Thermodynamic parameters of coaxial stacking on stacking hybridization of oligodeoxyribonucleotides

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Continuous stacking hybridization of oligodeoxyribonucleotides with the stem of a preformed minihairpin structure of a DNA template was studied by thermal denaturation in solution. The thermodynamic parameters ($\Delta H°$, $\Delta S°$, and $\Delta G°_{37}$) were determined for the formation of all 16 possible types of coaxial stackings (or cooperative interactions) $^{5´}\mathbf{X}^{*}\mathrm{p}\mathbf{Y}^{3´}/^{5´}\mathbf{ZZ}^{´3´}$ (an asterisk stands for a nick) between the terminal complementary base pairs of two adjacent duplexes formed on a common DNA template. The maximum efficacy $\Delta G°_{37}$ of coaxial stacking (1 *M* NaCl, pH 7.4) was observed for the G*pC/GC interaction (−2.76 kcal mol^{-1}), whereas the minimum efficacy was observed for the T*pA/TA interaction (−0.85 kcal mol^{-1}). In the general case, the efficacy of the cooperative interaction at the X*pY/ZZ´ junction does not correlate with the energy of formation of the corresponding unified XY/ZZ´ dinucleotide pair in the structure of native DNA. The formation of a stack by the terminal oligonucleotide bases upon their continuous stacking hybridization makes the major and governing contribution to the energy of cooperative interaction.

Key words: coaxial stacking, cooperative interaction, continuous stacking hybridization, thermal stability, DNA duplexes.

Continuous stacking hybridization of oligonucleotides or the formation of tandem complexes of oligonucleotides underlies a number of biological processes and methodological approaches based on nucleic acid interactions. The formation of tandem oligonucleotide complexes is used for the enhancement of efficacy of reverse template hybridization in microchip technology,[1,2] employed in chemical modifications of NA within complementary complexes,[3—7] realized in the assembly of composite primers for sequencing,[8,9] and serves as the first step in ligation of oligonucleotides on a DNA template.[10,11]

To gain an insight into the factors responsible for the efficacy of continuous stacking hybridization of oligonucleotides and to mimic optimum tandem complexes, it is necessary to preliminiraly estimate their stabilities.

The formation of tandem oligonucleotide complexes involves their binding to the adjacent sites of a DNA template accompanied by the appearance of a cooperative interaction (or a coaxial stacking) between the terminal base pairs of the contiguous duplex regions that are formed, which results in their mutual stabilization.[12—16] Because of this, an account for the formation of tandem complexes, unlike the description of the formation of individual *oligonucleotide • template* complexes, requires a knowledge of not only the individual hybridization properties of oligonucleotides, which are determined by the entropy and enthalpy of their binding to the DNA template, but also the thermodynamic parameters of cooperative contact between the contiguous duplex regions of the oligonucleotides upon their continuous stacking hybridization.

Continuous stacking hybridization of oligoribonucleotides have been studied in most detail[17—19] because coaxial interactions between duplex regions play an important role in the organization of the RNA secondary structure. Systematic thermodynamic studies of this type of interactions in DNA complexes have not been carried out. The characteristic features of the formation of particular tandem oligonucleotide complexes and properties of coaxial stackings in these complexes were studied.[4,16,20—24] All types of cooperative interactions that occur upon hybridization of a short DNA template with decamers immobilized on a microchip in polyacrylamide gel (PAAG) in the presence of auxiliary pentamers were examined in a recent study.[25]

Earlier,[26] we have analyzed the conditions of the formation of tandem complexes, which allow one to determine the thermodynamic parameters for the process within the framework of a two-state model, and estimated the efficacy of a number of cooperative interactions in the hybridization of oligodeoxyribonucleotides.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 7, pp. 1057—1066, July, 2002.

1066-5285/02/5107-1145 $27.00

It was shown that the formation of three- and four-component tandem complexes can be correctly described with the use of the thermodynamic parameters determined for the coaxial stackings at the G*pC/GC, A*pT/AT, A*pC/GT, C*pT/AG, and A*pG/CT* interfaces.

In the present study, we determined the complete set of the thermodynamic parameters for stacking interactions between all terminal base pairs of the duplex regions in the same nucleotide environment which are formed upon continuous stacking hybridization of oligodeoxyribonucleotides, and considered some factors responsible for the efficacy of coaxial stacking.

Experimental

Oligodeoxyribonucleotides were synthesized by the standard phosphoramidite protocol on an ASM-700 synthesizer (Biosset, Russia). After deprotection, the oligonucleotides were isolated by successive ion-exchange and reversed-phase HPLC on columns packed with Polysil SA-500 (Teoreticheskaya praktika [Theoretical Practice], Russia) and LiChrosorb RP-18 (Merck), respectively. The homogeneity of the oligonucleotides was checked by electrophoresis in 20% denaturing PAAG with staining of the nucleotide by Stains-all (Sigma). The homogeneity of the oligomers used in the study was higher than 98%.

Concentrations of oligonucleotides in water were determined by spectrophotometry on a Shimadzu 2100 spectrophotometer. The molar absorption coefficients of the oligonucleotides at the wavelength of 260 nm were calculated according to a known procedure.[27]

Optical melting curves of the complexes prepared from stoichiometric mixtures of oligonucleotides in a buffer containing NaCl (1 mol L^{-1}), sodium phosphate (0.01 mol L^{-1}), and Na_2H_2edta (0.1 mmol L^{-1}), pH 7.4, were recorded simultaneously at several (no less than four) wavelengths in the range of 230—300 nm as described previously.[28,29]

Determination of the thermodynamic parameters for the complex formation was carried out by optimization within the framework of a two-state model taking into account the slopes of the base lines with the use of several (no less than two) total concentrations of the oligonucleotide chains.[30] The ΔH° and ΔS° parameters for the formation of simple duplexes of heptamers and complexes upon their continuous hybridization were determined with the use of the Simplex program by minimization of the rms deviations between the experimental and calculated melting curves. In the minimization procedure, six temperature-independent parameters were varied.[30] Each melting curve was optimized taking into account all experimental temperature-dependent optical densities recorded with a step of at least 10 points deg^{-1}. The ΔH° and ΔS° values that have been determined by optimization of individual curves recorded at different wavelengths were averaged; the mean deviations were no larger than ±5% and ±6%, respectively.

* Hereinafter, an asterisk signifies a nick.

Determination of the thermodynamic parameters by the concentration method was performed by linearization of the plot T_m^{-1} *vs* $\ln(C_T)$ in the 30—40-fold dilution range (2—80 μmol L^{-1}). The temperatures T_m were calculated from ΔH° and ΔS° determined by the optimization method. In these cases, the linear correlation parameter (R^2) was no lower than 0.99.

