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THERMODYNAMIC ANALYSIS OF 3 → 2 TRANSITION IN OLIGONUCLEOTIDE TRIPLE–HELIX COMPLEXES dA_n-$2dT_m$

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The work considers a possibility for thermodynamical describing the transition in oligomeric complexes dA_{15}-$2dT_{10}$ and dA_{19}-$2dT_{19}$ from the triplex form to the duplex+single strand (3→2 transition), using the "Staggering Zipper" model and assuming the transition to be bimolecular reaction $A{\cdot}2B \Leftrightarrow AB+B$. It was shown that upon physically grounded values of the transition thermodynamic parameters (ΔH_{23}, ΔS_{23}, nucleation parameter β_{23} and others) the model describes adequately the experimental melting curves for triplexes with various lengths of oligonucleotide chains and different ionic conditions.

As well, the work considers effects of the dye covalently attached to dT_{10}, intercalating between A·T pairs of the duplex on the transition parameters. Possible causes of great dispersion of the experimental data obtained for ΔH_{32} by different methods (DSC, ITC, van't Hoff analysis and so on) are analyzed. It is shown that disordering of the triplex and duplex ends (the so called "end fraying") has an significant influence on the form of melting curves and on thermodynamic transition parameters.

KEY WORDS: oligonucleotide triplexes, helix-coil transition, stabilization of triplexes by dye, "Staggering Zipper" model.

In recent years, due to interest shown in studies on triplex structures of nucleic acids (NA), calculations of thermodynamic parameters conditioning the NA stability are of great importance [1-9]. Along with microcalorimetric measurements of heats of helix-coil transitions by methods of differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC), transitions enthalpies are calculated from melting curves determined by monitoring changes in ultraviolet absorption. But the great scatter of enthalpy values of the transition from triplex complex to the duplex one + single chain (3→2 transition) is observed for the results obtained by different methods [1]. In particular, there are some data [6,7] evidencing nonadequacy of the two-state model for the thermodynamic description of such transitions. Therefore a necessity arises to apply more perfect approximations to calculate thermodynamic characteristics. The "Staggering Zipper" model developed by Zimm [10] and Applequist and Damle [11,12] can serve a such approximation that was shown by Eigen and Pörschke [13,14] for transitions in ribooligonucleotide duplexes rA_n-rU_n.

In our previous work [15] we have performed calculations by "Staggering Zipper" model to describe transitions in deoxynucleotide duplexes dA_n-dT_m of different lengths as well as those in duplexes strengthened by covalent attachment of a phenazine dye to one of the pyrimidine strand ends. In the present paper we have applied this model to thermodynamic analysis of melting transitions in desoxyoligonucleotide triplexes dA_n–$2dT_m$. The model was modified to take into account effects of covalently attached phenazine dye and ionic strengths of solutions.

THEORETICAL MODEL

The process of the triplex formation in our oligonucleotide system may be considered as the attachment of the third strand, dT_m, to the already formed duplex dA_n-dT_m (dA_n-dT_m + $dT_m \Leftrightarrow dA_n$-$2dT_m$ reaction) (Fig. 1). In this case the process is the bimolecular reaction and, by the "Staggering Zipper" model approximation, may be described by the equilibrium constant:

$$K_{23} = \beta_{23}\sum_{n=1}^{N}(N-n+1)^2 \cdot s_{23}^{n} = \beta_{23}L(s) \qquad (1)$$

where β_{23} is the nucleation constant, N is the number of base pairs in the duplex (or bases in the third strand), n is the number of bound bases in the third chain; s_{23} is a constant of a new base attachment to the ordered section in the third chain (the third chain growth parameter):

$$s_{23} = \exp(-\Delta G_{23} / RT) \qquad (2)$$

where ΔG_{23} is the difference between free energies of ordered and disordered bases in the third chain. For the reaction of AB+B↔A2B type the fraction of the ordered bases in the third chain, f, can be calculated by formula [12,14]

$$f = \left[1 + \gamma_{23} L(s) - \left(1 + 2\gamma_{23} L(s)\right)^{1/2}\right] \cdot s_{23} L'(s) / \left(N \gamma_{23} \cdot L^2(s)\right) \tag{3}$$

where $\gamma_{23} = \beta_{23} \cdot C$ and C is the concentration of oligonucleotide strands.

