounds form such sites in DNA. The theoretical calculated results for the increase of melting temperatures for random distribution of SSSs are consistent with experimental data.

Introduction. – DNA interstrand crosslinks covalently link two DNA strands, thereby blocking the normal DNA metabolism [1–3]. Mitomicin C, nitrogen mustard, nitrosourea and their derivatives can form interstrand crosslinks, and this ability is the origin of their antitumor activity [3]. It is shown that the monofunctional analogs of bifunctional crosslinking agents are 1000 times less cytotoxic [4]. Various interstrand crosslinks induce a variety of structural distortions in DNA [3]. Usually interstrand crosslinks destroy the DNA structure at the sites of their location [3] and weaken the stabilizing effect of crosslinking [5,6]. However, all kinds of interstrand crosslinks stable to denaturation conditions restore the helical structure of long DNAs after the denaturation effect is removed [7] because they prevent strand separation after full melting and form centers of nucleation that enforce correct DNA recovery.

Resistance to these and other drugs often occurs after several cycles of tumor treatment [8,9]. It is of interest to find compounds with different mechanisms of antitumor activity that can be used against tumors with acquired resistance. Therefore in this study we try to reveal another type of compounds that do not locally decrease DNA stability but form strongly stabilized sites (SSSs). They can be effective against tumors with resistance acquired by traditional crosslinking agents.

After full melting of unmodified AT and GC base pairs, interstrand crosslinks prevent strand separation, but base pairs included in formation of interstrand crosslinks are melted as unmodified ones. Similar to an interstrand crosslink, a strongly stabilized site (SSS) prohibits strand separation. However, in contrast to an interstrand crosslink, SSS conserves its helicity at the temperature of full melting of unmodified AT and GC pairs. In theory, SSS can be imaginarily formed from ordinary modifications enclosing only one strand or from interstrand crosslink by a high local increase (δF) in the free energy of the helix-coil transition of L neighboring base pairs.

It is difficult to record in experiments the formation of short SSSs, which conserve their helicity after melting of ordinary base pairs. The problem gets complicated because SSSs stimulate the formation of helical regions of ordinary base pairs around them at temperatures of full melting of unmodified DNA [10]. Parameters that reflect

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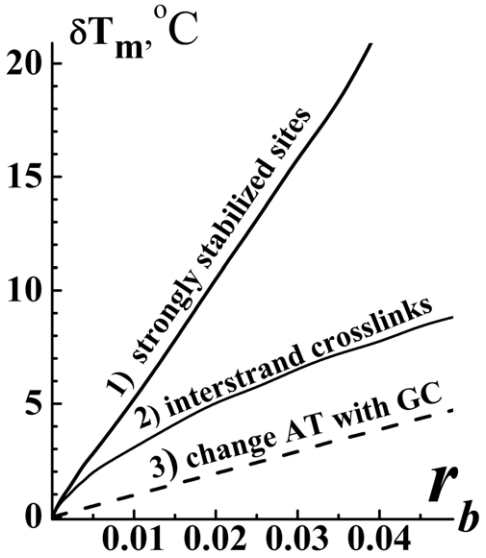

Fig. 1: The dependences of the shift in melting temperature for various types of randomly distributed chemical modifications on their relative concentration ($\delta T_m(r_b)$). $r_b = \omega/(2N)$, where N is the number of base pairs, and ω is the number of chemical modifications in the DNA chain. 1) SSSs ($L = 1$ bp); 2) interstrand crosslinks for $\delta F = 0$; 3) the replacement of ω AT base pairs with GC in the DNA chain. For a periodical distribution, curve 3 is the same, but curves 1 and 2 are located higher.

the formation of short SSSs in theoretical studies [10,11] are not measured in experiments. Usually only the melting temperature (T_m) less sensitive to the formation of SSSs rather than the full melting curve is presented in the literature [12–14]. However, T_m is also suitable for identification of SSSs because it differs for chemical modifications that give destabilization, moderate stabilization, interstrand crosslinks, and strongly stabilized sites (fig. 1).

For the study of SSSs, we use general approaches reviewed in [15,16]. For DNA with interstrand crosslinks, our method of calculation is used [5,17]. A new class of mesoscale models [18–20] which can take into account a detailed structure of DNA covered with histone molecules and DNA chemically modified with platinum compounds will be useful for such studies after taking away size restrictions. Moreover, these models are suitable for studies of DNA properties that cannot be simulated in the framework of traditional approaches [5,15–17].