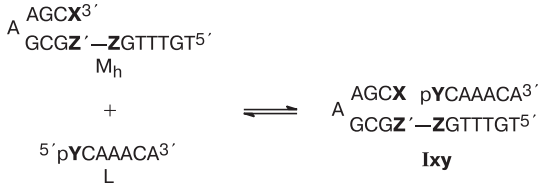
Determination of the overlapping area of the base surfaces in the dinucleotide step 5′-NN′-3′ was performed with the use of the HyperChem 6.03 program package (Hypercube Inc). The total surfaces accessible to solvent for both the individual bases (Σ_N) and vertical stacks of one chain within the classical B-form DNA double helix (Σ_{NN}) were calculated according to a known procedure[31] using a spherical particle with a radius of 1.4 Å, which corresponds to the average radius of the water molecule. The surface of the bases, which was excluded from the contact with solvent upon formation of the corresponding dinucleotide stack, was taken as the overlapping area of the base surfaces in the stack ($\Delta\Sigma_{NN}$). This parameter was calculated according to the equation

$$\Delta\Sigma_{NN} = (\Sigma_N + \Sigma'_N)/2 - \Sigma_{NN}.$$

Results and Discussion

Determination of the thermodynamic parameters of coaxial stackings

The thermodynamic parameters of coaxial stackings were studied by examining melting of complexes **Ixy** formed by the heptanucleotide p**Y**CAAACA (L) with the complementary single-stranded region TGTTTGZ of a 16-mer DNA template (M_h). The template M_h contains the single-stranded region along with the sequence **Z**′GCGAAGC**X**[32] capable of forming a very stable minihairpin with T_m above 75 °C (see, for example, Fig. 1, *a*). Hybridization of the heptanucleotide with the single-stranded region of this template results in the formation of a X*pY/ZZ′ cooperative contact at the interface of two duplex regions:



X, Y = A, T, G, C,
Z, Z′ are the corresponding complementary nucleotides.

Previously,[26] we have demonstrated that if the hairpin structure of the template is retained over the entire temperature range of melting of intermolecular complex **Ixy**, the formation of the tandem heptanucleotide complex can be described within the framework of a two-

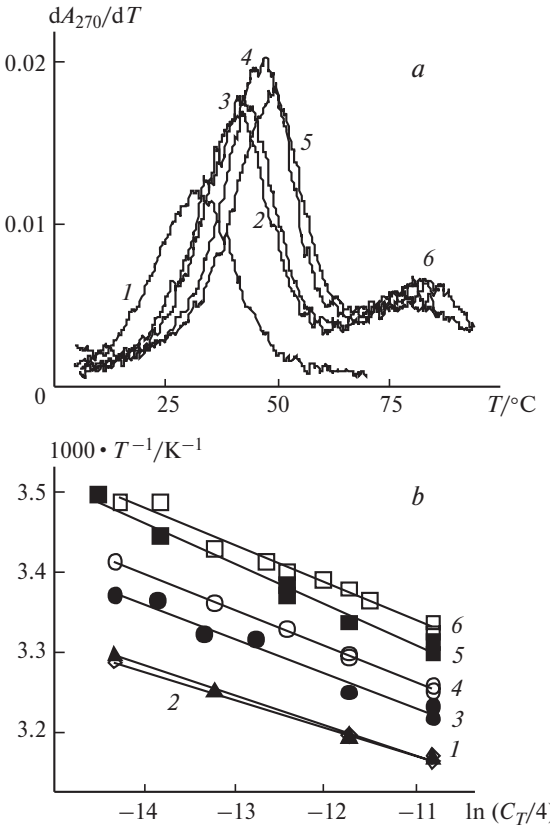


Fig. 1. Thermal denaturation of heptanucleotide complexes **1y** and **Ixy**. *a*. The differential melting curves for complexes **1c** (*1*), **Icc** (*2*), **Itc** (*3*), **Iac** (*4*), and **Igc** (*5*); *6*, the melting region of the hairpin structures of the templates M_h (C_T = 2 μmol L^{-1}). *b*. The plot of the reciprocal melting points of complexes **Iaa** (*1*), **Itc** (*2*), **1g** (*3*), **1c** (*4*), **1a** (*5*), and **1t** (*6*) *vs*. the total concentration of the components. (The melting conditions are given in the Experimental section.)

state model. The degree of association (α) of intermolecular complex **Ixy** may be represented as follows:

$$M_h + L \overset{K^{eff}}{\rightleftharpoons} \mathbf{Ixy},$$

$$\alpha = 1 + \frac{1}{K^{eff}C_T} - \sqrt{\left(1 + \frac{1}{K^{eff}C_T}\right)^2 - 1}, \qquad (1)$$

where C_T is the total concentration of the oligonucleotides in the stoichiometric mixture, $K^{eff} = K_{12}K$, K is the constant of the formation of the individual heptanucleotide complex, and K_{12} is the constant of formation of the stacking interaction between the terminal X*pY/ZZ′ base pairs of the heptanucleotide duplex and the hairpin stem.

The effective constant of continuous hybridization of the heptanucleotide to the hairpin stem of the template (K^{eff}) is a product of the constant of formation of a simple *heptanucleotide • template* duplex region within tandem complex **Ixy** and the constant of the cooperative interaction occurring in the nick (K and K_{12}, respectively). In this case, the free energy of formation of the tandem heptanucleotide complex **Ixy** ($\Delta G^\circ_{\mathbf{Ixy}}$) includes the energy components ΔG° and ΔG°_{12} of the corresponding processes:

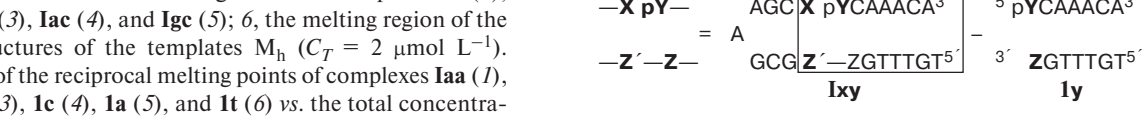
$$-RT\ln(K_{12}K) = \Delta G^\circ_{\mathbf{Ixy}} = \Delta G^\circ + \Delta G^\circ_{12} =$$
$$= (\Delta H^\circ + \Delta H^\circ_{12}) + T(\Delta S^\circ + \Delta S^\circ_{12}) =$$
$$= \Delta H^\circ_{\mathbf{Ixy}} + T\Delta S^\circ_{\mathbf{Ixy}}. \qquad (2)$$

Taking the energy of formation of control blunt heptanucleotide complex **1y** (in the absence of a hairpin structure) as the free energy of formation of the individual heptanucleotide duplex region (ΔG°) within tandem complex **Ixy**, one can determine the thermodynamic parameters characterizing the efficacy of the cooperative interaction at the X*pY/ZZ′ interface as follows:

$$\Delta G^\circ_{12} = \Delta G^\circ_{\mathbf{Ixy}} - \Delta G^\circ_{\mathbf{1y}},$$

$$\Delta H^\circ_{12} = \Delta H^\circ_{\mathbf{Ixy}} - \Delta H^\circ_{\mathbf{1y}},$$

$$\Delta S^\circ_{12} = \Delta S^\circ_{\mathbf{Ixy}} - \Delta S^\circ_{\mathbf{1y}}, \qquad (2a)$$