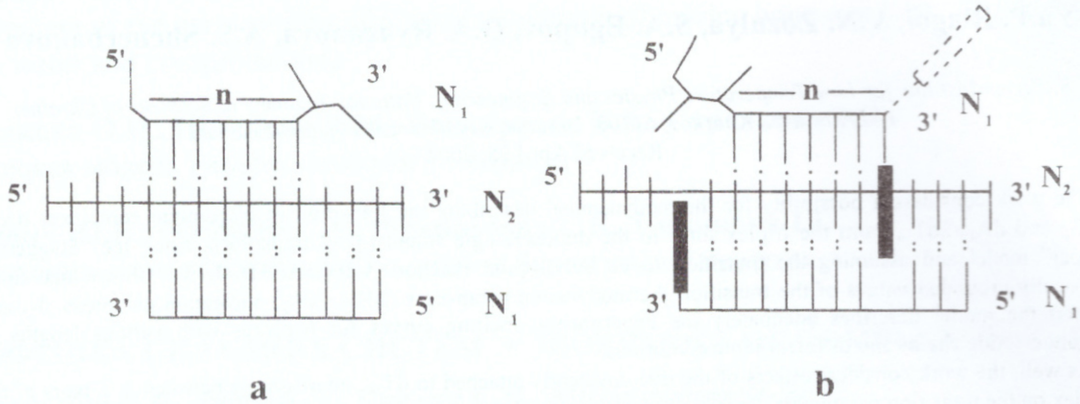

Fig.1. Schemes of oligonucleotide triplex complexes: (a) – dA_{15}-$2dT_{10}$, (b) - dA_{15}-$2dT_{10}Ph$. It is supposed that third strand (dT_{10} or $dT_{10}Ph$) attaches to completely formed duplex and dye intercalates between base pairs of duplex. Possible disordered end bases of Hoogsteen strand are shown. States with disrupted phenazine intercalation (dotted line) are noticed.

But it should be noted that such an approach simplifies the situation because the possible partially disordered duplex states (the "end fraying") are not taken into account. This may results in some quantitative changes in the properties being calculated but don't change the picture qualitatively.

As in the case of duplexes, the model permits to calculate the fraction of the completely separated triplexes (into duplexes and single strands)

$$f_0 = \left[\left(1 + 2\gamma_{23} \cdot L(s)\right)^{1/2} - 1\right] / \left(\gamma_{23} \cdot L(s)\right) \tag{4}$$

and to determine the ordered segments distribution on lengths (that is to carry out the "population" analysis)

$$f_n = \frac{1}{2} \gamma_{23} \cdot f_0^2 \cdot (N - n + 1)^2 \cdot s_{23}{}^n \tag{5}$$

The change in the free energy, ΔG_{23}, can be calculated by formula [14,16]

$$\Delta G_{23} = \Delta H_{23} - T\left(\Delta S_{13} - RC_1'\right) + T\left(\Delta S_{12} - RC_1\right) + RT \cdot \log \mu \cdot \left(C_2 - C_2'\right) \tag{6}$$

where $\Delta H_{23} = \Delta H_{13} - \Delta H_{12}$; C_1', C_2', C_1 and C_2 are empirical constants characterizing dependences of transition temperatures on ionic strength, μ , that are determined from "phase diagrams" for polymeric molecules.

The dye covalent attachment to one of the oligonucleotide ends increases the transition constant K_{23}, that may be taken into account by introducing the parameter of complex stabilization by dye (s_{ph}) [15]

$$s_{ph} = \exp\left\{\left(-\Delta H_{ph} + T\Delta S_{ph}\right) / RT\right\} \tag{7}$$

where ΔH_{ph} and ΔS_{ph} are changes in enthalpy and entropy upon the dye intercalation between the bases of oligonucleotide duplexes. As follows from Fig. 1(b), this provides additional bonds between oligonucleotides the consideration of which results in the expression for the binding constant K_{23}'

$$K_{23}' = \beta_{23} \sum_{n=1}^{N_1} \left\{s_{ph}\left(N_1 - n\right) + \left(N_1 - n + 1\right)^2\right\} \cdot s_{23}^n \tag{8}$$

When calculating s_{ph}, the experimental value of binding constant determined earlier for the free phenazine association with poly(dA)-poly(dT) [17] were used. For convenient calculations and to take into account the temperature dependence of this constant, formula (7) was transposed to

$$s_{ph} = s_{ph}^0 \exp\left\{-\alpha \cdot (T - 273)\right\} \tag{9}$$

where $\alpha = \Delta H_{ph} / R \cdot T_0 \cdot T$ and s_{ph}^0 is a value of the constant s_{ph} at T_0=273 K.