Our present computer modeling demonstrates that, in contrast to moderate stabilization, influence of SSSs is strongly dependent on their distribution (either, random or periodical). However, for each of these types of distribution, the universal dependence $\delta T_m(r_b)$ for the shift of the melting temperature caused by SSSs exists ($r_b = \omega/(2N)$; N is the number of base pairs; $2N$ the number of nucleotides; ω the number of chemical modifications in a DNA chain). These two $\delta T_m(r_b)$ functions are weakly dependent on DNA GC-content and on the size of SSSs (for short stabilized sites). We will show in this study that SSSs really exist: chemical modifications caused by some platinum and ruthenium compounds [12–14] give the shift of melting temperature corresponding to the dependence calculated for randomly distributed SSSs.

Methods. – In this work, we use a lattice model of linear DNA of N base pairs that contains ω SSSs or interstrand crosslinks at base pairs with numbers n_i where $i = 1, \ldots, \omega$. More precisely, n_i is the location of the left end of the i-th SSS. Every SSS includes L base pairs. At a stabilized site, the energy of helix-coil transition is increased by δF. We suppose in this study that interstrand crosslinks are ideal, *i.e.*, they do not change the free energy of helix-coil transition at the sites of their location ($\delta F = 0$). They only prohibit local and total strand separation.

For strongly stabilized sites, calculations were carried out using the Poland-Fixman-Freire method [21,22] and strand dissociation after full melting was taken into account [17,21]. Probabilities of base pairs to be helical at various temperatures (P_m) were calculated for intact DNA and for DNA with SSSs using the expression $P_m = \rho(m) \cdot \vartheta_{ext}$, where $\rho(m)$ is the probability for the base pair m to be helical in a non-dissociated DNA molecule, ϑ_{ext} is the fraction of non-dissociated (not fully melted) DNA molecules. For the calculation of the melting temperature and other thermodynamic parameters of DNA with interstrand crosslinks, the method described in works [5,17] was used.

The following DNA parameter values were used in this study: the total number of base pairs is $N = 5000$ bp; the fraction of GC base pairs is $X_{\rm GC} = 0$, 0.25, 0.5, 0.75, and 1 (for $X_{\rm GC} = 0.25$, 0.5, 0.75, the DNA sequences were produced with the random number generator); the loop entropy factor for a loop of L base pairs formed by boundaries of internal melted regions and/or by interstrand crosslinks is $\delta(L) = (L+1)^{-1.7}$ [15]; the cooperativity factor (statistical weight assigned to the boundaries of an internal melted region bordered by helical base pairs) is $\sigma = 5 \cdot 10^{-5}$; the strand association parameter β is taken to be equal to σ [23]; enthalpy, entropy and melting temperatures of AT and GC base pairs are $\Delta H_{\rm AT} = 8.41$ kcal/(mole bp), $\Delta H_{\rm GC} = 9.47$ kcal/(mole bp), $\Delta S_{\rm AT} = \Delta S_{\rm GC} = 24.85$ cal/deg, $T_{\rm AT} = 65.2\,^\circ$C and $T_{\rm GC} = 107.8\,^\circ$C, respectively [16]. It was assumed in all calculations that DNA strands are non–self-complementary. As follows from the parameterization given above, we use the model with only two stability parameters (AT and GC base pairs) for unmodified DNA. This simplification does not influence the effects considered in this work and is applied in various melting studies [16]. For a modified DNA, the four stability parameters corresponding to unmodified AT and GC base pairs and modified ones are used.

Results and discussion. – Experimental studies demonstrate that the chemical modification of the double helix with some compounds result in a strong increase

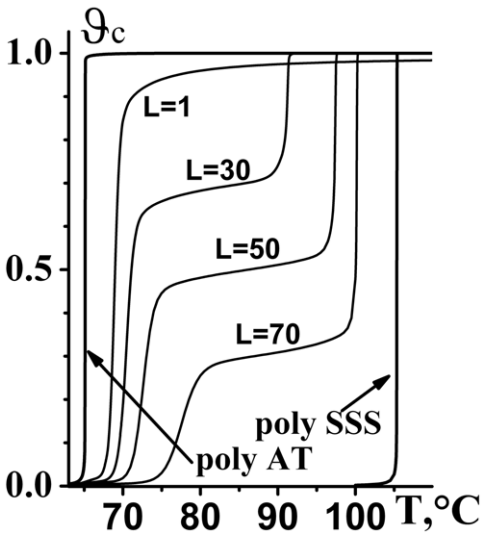

Fig. 2: Two-step character of the helix-coil transition of DNA (poly AT) with periodical distribution of long SSSs. $N = 5000$ bp; $\delta F/L = 1$ kcal; $r_b = 0.005$; $\omega = 50$ SSSs. $L = 30$ bp ($\delta F = 30$ kcal), $L = 50$ bp ($\delta F = 50$ kcal) and $L = 70$ bp ($\delta F = 70$ kcal). Melting curves of poly (AT) without SSSs, with short SSSs ($L = 1$ bp, $\delta F = 25$ kcal) and of SSS homopolymer (poly SSS) are shown for comparison.