The thermodynamic parameters for the formation of complexes **Ixy** ($\Delta H^\circ_{\mathbf{Ixy}}$ and $\Delta S^\circ_{\mathbf{Ixy}}$) and **1y** ($\Delta H^\circ_{\mathbf{1y}}$ and $\Delta S^\circ_{\mathbf{1y}}$) were determined by optimization of the experimental melting curves of the corresponding complexes within the framework of a two-state model and (in some cases) additionally by the concentration method using linearization of the plot of the reciprocal melting point of the duplex *vs*. the total concentration of the oligonucleotides (T_m^{-1} *vs* $\ln(C_T/4)$) according to the equation:[30]

$$\frac{1}{T_m} = \frac{R\ln(C_T/4)}{\Delta H^\circ} + \frac{\Delta S^\circ}{\Delta H^\circ}. \qquad (3)$$

In all cases, the optical density in the course of thermal denaturation was recorded simultaneously at several (no less than four) wavelengths in the range from 230 to 300 nm.[28,29] The six-parameter optimization, which took into account the temperature dependence of the base lines, was carried out for the individual melting curves of the complexes, which were recorded at several wavelengths, and also for the curve obtained by averaging all experimental optical densities. For each complex, the thermodynamic parameters (ΔH° and ΔS°) thus deter-

mined are virtually identical. In all cases, the standard deviations of the melting points calculated with the use of these parameters were at most 0.3 °C. The averaged ΔH° and ΔS° values determined by the optimization method are, in turn, in good agreement with the values determined by the concentration method from the plot $1/T_m$ *vs* $\ln(C_T/4)$ (the deviations are no larger than 6%).

The differential melting curves for complexes **Ixc** forming the X*pC/GZ interfaces and the control complex of heptanucleotide **1c** with the 5′-terminal C/G pair are shown in Fig. 1, *a*. It can be seen that continuous hybridization of the heptanucleotide p**C**CAAACA to the preformed hairpin stem leads to an increase in the melting point of its duplex from 30.1 to 38.5—46.7 °C depending on the type of the cooperative contact formed. The linear dependence of the reciprocal melting points of heptanucleotide complexes **1y**, **Iaa**, and **Itc** on the concentration is indicative of the applicability of the two-state model to the description of the duplexes under consideration (Fig. 1, *b*).

The thermodynamic parameters for the formation of both the control heptamer complexes (**1y**) and heptamer complexes formed upon continuous hybridization (**Ixy**) as well as the parameters of the cooperative interactions formed at the X*pY/ZZ′ interfaces calculated according to Eq. (2a) are listed in Table 1.

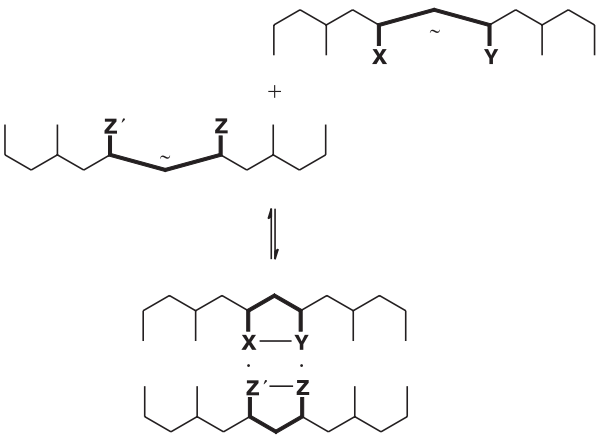
The ΔG°_{12} values for the cooperative interactions at the X*pY/ZZ′ interfaces occurring in complexes **Ixy** increase from −0.85 (T*A/TA) to −2.76 kcal·mol^{-1} (G*C/GC) on going from the 5′-pyrimidine*purine-3′ interfaces in the nick to the 5′-purine*pyrimidine-3′ interfaces.

The energy contributions of the coaxial stacking ΔG°_{12} for the X*pY/ZZ′ interactions determined in the present study are in good agreement with a number of parameters estimated by us previously[26] under analogous conditions and with those published in the literature[22] (−1.90 kcal·mol^{-1} for the AG*pGA/TCCT interface). However, these characteristics differ from those for the interactions of the Xp*Y/ZZ′ type evaluated for the heterophase conditions.[25] This difference may be due to both the characteristic features of hybridization of oligonucleotides in gel and related procedures for the determination of the experimental values[25,33] and the difference in the structure of the contact (5′- or 3′-phosphate in the nick) and of the nearest neighbors contiguous to the terminal stacking bases (CX*pYC or AXp*YG). Interestingly, the most substantial differences are observed for the G*pX and Gp*X interfaces.

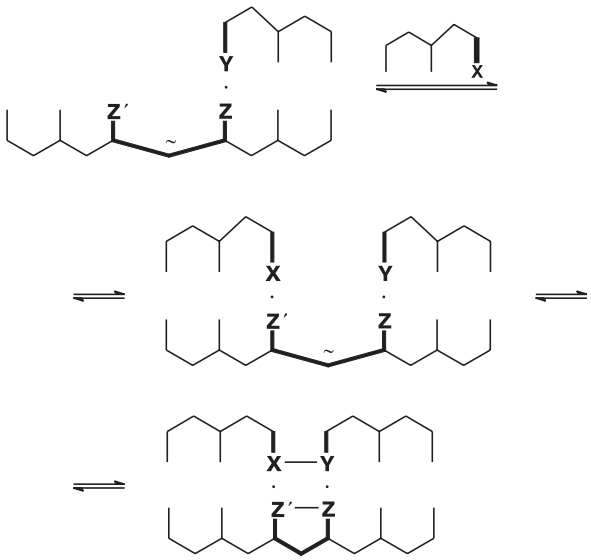
Formally, a cooperative interaction at the interface of two duplex regions of a tandem complex may be represented as a dinucleotide pair with a nick. Hence, the characteristic features of the formation of tandem complexes can be revealed by comparing the free energy of the intraduplex dinucleotide pair with that of the coaxial stacking. The differences may be associated with different types of major interactions, which are reflected in their thermodynamic parameters, and also may result from the difference in the energy levels of the formal initial states of the components involved in the formation of the continuous dinucleotide pair or that containing a nick (Scheme 1).

Scheme 1

Formation of a continuous dinucleotide pair



Formation of a dinucleotide pair with a nick



The stability of a complementary XY/ZZ′ dinucleotide pair is determined primarily by the number of hydrogen bonds between the X/Z′ and Y/Z base pairs.[34] The attendant formation of vertical stacks (along the axis of the helix) of two adjacent bases belonging to one chain, *viz.*, XY and ZZ′, takes place due to reorganization of their initial single-stranded state.