In all the cases, the calculation formulae include a number of unknown values which can serve as fitting parameters that diminishes a significance of the experimental test of the theory. Therefore, to restrict an arbitrary selection of parameters and to get uniqueness of calculated data, the additional conditions resulting from the physical meaning of the values considered were imposed. Since for the polymeric molecule the change in the free energy ΔG_{32} at the middle point of the transition is equal to zero, the relation between enthalpy and entropy of the transition is

$$\frac{\Delta H_{23}}{\Delta S'_{23}} = T_m \tag{10}$$

where $\Delta S'_{23} = \Delta S_{13} - RC'_1 - \left(\Delta S_{12} - RC_1\right)$.

The nucleation parameter β is conditioned by an entropy loss upon the chain combining [11]. Upon nucleation of the triplex structure, one of its constituents - duplex is in the ordered state. Therefore, on the chain ordering, the entropy change should be less compared with the double helix formation [18]. Taking this into consideration upon calculations of triplexes, higher values of β_{32} than for duplexes must be used.

RESULTS AND DISCUSSION

To approve the present model approximation, the 3→2 transition curves were calculated for the dA_{15}-$2dT_{10}$ and dA_{15}-$2dT_{10}Ph$ systems studied earlier [19]. The transitions were determined from UV-absorption measurements at $\lambda = 284nm$ in cacodylate buffer, pH 7.0, at ionic strength of solution $\mu = 1$. Concentration of each strand in the triplex studied was 7μM. Synthesis of decathymidylate-phenazine conjugate ($dT_{10}Ph$), methods and conditions of measurements as well as optical properties of the phenazine dye are earlier described in detail in [17,19,20]. Besides, melting curves were calculated for the dA_{19}-$2dT_{19}$ system studied in [6,21]. To get more reliable calculations, upon the selection of thermodynamic parameters, some experimental data were used, first of all, values of transition enthalpy ΔH_{32}.

In calculations ΔH_{32} equal to 4.9 кcal/mole obtained for polymeric molecules [22,23] can be used. As was mentioned above, great dispersion among the available literature data for ΔH_{32} is observed. The values obtained by ITC and DSC methods are considered as the most reliable ones. But in this case ΔH_{32} also has a great scattering of values ranged from 3.0 кcal/mole [24] to 4.1 кcal/mole [8]. These differences can be conditioned not only by various pH values and ionic conditions used, but by the effect of the buffer system applied in experiments [24]. Therefore the calculations were fulfilled with several ΔH_{32} values and corresponding $\Delta S'_{23}$ values derived from relation (10).

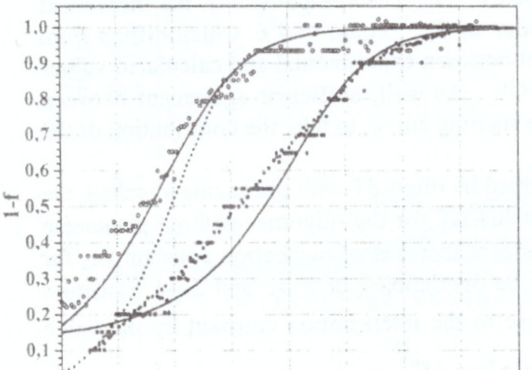

Fig.2. Melting 3→2 transitions in triplexes dA_{15}-$2dT_{10}$ (○), dA_{15}-$2dT_{10}Ph$ (●) - experimental values obtained at μ=1 and concentration of each strand 7μM. Calculations by "Staggering Zipper" model: solid lines – fractions of disordered bases in Hoogsteen strand; dotted line – fraction of completely separated strands.

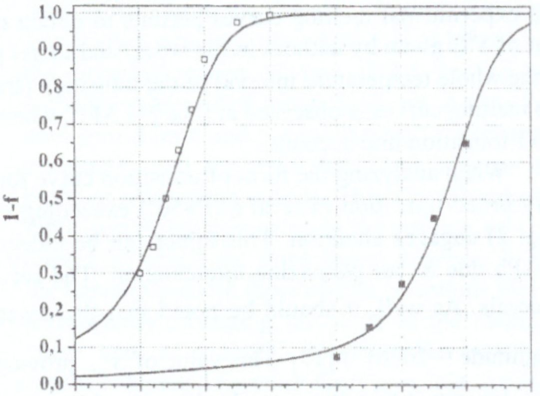

Fig.3. Melting 3→2 transitions in triplex dA_{19}-$2dT_{19}$. Symbols - experimental data from [21]: (□) - [Na$^+$] = 0.2 M, (■) - [Na$^+$] = 1 M. Solid lines - calculations by "Staggering Zipper" model. Values of calculation parameters are given in Table 1.

