$\delta T_{mod}(\delta G)$, *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 separation gives rise to an increase in melting temperature due to the formation of additional loops in melted regions that decreases their entropy. Short oligonucleotide duplexes do not form loops and insertion of a single crosslink does not give rise to the loop formation. A well-known strong increase in the melting temperature (T_m) after crosslinking is caused by prohibition of total strand separation.

For evaluation of the thermal effect caused by structural distortions in nucleotide duplexes (δT_{mod}), we use imaginary introduction into a real unmodified DNA duplex of an ICL that do not cause structural distortions, but prevent strand separation (Figure 1.I, A→B). We call such an ICL as "ideal". The calculated melting temperature T_{cr_id} of the imaginary duplex with an ideal ICL can be considered as a zero reference for the real duplexes of the same sequence that contains a real ICL (Figure 1.I.C). Higher or lower melting temperature of a real crosslinked duplex (T_{cr}) relative to ideally crosslinked (T_{cr_id}) means local thermal stabilization or destabilization, respectively. Thus, the thermal effect can be calculated in the following way.

$$\delta T_{mod1} = T_{cr} - T_{cr\_id} \qquad (1)$$

Another less obvious procedure for calculation of δT_{mod} is "uncrosslinking" of real crosslinked oligonucleotide duplex that means an imaginary deletion of the crosslinking from a real crosslinked DNA duplex while conserving the local distortion at the site of crosslinking (Figure 1.I, C→D), and calculating melting temperature of the uncrosslinked duplex (T_{uncr}). Higher or lower calculated melting temperature of the uncrosslinked duplex (T_{uncr}) relative to unmodified one (T_{unm}) demonstrates stabilization or destabilization at sites of crosslinking, respectively (Figure 1.I). The thermal effect of local structural changes at the site of crosslinking on melting temperature (δT_{mod2}) is equal to the difference between melting temperatures of an imaginary uncrosslinked duplex (T_{uncr}) (Figure 1.I.D) and real unmodified duplex (T_{unm}, Figure 1.I.A):

$$\delta T_{mod2} = T_{uncr} - T_{unm} \qquad (2)$$

Illustration of both methods of calculation is given in Figure 1.I.

Using Breslauer's approach, we have obtained the following expressions for δT_{mod1} and δT_{mod2}:

$$\delta T_{mod1} = T_{cr} - T_{unm} + \frac{T_{unm}^{2} \cdot R \cdot \ln(C_t/4)}{\Delta H_{unm\_cal} + R \cdot T_{unm} \cdot \ln(C_t/4)} \qquad (3)$$

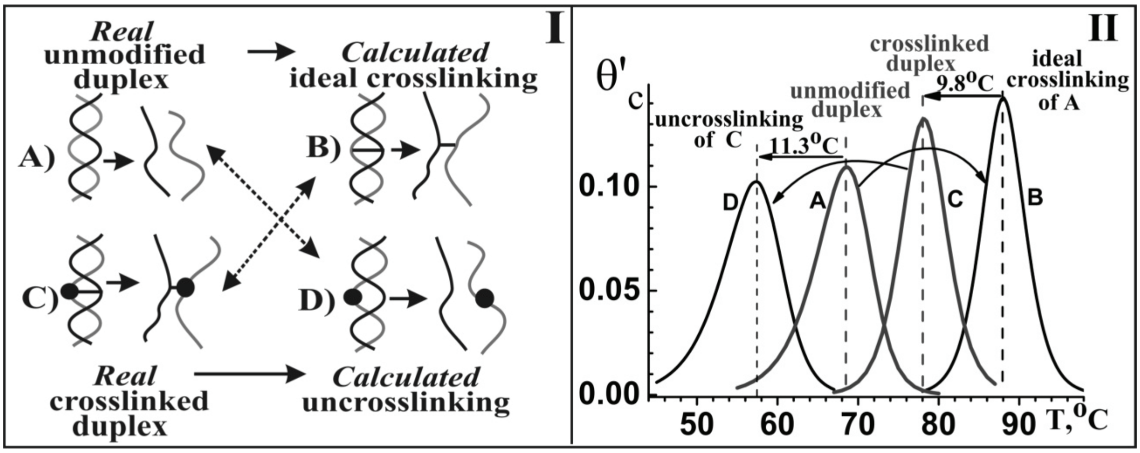

Figure 1 – (**I**) Illustration of temperature induced melting of a duplex with various chemical modifications. **A**) A real unmodified non-self-complementary duplex; **B**) The same duplex **A** with an imaginary ideal interstrand crosslink; **C**) A real crosslinked duplex (duplex **A** with a single interstrand crosslink); **D**) An imaginary "uncrosslinked duplex" originated from the real crosslinked duplex **C** by deletion of the interstrand crosslinking. Solid arrows **A**$\rightarrow$**B** and **C**$\rightarrow$**D** are directed from real duplexes **A, C** to corresponding imaginary duplexes **B, D** characterized by the same internal entropy and enthalpy as real. Dashed arrows show pairs of duplexes used for evaluation of the influence of local distortions at sites of the interstrand crosslinking on melting temperatures (T_{uncr}(**D**)-T_{unm}(**A**), T_{cr}(**C**)-T_{cr_id}(**B**)). (**II**) Modeling of differential melting curves using direct calculation for various chemical modifications depicted in Figure 1.I

$$\delta T_{mod2} = T_{cr} - T_{unm} + \frac{T_{cr}^{2} \cdot R \cdot \ln(C_t/4)}{\Delta H_{cr\_cal} - T_{cr} \cdot R \cdot \ln(C_t/4)} \qquad (4)$$

where C_t is the total molar concentration of strands; R is the universal gas constant per mole; ΔH_{unm_cal} and ΔH_{cr_cal} are the total enthalpy of unmodified and crosslinked duplex, respectively.

Using the described method and experimental data [1,2] for cisplatin and transplatin ICLs, we have determined both constituents of the influence. For the duplex of 20 bp in length, a single ICL in middle formed by cisplatin causes the total increase in melting temperature by 8.7°C. However, ideal interstrand crosslinking (δG=0) gives a 20.3°C increase. It means that local distortions decrease melting temperature by 11.6°C. Procedure of "uncrosslinking" gives a close value of 12.8°C. The average value of the decrease in melting temperature is 12.2^oC. The distortion caused by the transplatin ICL gives an average decrease in melting temperature by 14.6^oC (Table 1). We also considered another duplex of 15 bp crosslinked with transplatin [2]. In this case a decrease in melting temperature caused by local distortions relative to ideal ICL is slightly higher (12.9^oC).

Table 1 – The effect of a structural distortion caused by an ICL located in the middle of duplexes of 20 and 15 base pairs on the thermal stability

Crosslinking agent	T_{unm}, ^oC	T_{cr_id}, ^oC	T_{cr}, ^oC	T_{uncr}, ^oC	T_{cr}-T_{cr-id}, ^oC	T_{uncr}-T_{unm}, ^oC	Average effect, ^oC
Cisplatin (20 bp) [1]	68.8	89.1	77.5	56.0	-11.6	-12.8	-12.2
Transplatin (20 bp) [1]	68.8	89.1	73.8	55.0	-15.3	-13.8	-14.6
Transplatin (15 bp) [2]	67.7	92.2	78.8	55.4	-13.4	-12.3	-12.9

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