Table 1. Thermodynamic parameters (ΔS°/kal (mol K)$^{-1}$, ΔH°/kcal mol^{-1}, and ΔG°/kcal mol^{-1}) of the complementary heptanucleotide complexes with the heptameric DNA template and of those formed upon continuous hybridization on the M_h template (**1y** and **Ixy**, respectively), the thermodynamic characteristics (ΔS°_{12}, ΔH°_{12}, and ΔG°_{12}) of the corresponding cooperative interactions X*pY/ZZ′, and the overlapping areas ($\Delta\Sigma_{XY}$) of the bases in the XY stacks

	Complex	Type of interaction X*pY/ZZ′	ΔS°	ΔH°	ΔG° (37 °C)	ΔS°_{12}	ΔH°_{12}	ΔG°_{12} (37 °C)	$\Delta\Sigma_{XY}$
1t	5′pTCAAACA AGTTTGT		−120.7	−42.7	−5.3				
Igt	AAGCG pTCAAACA3′ GCGC−AGTTTGT	G*pT/AC	−150.4	−54.1	−7.4	−30.1	−11.4	−2.10	83.3
Iat	AAGCA pTCAAACA3′ GCGT−AGTTTGT	A*pT/AT	−149.7	−53.6	−7.1	−29.0	−10.9	−1.89	79.7
Ict	AAGCC pTCAAACA3′ GCGG−AGTTTGT	C*pT/AG	−148.6	−52.5	−6.4	−27.9	−9.8	−1.19	71.4
Itt	AAGCT pTCAAACA3′ GCGA−AGTTTGT	T*pT/AA	−146.7	−52.0	−6.5	−26.0	−9.3	−1.22	76.2
1g	5′pGCAAACA CGTTTGT		−126.5	−45.9	−6.7				
Igg	AAGCG pGCAAACA3′ GCGC−CGTTTGT	G*pG/CC	−147.9	−54.5	−8.7	−21.4	−8.6	−1.96	87.4
Iag	AAGCA pGCAAACA3′ GCGT−CGTTTGT	A*pG/CT	−141.7	−52.0	−8.0	−15.3	−6.1	−1.35	82.3
Icg	AAGCC pGCAAACA3′ GCGG−CGTTTGT	C*pG/CG	−141.3	−51.7	−7.9	−14.9	−5.8	−1.17	68.9
Itg	AAGCT pGCAAACA3′ GCGA−CGTTTGT	T*pG/CA	−160.3	−57.6	−7.9	−33.8	−11.7	−1.20	71.0
1c	5′pCCAAACA GGTTTGT		−123.4	−44.5	−6.2				
Igc	AAGCG pCCAAACA3′ GCGC−GGTTTGT	G*pC/GC	−159.0	−58.3	−9.0	−35.6	−13.8	−2.76	81.7
Iac	AAGCA pCCAAACA3′ GCGT−GGTTTGT	A*pC/GT	−159.1	−57.8	−8.5	−35.6	−13.3	−2.29	78.4
Itc	AAGCT pCCAAACA3′ GCGA−GGTTTGT	T*pC/GA	−148.8	−53.8	−7.7	−25.4	−9.3	−1.46	74.1
Icc	AAGCC pCCAAACA3′ GCGG−GGTTTGT	C*pC/GG	−144.0	−52.1	−7.5	−20.6	−7.6	−1.25	70.8
1a	5′pACAAACA TGTTTGT		−113.3	−40.8	−5.6				
Iga	AAGCG pACAAACA3′ GCGG−TGTTTGT	G*pA/TC	−158.9	−57.1	−7.9	−45.6	−16.3	−2.21	83.1
Iaa	AAGCA pACAAACA3′ GCGT−TGTTTGT	A*pA/TT	−161.6	−57.7	−7.6	−48.3	−16.9	−1.93	79.6
Ica	AAGCC pACAAACA3′ GCGG−TGTTTGT	C*pA/TG	−145.0	−51.7	−6.7	−31.7	−10.9	−1.06	67.3
Ita	AAGCT pACAAACA3′ GCGA−TGTTTGT	T*pA/TA	−141.5	−50.4	−6.5	−28.2	−9.6	−0.85	69.8

In the case of X*pY/ZZ′ contact formation in the nick, complementary interactions of the bases in the X/Z′ and Y/Z pairs are unlikely to play a substantial role because they are formed (and are taken into account) upon the formation of individual oligonucleotide complexes serving as the initial state. In our opinion, the interplanar interactions must be of much greater importance because the formation of a tandem complex gives

rise to an essentially different X*pY stack between the terminal bases of two different, initially remote oligonucleotides. As in continuous dinucleotide pairs, the ZZ´ base stacking interaction in the template chain occurs against the background of the preorganized structure.

The only energies of formation of continuous intraduplex pairs estimated so far are those determined by statistical processing of the experimental thermodynamic parameters for the formation of oligonucleotide complexes within the framework of a nearest-neighbor model.[35,36] These parameters enable one to adequately describe the formation of oligonucleotide complexes.[36]

However, in spite of the averaged formalized values, the parameters ΔG°_{NN} for dinucleotide pairs appeared to be proportional to the number of hydrogen bonds between the complementary bases in these pairs (Fig. 2) ($R^2 = 0.89$). This suggests that the parameters ΔG°_{NN} reflect to a certain extent the physical sense and the essence of the interactions responsible for the formation of the XY/ZZ´ dinucleotide pairs. Therefore, we compared these parameters with ΔG°_{12} for the corresponding cooperative interactions at the X*pY/ZZ´ interface estimated in the present study.

The efficacy of the cooperative interaction ΔG°_{12} at the X*pY/ZZ´ interface is independent of the number of hydrogen bonds in the X/Z´ and Y/Z base pairs (Fig. 2) ($R^2 = 0.04$). Therefore, it is not surprising that there is no correlation between ΔG°_{12} and ΔG°_{NN} ($R^2 = 0.10$).

The ΔG°_{12} and ΔG°_{NN} values are of the same order of magnitude ($-2.76 \le \Delta G^\circ_{12} \le -0.85$ kcal mol^{-1} and $-2.23 \le \Delta G^\circ_{NN} \le -0.59$ kcal mol^{-1}). The averaged contribution of the coaxial stacking in the nick is higher than the averaged energy contribution of the unified internal pairs by -0.21 kcal mol^{-1}.

It is essential that a nick disturbs the symmetry of the energy contributions of the dinucleotide steps in the double helix. If non-self-complementary continuous dinucleotide pairs (for example, AC/GT and GT/AC) are

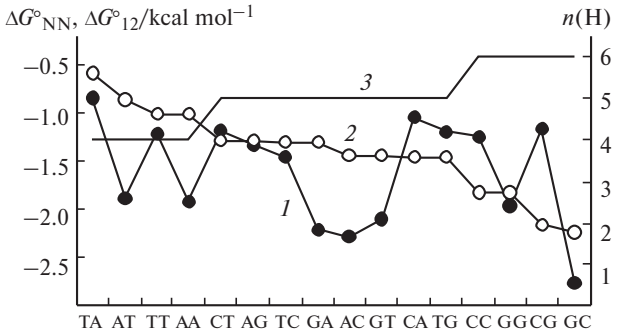


Fig. 2. Correlations of the energy contributions of cooperative interactions at the X*pY/ZZ´ interfaces (ΔG°_{12}) (*1*) and of the continuous unified XY/ZZ´ dinucleotide pair (ΔG°_{NN})[36] (*2*) with the total number of hydrogen bonds n(H) (*3*) between the corresponding complementary bases.