Fig. 2 presents experimental 3→2 transition curves obtained at $\mu=1$ for the triplex with oligo-dT_{10} and with conjugate $dT_{10}Ph$ in comparison with calculated curves. Thermodynamic parameters used in calculations are given in the Table 1. The table also shows experimental values of the transition temperature, T_m, and of the transition interval width, ΔT, for the systems studied as well as the values calculated by the model used. As the results given show, with the comparably good agreement between calculated and experimental values of T_m the widths of transition intervals differ significantly. The higher experimental values of ΔT may be due to the partial overlapping of temperature intervals of 3→2 and 2→1 transitions, that results in broadening of 3→2 transition.

The significantly better agreement between theoretical and experimental data is observed for the dA_{19} - $2dT_{19}$ system (Fig. 3) in which 3→2 transitions were measured by the method of UV absorption [21] in the Na^+ concentration range 0.2 - 1.0 M.

TABLE 1. Thermodynamic parameters used upon the approximation of 3 → 2 transition in triplexes $dA_n\cdot\ 2dT_m$ by Eq.(3) of "Staggering Zipper" model and experimental values of temperature parameters of the transitions.

	$[Na^+]$, M	T_m, °C		ΔT, °C		$\gamma_{23}\cdot 10^6$	$-\Delta H_{23}$, $\frac{kcal}{mol}$	$-(\Delta S_{13}-RC_1')$ $\frac{cal}{mol\cdot K}$
		Theor.	Exper.	Theor.	Exper.			
dA_{15}-$2dT_{10}$	0.1	~ -15	< 0	-	-			
	1	18	16.5±2	26	37±3	40	4.9	44.6
		19		34		4	4.0	41.8
		16		34		4	3.8	41.2
dA_{15}-$2dT_{10}Ph$	1	33.5	32±2	46	50±5	40	4.9	44.6
		36		40		4	3.8	41.2
dA_{19}-$2dT_{19}$	0.2	8	8 [21]	16	18±2	0.8	4.9	44.6
		4		17.5		4	4.0	41.8
	1	47	45 [21]	19	~23	8	4.9	44.6
		51		24.5		4	4.0	41.8

Upon the approximation the following constants were used:

$C_2 = 0.69$; $C_2' = 2.2$, $\Delta S_{12} - RC_1 = -30.7$ cal/mol·K.

The especially good agreement is observed at $[Na^+]=0.2$ M when 3→2 and 2→1 transitions are distinctly separated by temperature. In this case the 3→2 transition begins below 0^0C. The comparison of the theoretical and experimental melting curves permits to obtain more correct value of T_m [*)] equal to 4^0C which differs from that of 8^0C given by authors in the work, and to get good agreement between experimental and calculated values in the whole temperature interval of the transition (from 0^0C to $\sim 20^0C$). As well, sufficient agreement between the melting curves is observed at $[Na^+]=1$ M if, when calculating the melting curve, to take the contribution of the 2→1 transition into account.

When analyzing the form of transition curve for the triplex formed by oligo-dT with phenazine attached, the very broad transition interval ($\Delta T>50^0$) exceeding essentially the value ΔT for the oligomer without phenazine (Fig. 2) engages attention. This effect can be induced by the significant increase of nucleation constant β_{32} for $dT_{10}Ph$ due to the phenazine intercalation. The second cause may be overlapping of 3→2 and 2→1 transition intervals. As well, it should be noted that the constant s_{ph} is close to the intercalation constant by the value magnitude ($\sim 10^3\ M^{-1}$) [17]. This value of s_{ph} provides the T_m increase by $\sim 15^0$.

Important transition peculiarities may be revealed, when considering the data of population analysis. Figs 4 and 5 give distributions of triplex formations by lengths for some systems studied. Fractions of completely dissociated triplexes correspond to values n=0. The remaining complexes are characterized with the broad distribution of the ordered triplex structures by lengths. Thus, calculations for the systems considered reveal the significant "fraying" of ends (Fig. 1) increasing with temperature. This effect has an influence on the form of

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[*)] It is interesting to note that this value of the melting temperature T_m lies well onto the linear dependence of T_m on the log (Na^+ activity).