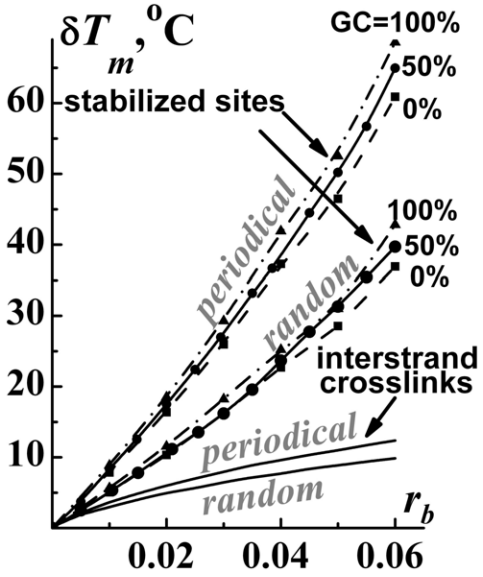

Fig. 3: Influence of short SSSs ($L = 1$ bp, $\delta F = 25$ kcal) periodically and randomly distributed along DNA chain on the melting temperature of AT (GC = 0%) and GC (GC = 100%) homopolymers, and DNA (GC = 50%). A shift of melting temperature caused by ideal interstrand crosslinks is shown for comparison.

in the DNA stability [12–14,24]. It means that they strongly stabilize the double helix at the sites of their location. In our previous studies, we have considered the influence of chemical modifications that give rise to strongly stabilized sites (SSSs). These sites conserve the helical structure even after almost full melting of ordinary AT and GC base pairs [5,10]. The two very different types of stabilized sites exist: "*long*" (more than 20 bp) and "*short*" (1–5 bp). Long SSSs give rise to the two-step melting curves (fig. 2). The short ones only increase melting temperature and the temperature melting range.

Long strongly stabilized sites. The analysis of the literature on melting of DNA complexes demonstrates that long SSSs really exist. The most well known and clear examples are DNA complexes with histones, protamines, polylysine and polyarginine. At low ionic strength, they increase melting temperature of covered regions by 20–40 °C. There is only a small elevation of T_m for uncovered free DNA regions [25–28]. In agreement with the SSS definition, the uncovered DNA regions are melted out before melting of the covered ones. An example of melting curves for periodical SSS distribution is exhibited in fig. 2. The general melting behavior is the same for random and periodical distribution of SSSs (not shown).

The SSSs can be also formed by long blocks (more than 20 bp) strongly enriched in GC base pairs [11]. A change of AT with GC base pair can be considered as a chemical modification. If this change of AT with GC is done in separate points of the DNA chain ($L = 1$ bp), then it will increase the melting temperature but will not cause formation of SSSs. If this change forms long GC blocks ($L > 20$ bp), then these blocks save their helicity (fully or in part) until the melting of base pairs located out of the blocks occurs.

Short strongly stabilized sites. Chemical modification of the double helix with some metal-based compounds result in a strong increase in the DNA melting temperature (T_m) [12–14]. They can give rise to SSSs that cause an effect similar to interstrand crosslinking. As follows from fig. 3, SSSs strongly increase the melting temperature. The shift of melting temperature ($\delta T_m(r_b)$) is strongly dependent on the type of SSS distribution, and it is higher for the periodical case. For both types of SSS distribution, the dependence of $\delta T_m(r_b)$ on GC is weak. There is some small difference in $\delta T_m(r_b)$ for AT and GC homopolymers, but the dependences calculated for the GC interval 25–75% coincide. The function $\delta T_m(r_b)$ was also computed for ideal interstrand crosslinks, which do not change the energy of the helix-coil transition at the sites of their location ($\delta F = 0$) (fig. 3). It is seen that SSSs give much higher $\delta T_m(r_b)$ than ideal interstrand crosslinks for both periodical and random distributions. However, ideal interstrand crosslinks also give high stabilization of the double helix. Their effect is much stronger than the effect caused by a change of the same number ω of AT base pairs with a more stable GC type (fig. 1). This last case also shows considerable stabilization: the difference in the melting temperatures of AT and GC homopolymers is higher than 40 °C.