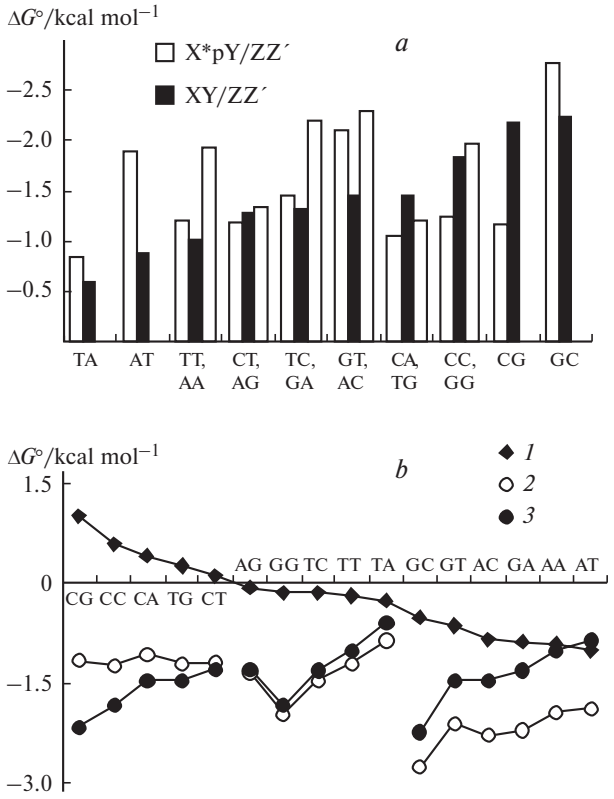


Fig. 3. Energy contributions ΔG° of the unified XY/ZZ´ dinucleotide pair [36] and the cooperative interactions at the corresponding X*pY/ZZ´ interfaces (*a*) and the differences between these contributions ($\Delta\Delta G^\circ$) (*b*): *1*, $\Delta\Delta G^\circ$; *2*, ΔG°X*pY/ZZ´; *3*, ΔG°XY/ZZ´.

energetically equivalent, and pairs with identical compositions (for example, AC/GT with CA/TG) have similar ΔG°_{NN} values, the coaxial stackings differ substantially in efficacy in the presence of a nick in these pairs (Fig. 3, *a*).

The difference between ΔG°_{NN} and ΔG°_{12} varies from 1.00 (C*pG/CG) to -1.02 kcal mol^{-1} (A*pT/AT). The T*pG/CA and C*pY/ZA interactions ($0.09 \le \Delta\Delta G^\circ \le 1.00$ kcal mol^{-1}) are less efficient as compared to the corresponding continuous dinucleotide pair. The energy contributions ΔG°_{12} of the contacts containing purine at the 5´-terminus and any base, except for G, at the 3´-terminus (Pu*pA/TPy, Pu*pT/APy, Pu*pC/GPy) are substantially larger than ΔG°_{NN} for the corresponding pairs ($-1.02 \le \Delta\Delta G^\circ \le -0.53$ kcal mol^{-1}).

There is no correlation between the energy contributions ΔG°_{12} of the X*pY/ZZ´ interactions and the number of hydrogen bonds formed by the X/Z´ and Y/Z pairs, which suggests that the horizontal interactions, even though they may contribute to the energy of the cooperative interaction, are different in nature from that of the direct hydrogen bonding, which is typical of the formation of complementary pairs.

According to the NMR spectroscopic data, the terminal base pairs, which are brought together in a nick upon the formation of a tandem complex, are less accessible to the destabilizing effect of water as compared to the terminal bases of individual duplexes.[37,38] Therefore, possible additional effects of horizontal interactions that appear at the interfaces of the duplex regions in the formation of the cooperative interaction result from the displacement of water molecules, which are localized in the hydrogen-bonding region of the terminal complementary pairs of the individual duplexes, once these pairs are brought into proximity due to formation of a tandem complex. In other words, the contribution of horizontal interactions to the efficacy of the cooperative interaction may be associated with the shift of the dynamic equilibrium from the partially untwisted state of these terminal oligonucleotide pairs stacked in the nick in the individual duplex regions to a more structured state in the tandem complex.[38]

Within the framework of the nearest-neighbor model, destabilization of the terminal pairs of the duplexes exposed to a medium is described by terminal corrections.[36] Previously, by formally comparing the cooperative interactions with the parameters of the dinucleotide steps, we have demonstrated[26] that these differences cannot be larger than the total effect of rejection of the terminal corrections for each pair involved in the formation of coaxial stacking.

The formation of base stacks or the stacking interaction as such is the second major type of interactions responsible for the efficacy of the cooperative interaction. The interplanar π—π interactions giving rise to the formation of nitrogen-base stacks involve Coulomb interactions and the dispersion, hydrophobic, and solvation effects and are determined to a large extent by the degree of overlap of the interacting base surfaces.[31,39,40]

When analyzing the contributions of the vertical interactions to the efficacy of cooperative interactions at the X*pY/ZZ′ interfaces, we assumed that the structure of the intraduplex continuous XY/ZZ′ dinucleotide pair is identical with that of the X*pY/ZZ′ pair containing a nick because the results of X-ray diffraction study[41] and NMR spectroscopy[38,42,43] showed that these pairs are structurally similar. Besides, we ignored interchain interactions in the duplex structure assuming that these interactions are the same in both the continuous dinucleotide pair and the pair containing a nick. We also did not take into account the possible effect of the nearest neighbors because all the interfaces under study have a similar environment.

When the cooperative interaction occurs at the X*pY/ZZ′ interface, the stacking interaction between the terminal bases of different oligonucleotides X and Y must make a much more substantial energy contribution. Hence, we initially compared the correlation between the determined efficacy ΔG°_{12} and the overlapping area $\Delta\Sigma_{XY}$ of the corresponding bases in the nick. The overlapping area of the bases in the stacks of one chain inherent in the canonical B-form DNA helix was calculated according to a known procedure[31] (Table 1; $\Delta\Sigma_{XY}$ and $\Delta\Sigma_{ZZ'}$).

As can be seen from Fig. 4, *a*, an increase in efficacy of the cooperative interaction is actually directly proportional to an increase in the overlapping area of the stack formed by the terminal bases of the oligonucleotides