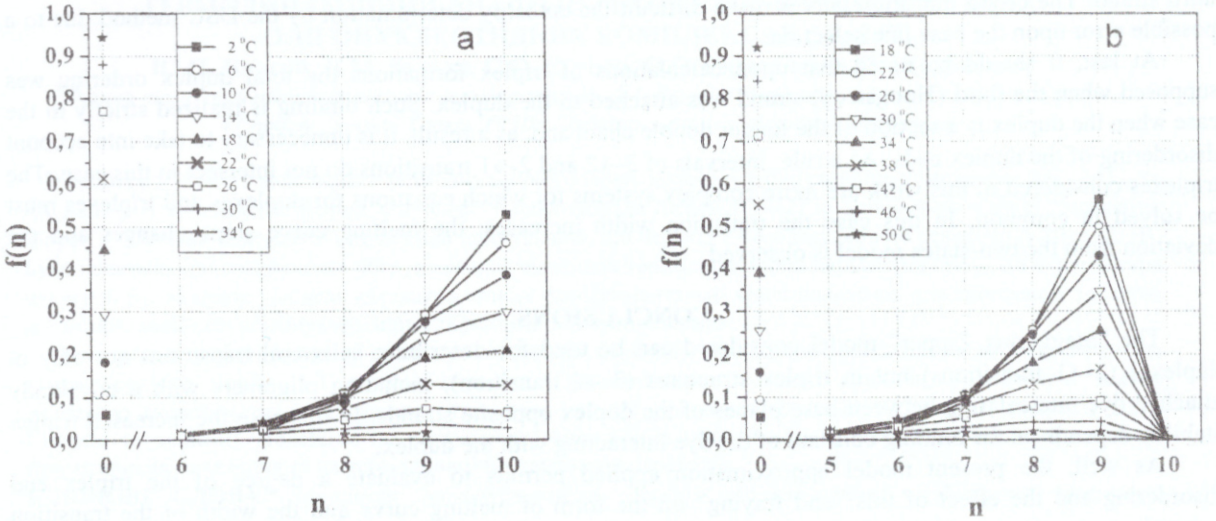

Fig4. Population analysis of ordered bases for triplex systems: (a) $dA_{15}-2dT_{10}$, (b) $dA_{15}-2dT_{10}Ph$, $\gamma = 8\cdot 10^{-7}$, $\mu=1$, $\Delta H_{32} = 4.9$ kcal/mole, $\Delta S_{31} = 44.6$ cal/mol· K.

melting curves, resulting in the formation of long low-temperature "tails", in the slope decrease and asymmetry increase of the curves. This effect is especially pronounced in the case of oligonucleotides with the dye attached.

The values calculated for thermodynamic characteristics and compared with experimental results permit to draw some conclusions on transition mechanisms in triplex structures. The main advantage of the "Staggering Zipper" model used is in the possibility to obtain thermodynamic parameters nondependent on temperature, strand lengths and ionic conditions that permits to predict the stability of duplex and triplex structures and to determine the character of conformational transitions between these structures. The results obtained in the present work clarify a number of controversial questions concerning effects of intermolecular interactions on the complex structure.

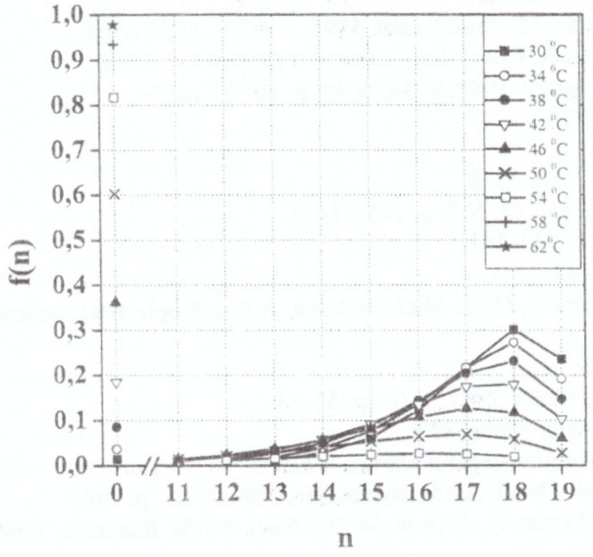

Fig.5. Population analysis of ordered bases for triplex systems $dA_{19}-2dT_{19}$ [21], $\mu = 1$, $\gamma = 8\cdot 10^{-7}$, $\Delta H_{32} = 4.9$ kcal/mole, $\Delta S_{31} = 44.6$ cal/mol· K.