Comparison of experiment with the results of the calculation is difficult because an increase in melting temperature caused by SSSs in general depends upon

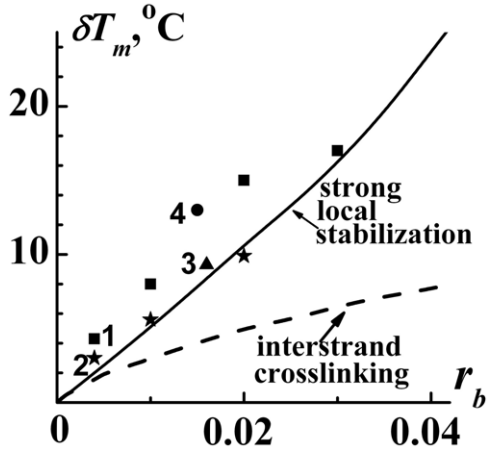

Fig. 4: The dependence $\delta T_m(r_b)$ for DNA that contains randomly distributed SSSs (solid line) ($L = 1$ bp). Experimental data for platinum and ruthenium compounds are also exhibited in the figure. A shift of melting temperature caused by interstrand crosslinks is shown for comparison. 1 (■): [{cis-PtCl $(NH_3)_2\}_2H_2N(CH_2)_4NH_2]^{2+}$ [12]; 2 (★): [{cis-PtCl$(NH_3)_2\}_2$ $H_2N(CH_2)_6NH_2]^{2+}$ [12]; 3 (▲): [{trans-PtCl$(NH_3)_2\}_2H_2N$ $(CH_2)_4NH_2]^{2+}$ [13]; 4 (●): $[Ru(phen)_2(OH_2)_2]^{2+}$ [14].

many parameters: relative concentration of SSSs (r_b), character of distribution (random or periodical), free energy of stabilization per mole of SSSs (δF), and SSS length (L). The melting temperature shift can also be dependent on the DNA sequence. However, some properties of SSSs we have found and some general considerations strongly simplify the situation: 1) as was shown in this and other [29] studies, the increase in melting temperature δT_m caused by short SSSs ($L \sim 1$ bp) is not changed with δF for $\delta F > 12$ kcal; 2) *in vitro* chemical modification of natural DNAs gives a random distribution of modifications rather than a periodical one; 3) redistribution of δF from one base pair ($L = 1$ bp) to several neighboring base pairs ($L = 2$–5 bp) does not change $\delta T_m(r_b)$; 4) for the same type of the SSS distribution (either, random or periodical), the difference in $\delta T_m(r_b)$ for DNA of various GC-content is small (fig. 3). Moreover, $\delta T_m(r_b)$ is almost the same for GC = 25–75%.

All these properties of SSSs simplify the comparison of the calculated dependence $\delta T_m(r_b)$ with the experiment, and allow us to conclude that the curve $\delta T_m(r_b)$ calculated for random distribution of SSSs, $L = 1$ bp, GC = 50%, and $\delta F = 25$ kcal per mole of stabilized sites is universal for any natural DNA and any type of short SSSs.

The simplest way to search for SSSs is to compare this universal dependence $\delta T_m(r_b)$ with experimental data for chemical modifications that give rise to a strong increase in T_m at low r_b. Some of these stabilizing compounds can form interstrand crosslinks [12,13]. This formation does not hinder the use of the described approach. Our calculations demonstrate that the interstrand crosslinking itself does not influence the melting temperature if the increase in the free energy (δF) of the helix-coil transition at the sites of their location is sufficiently large [5].

In fig. 4, the calculated universal dependence $\delta T_m(r_b)$ is shown together with experimental data found in the literature [12–14] for four metal-based platinum and ruthenium compounds that give rise to a strong increase in melting temperature. It is seen that all experimental points coincide with the calculated dependence $\delta T_m(r_b)$ or are located slightly above it. Higher shifts in melting temperature can be caused by a slight deviation of the SSSs distribution from the random one. This comparison demonstrates that strongly stabilized sites exist.

It is known that at least three of these compounds form interstrand crosslinks [12,13]. However, ideal interstrand crosslinks, for which $\delta F = 0$ at sites of crosslinking, demonstrate much lower $\delta T_m(r_b)$ in comparison with SSSs (figs. 1, 3, 4). This means that the considered compounds additionally stabilize DNA beyond the crosslinking effect. In contrast to these compounds, interstrand and intrastrand crosslinks formed by an antitumor compound, cisplatin, locally destabilize the DNA structure [30–32]. This might explain the different antitumor activity of these platinum compounds [12,13] in comparison with cisplatin, and their antitumor effect in cisplatin-resistant cell lines [33,34].

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