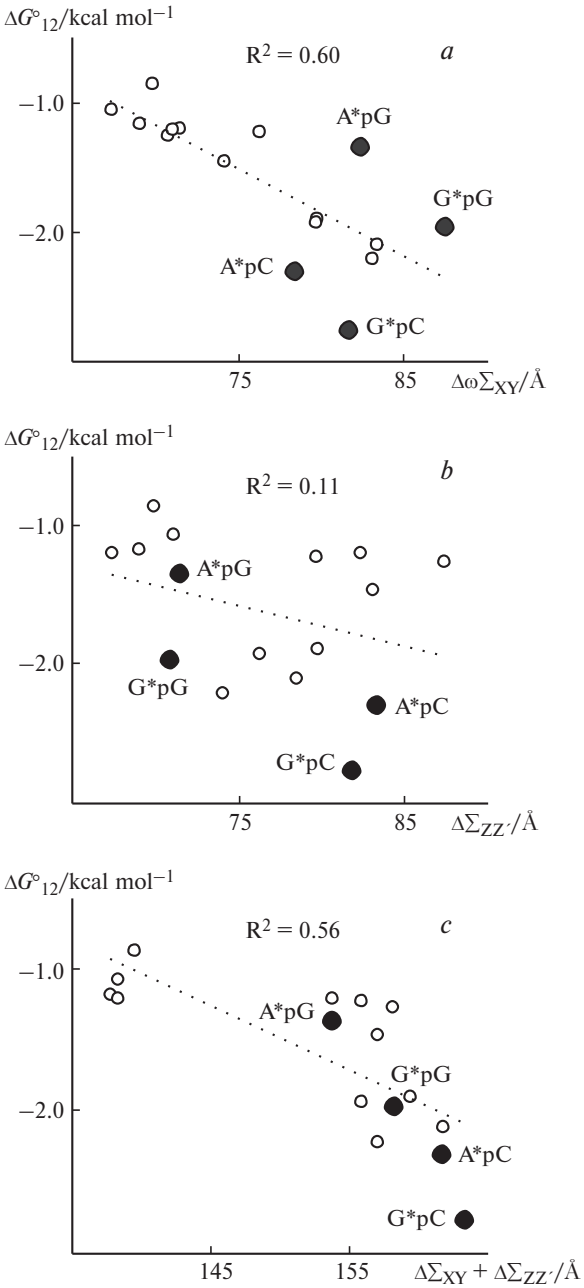


Fig. 4. Plots of the energy contribution ΔG°_{12} of the cooperative interaction X*pY/ZZ′ *vs.* the overlapping area of the bases in the nick (*a*), in the template chain (*b*), and their sum (*c*).

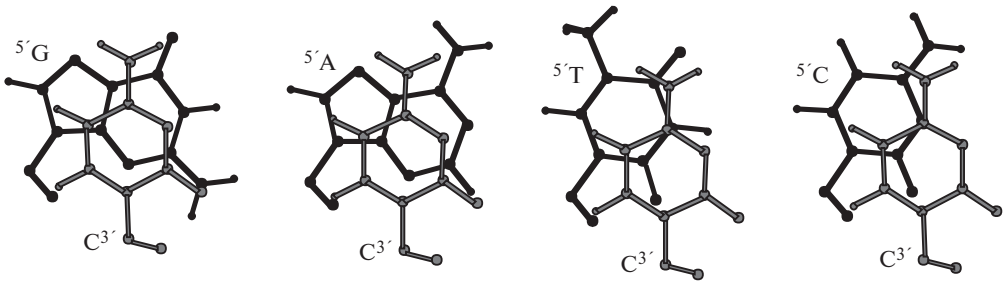


Fig. 5. Projections of the base stacks of one chain in the classical double helix of the B-form DNA along the 3′C→X5′ direction.

adjacent to the template. The good general correlation between ΔG°_{12} and the corresponding $\Delta\Sigma_{XY}$ values is violated by four cooperative interactions. Thus the A*pG/CT and G*pG/CC interactions are much weaker, whereas the A*pC/GT and G*pC/GC interactions are, on the contrary, stronger than those expected taking into account the overlap of the bases in the corresponding AG, GG, AC, and GC stacks. At the same time, there is no correlation between ΔG°_{12} of the cooperative interaction and the overlapping area of the base stack $\Delta\Sigma_{ZZ'}$ in the template chain (Fig. 4, *b*).

As can be judged from the $\Delta\Sigma_{XY}$ values (see Table 1), the overlapping area in the stack is determined primarily by the 5′-terminal base (for example, in the series of base stacks GC > AC > TC > CC, Fig. 5). Hence, we examined the dependence of stability of the interaction on the overlap efficacy of the bases in the stack containing a nick for groups with the same 3′-terminal base.

In virtually all groups (X*pG/CZ′, X*pA/TZ′, X*pT/AZ′, X*pC/GZ′), the efficacy of the cooperative interactions ΔG°_{12} is increased as 5′-X changes in the series C < T < A < G (Fig. 6, *a*) and correlates well with the corresponding overlapping areas of the bases $\Delta\Sigma_{XY}$ (R^2 = 0.76, 93, 85, and 97, respectively). In addition, the ΔG°_{12} parameters of the cooperative interactions in the groups are described by strict linear dependences (Fig. 6, *b*).

By contrast, in the groups of interactions with the same 5′-terminal base (C*pY/ZG, T*pY/ZA, A*pY/ZT, G*pY/ZC) (Fig. 7, *a*), an increase in the efficacy of interactions ΔG°_{12} in parallel with an increase in the overlapping area of the corresponding bases $\Delta\Sigma_{XY}$ is observed only for the C*pY/ZG and T*pY/ZA interfaces in which the coaxial stacking strengthens in the series:

Py*pA < Py*pG < Py*pT < Py*pC.

On the contrary, an inverse dependence is observed for the A*pY/ZT and G*pY/ZC interactions in each group, *viz.*, the cooperative interaction strengthens as $\Delta\Sigma_{XY}$ decreases in the series:

Pu*pG < Pu*pT < Pu*pA < Py*pC.

Nevertheless, the parameters ΔG°_{12} for the interactions in the A*pY/ZT and G*pY/ZC groups and also in the C*pY/ZG and T*pY/ZA groups are linearly dependent and correlate well with each other (Fig. 7, *b*).

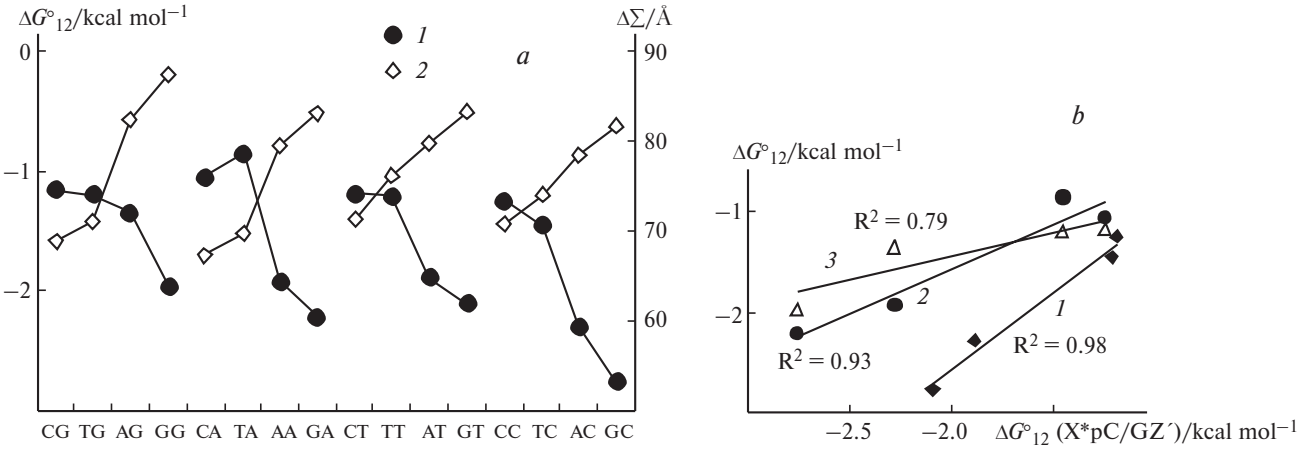


Fig. 6. *a*. Changes in the efficacy of coaxial stacking (ΔG°_{12}) (*1*) and the overlapping areas of the corresponding bases in the stacks ($\Delta\Sigma_{XY}$) (*2*) in the series of the X*pG/CZ′, X*pA/TZ′, X*pT/AZ′, and X*pC/GZ′ interactions. *b*. The plot of ΔG°_{12} for the X*pT/AZ′ (*1*), X*pA/TZ′ (*2*), and X*pG/CZ′ (*3*) interactions *vs.* ΔG°_{12} for the corresponding X*pC/GZ′ interactions.

