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DETERMINATION OF TWO COSTITUENTS OF THE INFLUENCE OF INTERSTRAND CROSSLINKS ON DNA STABILITY

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Various compounds form interstrand crosslinks (ICLs) in DNA. For mitomycin C, nitrogen mustard, nitrosourea and their derivatives, this ability is the origin of their antitumor activity. Besides crosslinking in itself, various ICLs induce a variety of local structural distortions in DNA. Those various structural features of ICLs can influence a number of important secondary effects. For example, despite sharing the same target sequence for interstrand crosslinking, the nitrogen mustards are effective antitumor agents, whereas diepoxyalkanes cause cancer. Therefore, the genomic site of crosslinking alone cannot explain why some crosslinking agents act as antitumor drugs and others as toxins. Local structure and stability alterations in the double helix at sites of crosslinking might determine some peculiarities of anticancer activity. During the DNA helix-coil transition caused by an increase in temperature, local and then total strand separation occurs. Interstrand crosslinking and local distortions caused by ICLs strongly influence these types of strand separation.

There are no "pure" crosslinking agents that form only ICLs in long DNA chains. Interstrand crosslinks usually account for a small fraction relative to

monofunctional adducts and/or intrastrand crosslinks, which also influence the melting behavior and hide the effect of ICLs. In the case of oligonucleotide duplexes (short DNAs), the problem is overcome by synthesis of sequences that allow formation of a single interstrand crosslink at a given site of a duplex. For long DNAs, only computer modeling can help to investigate pure effect of ICLs, and then compare it with the influence of monofunctional adducts and intrastrand crosslinks to reveal thermodynamic peculiarities of interstrand crosslinking. We carry out such modeling in this work using our method for calculation of melting curves for DNA modified with ICLs [1,2]. The total thermal effect of ICLs along with separate estimation of the influence of crosslinking in itself and of local distortions is determined.

Chemical modifications that do not form ICLs locally disturb DNA structure and cause a local change in the free energy of the helix coil transition by δG. In this way, they change DNA thermal stability (melting temperature, T_m). In addition to these effects, modifications that generate ICLs decrease the loop entropy factors of melted regions and prohibit total strand separation.

In Figure 1, the thermal effect of chemical modifications with ICL formation (δT_{cr}) and without ICL formation (δT_{mod}) is evaluated relative to the melting temperature of unmodified DNA (T_{unm}), *i.e.*, $\delta T_{mod}= T_{mod} - T_{unm}$ for modifications without ICL formation, and $\delta T_{cr}=T_{cr}-T_{unm}$ for DNA with ICLs.

However, δT_{cr} expresses the effect of ICLs that includes the two constituents: the effect of crosslinking in itself and the effect of structural distortions at the site of ICLs. To obtain the crosslinking thermal effect (δT_{crng}), the local effect of chemical modifications that form crosslinks (δT_{mod}) should be subtracted from the total effect caused by crosslinks (δT_{cr}) calculated for the same sites and δG:

$$\delta T_{crng}(\delta G,r_{cr})=T_{cr}(\delta G,r_{cr})-T_{mod}(\delta G,r_{cr})= \delta T_{cr}(\delta G,r_{cr}) -\delta T_{mod}(\delta G,r_{cr}) \quad (1)$$

Only for ideal ICLs ($\delta G=0$), the crosslinking effect equals the effect of crosslinks because $\delta T_{mod}=0$ for $\delta G=0$.