One of the main characteristics of the triplex stability is the transition enthalpy $\Delta H_{32} = \Delta H_{31}-\Delta H_{21}$, determining a change in enthalpy upon the attachment of the third strand to the duplex. As was noted above, very great dispersion is observed between the literature data obtained by different methods (DSC, ITC, by van't Hoff - from the slope of transition curves). The triplex structure disordering may be one of the principal cause of low (2-2,5 kcal/mole) enthalpy values cited in the literature. As can be seen from Figs 4 and 5, "end fraying" contributes essentially into the total disordering degree of bases of pyrimidine strands. This effect becomes more increased due to ends disordering of the duplex to which the third strand attaches. With the temperature rise these effects contribute more significantly, changing the slope of the melting curve and, as a result, distorting the enthalpy results calculated by van't-Hoff principle. As can be seen from Figs 2 and 3 at low temperatures (in the beginning of the melting curve) "end fraying" makes a great contribution into the helix-coil transition that induces strong asymmetry of the curves, long low temperature "tails" appeared in these curves and strong deviation from the two-state model. As Fig. 2 shows, the slope of the calculated curve corresponding to the completely separated strands for dA_{15}-$2dT_{10}$ system differs from the experimental dependence by (30-40)%. This effect is even higher in triplexes stabilized by the dye attached to the third strand. The broad transition curves make difficult the enthalpy determination by the DSC method due to a possible error upon the base line selection.

At last, it should be noted that upon calculations of triplex formations the total duplex ordering was supposed when the third (Hoogsteen) strand was attached to the duplex. Such binding is realized strictly in the case when the duplex is a section of the longer double chain and, as a result, it is unnecessary to take into account disordering of the duplex ends. As a rule, intervals of 3→2 and 2→1 transitions do not intersect in this case. The triplexes considered in this work are more complex systems for which equations for duplexes and triplexes must be solved in common. In this case the transition width increases, the melting curve slope changes and the deviation from the two-states model is observed.

CONCLUSIONS

The "Staggering Zipper" model considered can be used for describing helix-coil transitions not only in duplexes (2→1 transitions) but in triplex structures (3→2 transition), including oligomers with a covalently attached dye intercalating between base planes of the duplex opposite strands. In this case the increased triplex stability depends on the binding constant of the dye interacting with the duplex.

As well, the present model approximation applied permits to evaluate a degree of the triplex end disordering and the effect of this "end fraying" on the form of melting curve and the width of the transition interval.

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ТЕРМОДИНАМІЧНИЙ АНАЛІЗ ПЕРЕХОДУ 3→2 В ТРИСПІРАЛЬНИХ ОЛІГОНУКЛЕОТИДНИХ КОМПЛЕКСАХ dA_n-$2dT_m$

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В роботі розглянуто можливість термодинамічного опису переходу в олігомерних комплексах dA_{15}-$2dT_{10}$ та dA_{19}-$2dT_{19}$ із триплексної форми в дуплекс + одна спіраль (перехід 3→2) за допомогою моделі "Staggering Zipper" ("Застібка-блискавка") та за умов припущення, що перехід є бімолекулярною реакцією $A{\cdot}2B \Leftrightarrow AB+B$. Було показано, що при фізично обгрунтованих значеннях термодинамічних параметрів (ΔH_{23}, ΔS_{23}, параметра нуклеації β_{23} та інших) модель адекватно описує експериментальні криві плавлення для триплексів з різною довжиною ланцюгів олігонуклеотидів і за різними іонними умовами.

Крім того, в роботі вивчено вплив на параметри переходу ковалентно приєднаного до dT_{10} барвника, інтеркалюючого поміж A·T парами дуплекса. Проаналізовано можливі причини, зумовлюючі значну дисперсію експериментальних даних, отриманих для ΔH_{32} різними методами (DSC, ITC, аналіз за ван'т Гоффом та іншими). Показано, що розупорядкування кінців триплексів і дуплексів має значний вплив на форму кривих плавлення та на термодинамічні параметри переходів.

КЛЮЧОВІ СЛОВА: триплекси олігонуклеотидів, перехід спіраль-клубок, стабілізація триплексів барвником, модель "Застібка-блискавка".