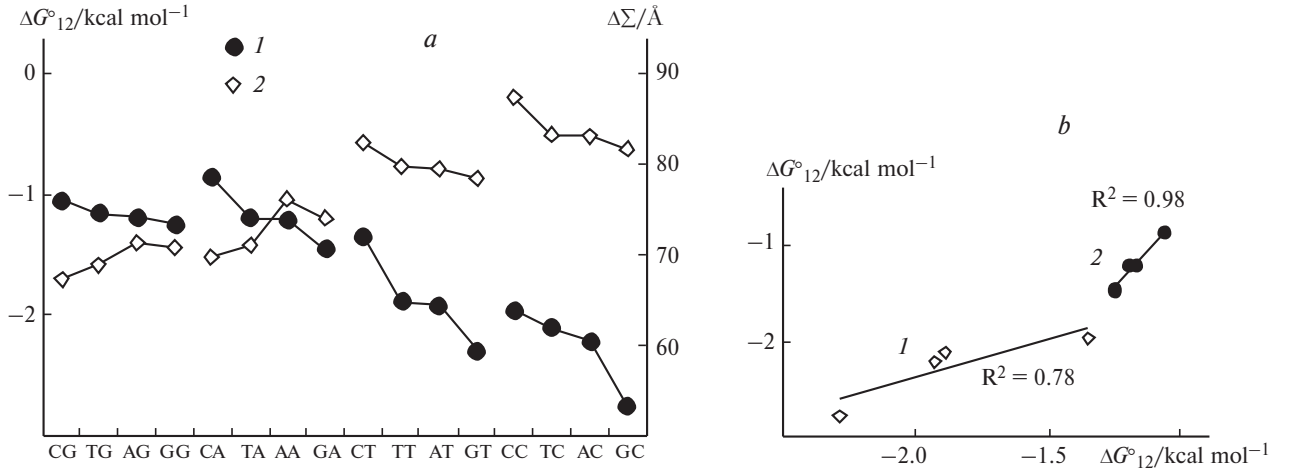


Fig. 7. *a*. Changes in the efficacy of coaxial stacking ($\Delta G°_{12}$) (*1*) and the overlapping areas of the corresponding bases in the stacks ($\Delta\Sigma_{XY}$) (*2*) in the series of the C*pY/ZG, T*pY/ZA, A*pY/ZT, and G*pY/ZC interactions. *b*. The plot of $\Delta G°_{12}$ for the G*pY/ZC (*1*) and T*pY/ZA (*2*) interactions *vs.* $\Delta G°_{12}$ for the A*pY/ZT and C*pY/ZG interactions, respectively.

In spite of the absence of a correlation between $\Delta G°_{12}$ and $\Delta\Sigma_{ZZ'}$, the interplanar interactions between the bases in the template continuous chain ZZ´ do make a contribution to the total energy of coaxial stacking as evidenced by violation of symmetry of the realtionship between the energy contribution of the cooperative interaction ($\Delta G°_{12}$) at the X*pY/ZZ´ interfaces and the overlapping area of the stacks of the broken chain $\Delta\Sigma_{XY}$ in the 5´→3´ and 3´→5´ directions.

When the overlapping area of the surfaces of both stacks is taken into account, the correlation between $\Delta G°_{12}$ and $\Delta\Sigma_{XY} + \Delta\Sigma_{ZZ'}$ in the series with different directions becomes virtually symmetrical. In both groups of interactions, $\Delta G°_{12}$ increases in parallel with $\Delta\Sigma_{XY} + \Delta\Sigma_{ZZ'}$ (Fig. 8).

The significant dependence of $\Delta G°_{12}$ on $\Delta\Sigma_{XY}$ and the correlation between $\Delta G°_{12}$ and $\Delta\Sigma_{XY} + \Delta\Sigma_{ZZ'}$ in the groups of interactions (linear dependence in the common series is characterized by $R^2 = 0.60$ and 0.56, respectively, Fig. 4, *c*) indicate that the efficacy of cooperative interactions is determined primarily by the occurrence of stacking interactions between bases of both chains, *i.e.*, between the terminal bases of the adjacent duplex regions. However, in this case the formation of stacks in the template is of lesser importance because the energy of coaxial stacking $\Delta G°_{12}$, though demonstrating the correlation in the common series with the overlapping areas of the stacks in the newly formed chain $\Delta\Sigma_{XY}$, is independent of the overlapping surface of the stacks in the template chain $\Delta\Sigma_{ZZ'}$.

Evaluation of the contribution of the stack formation in the template chain demonstrates that the best correlation between the efficacy of the X*pY/ZZ´ coaxial stacking and the total overlapping surface of the bases is achieved where as much as 40 to 60% of the overlapping area of the bases in the template chain is taken into account.

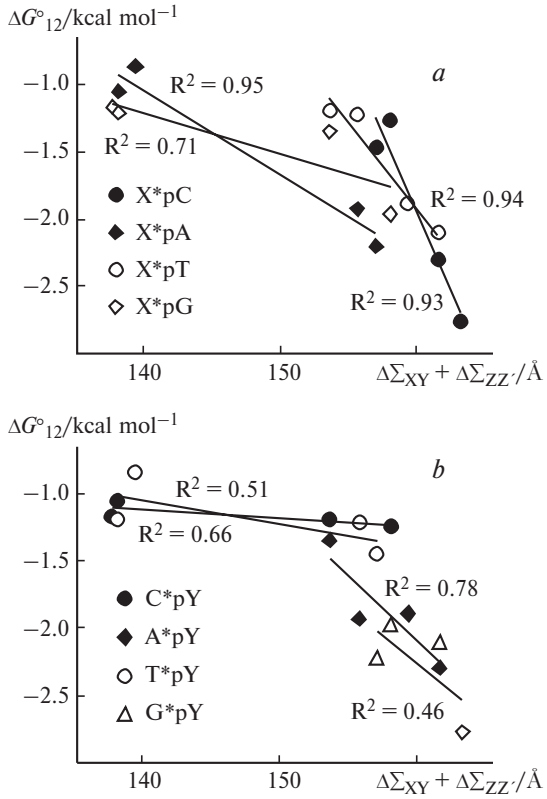


Fig. 8. Plot of the efficacy of cooperative interactions in the groups determined by the 3´-terminal base in the nick (X*pT/AZ´, X*pC/GZ´, X*pG/CZ´, and X*pA/TZ´) (*a*) and in those determined by the 5´-terminal base in the nick (T*pY/ZA, C*pY/ZG, G*pY/ZC, and A*pY/ZT) (*b*) *vs.* the total overlapping areas of the corresponding base stacks.