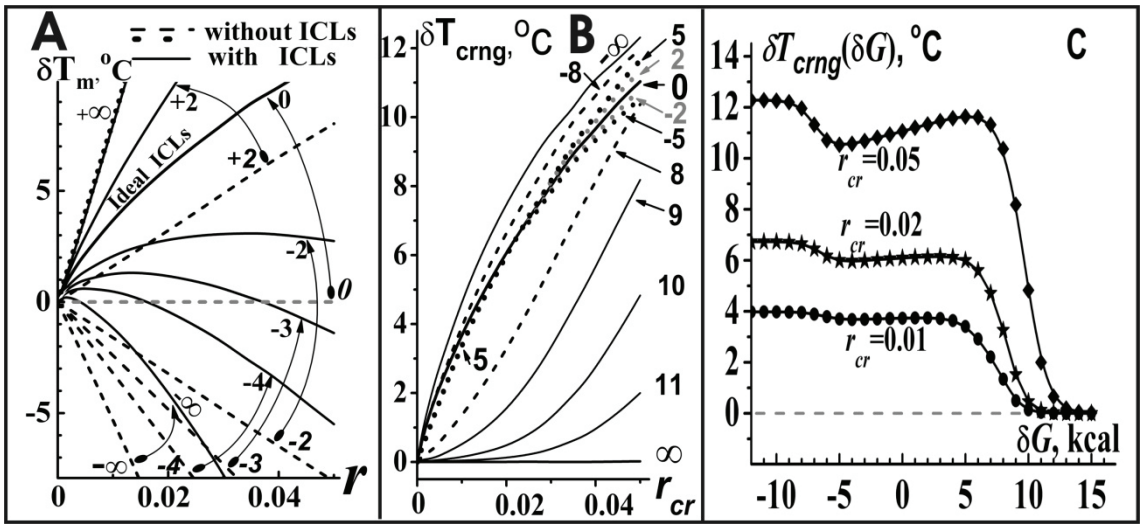

Figure 1 – A) The dependences of the shift of DNA melting temperature (δT_m) on relative (per nucleotide) molar concentration of periodically arranged chemical modifications (r) without (dotted lines) and with (solid lines) interstrand crosslinking. The alteration of the free energy of the helix-coil transition (δG, kcal/ mole of modifications) at sites of modifications is shown in the figure. The arrows show curve transformation after introduction of interstrand crosslinking at sites of modifications. The calculation was carried out using the theoretical method developed for DNA with ICLs [1,2]. B) The thermal effect of interstrand crosslinking at sites of modification $\delta T_{crng}(\delta G, r_{cr})$. C) The dependence of the thermal effect of interstrand crosslinking at sites of modification ($\delta T_{crng}(\delta G, r_{cr})$) on local alteration of the free energy of the helix-coil transition per mole of modifications (δG) for r_{cr}=0.01, 0.02, 0.05

Our results (Figure 1) demonstrate that all DNA chemical modifications (with or without interstrand crosslinking) can increase or decrease DNA thermal stability depending on their relative concentration (r), and on a local change in the free energy of the helix-coil transition at sites of their location (δG). DNA interstrand crosslinks in per nucleotide concentration r_{cr} from 0 to 0.05 can increase melting temperature by 47°C or decrease by 17°C (Figure 1A), and the influence weakly depends on DNA sequence and GC content. However, the crosslinking effect in itself ($\delta T_{crng}=\delta T_{cr}(\delta G)-\delta T_{mod}(\delta G)$) is always positive (0-12°C) (Figure 1B). It is approximately the same for a given r_{cr} value if δG is lower than 5 kcal per mole of ICLs. For these δG values, the effect of real ICLs on melting temperature $\delta T_{cr}(\delta G)$ can be represented as the sum of the temperature shifts caused by ideal interstrand crosslinks $\delta T_{cr}(\delta G=0)$ and by a local change in DNA stability at the same sites

$\delta T_{mod}(\delta G)$, *i.e.*, interstrand crosslinking and structural distortions at sites of their location almost independently influence the thermal stability (δT_{cr}) if δG<5kcal/mole ICLs. As δG increases from 5 to 12 kcal/mole ICLs, then $\delta T_{crng}(\delta G)$ decreases from the value approximately corresponding to the ideal interstrand crosslinks to zero (Figure 1C).

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THE INFLUENCE OF STRUCTURAL DISTORTIONS AT A SITE OF INTERSTRAND CROSSLINKING ON THE STABILITY OF OLIGONUCLEOTIDE DUPLEXES

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An interstrand crosslink (ICL) introduced in long DNAs or in oligonucleotide duplexes influence the DNA thermal stability in two ways. Firstly, the crosslinking in itself stabilizes DNA prohibiting local and total strand separation. Secondly, structural distortions at sites of crosslinking can increase or decrease the thermal stability. Here we describe a procedure of determination of thermal effect caused by structural distortions in nucleotide duplexes (δT_{mod}) at the site of crosslinking. In long DNAs, prohibition of local strand