The underestimated contribution of the vertical interactions between the ZZ´ bases of the template chain to the energy of coaxial stacking is attributed to the fact that, as suggested above, the ZZ´ stack is not the newly formed one but appears due to reorganization of the initial mutual arrangement of these bases, *viz.*, 5´-Z in the single-stranded region of the template and 3´-Z´ in the double-stranded region of the template.

At first glance, the most evident initial structural organization of the bases in the template upon continuous hybridization of oligonucleotides involves dangling of the nucleotide over the duplex region. A comparison of the efficacy of stacking hybridization of a short oligoribonucleotide to 3´- and 5´-single-stranded regions of the preformed hairpin structure[19] led to the suggestion that the 3´-dangling, which exerts a more substantial stabilizing effect on riboduplexes, may have an adverse effect on the formation of coaxial stacking. When studying the continuous hybridization of oligodeoxyribonucleotides for the cooperative interaction at the A*pT/AT interface, we have demonstrated that different dangling types have virtually no effect on its efficacy.[26] The contributions of all possible oligonucleotide danglings to the free energy of ordinary hybridization of oligodeoxyribonucleotides were reported in the study.[44] If dangling as preorganization of the template structure plays a significant role in the formation of tandem oligonucleotide complexes, the efficacy of the cooperative interaction would be expected to depend on the type of the first dangling base in the single-stranded region of the template. However, a comparison of the complete set of ΔG° for the 5´-single-nucleotide danglings **Z**Z´/X with the energy contributions ΔG°_{12} of the coaxial stackings at the corresponding X*pY/ZZ´ interfaces revealed no correlation between these parameters. Thus the spatial arrangement of the 5´-dangling base of the template does not hinder (at least appreciably) the formation of the cooperative interaction upon continuous hybridization of oligonucleotides.

The results of analysis suggest that the efficacy of the cooperative contact at the X*pY/ZZ´ interfaces is determined primarily by the efficacy of stacking interactions in the stacks of bases involved in this contact. Violation of symmetry of the energy contributions of the dinucleotide steps caused by a nick results from the difference in contributions of the base stacks of the template and the stacks that are formed in the nick to the efficacy of the cooperative interaction. Bases with larger overlapping areas give rise to more favorable coaxial stacking (the exception is the AC/GT complementary pair).

Probably, the surprisingly low energy contributions of the cooperative interaction at the C*pY/ZG interfaces of the nearly continuous dinucleotide step of the DNA helix result from weak stacking in the C*pY stacks, whereas the unexpectedly strong A*pPy/PuT and Pu*pA/TPy interactions may be associated with substantial interplanar interactions in the broken chain. Even if the horizontal interchain interactions at the junction of the duplex structures, which arise from the shift of the dynamical equilibrium of the terminal pairs toward "ordering," are of importance, their effects are, most likely, associated with the strength of interplanar interactions between the bases in the nick. For weak C*pY/ZG interactions, these effects are apparently of least importance, whereas stronger interactions have a certain effect whose contribution depends on the type of interface.

To summarize, we determined for the first time the complete set of the thermodynamic parameters (ΔG°, ΔS°, and ΔH°) of coaxial stackings for all 16 types of interactions taking place upon continuous hybridization of oligonucleotides in solution on a common template. The formation of the vertical stack by the terminal bases of the oligonucleotides pairing in the nick was demonstrated to make the major and decisive contribution to the energy of the cooperative interaction. The parameters determined in the present study allow one to make a preliminary estimate of thermal stability of tandem complexes in relation to the type of cooperative interactions thus appeared.

We thank T. Yu. Bushueva for technical assistance.

This study was in part financially supported by the Russian Academy of Sciences (Program of the Scientific Projects of Young Scientists of the Siberian Branch of the Russian Academy of Sciences, D. V. Pyshnyi's Personal Project, VI Competition of Research Projects of Young Scientists of the Russian Academy of Sciences, 1999, Project No. 230).

References

1. S. Parinov, V. Barsky, G. Yershov, E. Kirillov, E. Timofeev, A. Belgovskiy, and A. Mirzabekov, *Nucleic Acids Res.*, 1996, **24**, 2998.
2. R. Maldonado-Rodriguez, M. Espinosa-Lara, P. Loyola-Abitia, W. G. Beattie, and K. L. Beattie, *Molecular Biotechnology*, 1999, **11**, 13.
3. I. V. Kutyavin, M. A. Podyminogin, Yu. N. Bazhina, O. S. Fedorova, D. G. Knorre, A. S. Levina, S. V. Mamayev, and V. F. Zarytova, *FEBS Lett.*, 1988, **238**, 35.
4. D. V. Pyshnyi, S. G. Lokhov, E. M. Ivanova, and V. F. Zarytova, *Bioorg. Khim.*, 1998, **24**, 201 [*Russ. J. Bioorg. Chem.*, 1998, **24** (Engl. Transl.)].
5. A. Adina-zada and O. S. Fedorova, *Bioorg. Khim.*, 1995, **21**, 703 [*Russ. J. Bioorg. Chem.*, 1995, **21** (Engl. Transl.)].
6. M. I. Dobrikov, S. A. Gaidamakov, A. A. Koshkin, and V. V. Vlassov, *Dokl. Akad. Nauk,* 1996, **351**, 687 [*Dokl. Chem.*, 1996 (Engl. Transl.)].
7. Z. A. Sergeeva, A. G. Venyaminova, and V. F. Zarytova, *Nucleosides Nucleotides*, 1998, **17**, 2153.
8. J. Kieleczawa, J. J. Dunn, and F. W. Studier, *Science*, 1992, **258**, 1787.

9. D. G. Knorre, D. V. Pyshnyi, E. M. Ivanova, A. A. Bondar´, I. V. Morozov, N. P. Mertvetsov, and V. F. Zarytova, *Dokl. Akad. Nauk,* 1996, **350**, 119 [*Dokl. Chem.*, 1996 (Engl. Transl.)].
10. M. J. Engler and C. C. Richardson, in *The Enzymes*, Ed. P. D. Boyer, Academic Press, New York, 1982, **15**, 3.
11. D. V. Pyshnyi, A. A. Krivenko, S. G. Lokhov, E. M. Ivanova, G. M. Dymshits, and V. F. Zarytova, *Bioorg. Khim.*, 1998, **24**, 25 [*Russ. J. Bioorg. Chem.*, 1998, **24** (Engl. Transl.)].
12. J. Brams, A. M. Michelson, and K. E. van Hold, *J. Mol. Biol*, 1966, **15**, 467.
13. E. R. Kandimalla, A. Manning, C. Lathan, R. A. Byrn, and S. Agrawal, *Nucleic Acids Res.*, 1995, **23**, 3578.
14. T. V. Abramova, V. V. Gorn, I. V. Kutyavin, A. V. Lebedev, S. G. Lokhov, and M. A. Podyminogin, *Dokl. Akad. Nauk,* 1990, **315**, 1485 [*Dokl. Chem.*, 1990 (Engl. Transl.)].
15. D. V. Pyshnyi, I. A. Pyshnaya, S. G. Lokhov, E. M. Ivanova, and V. F. Zarytova, *Bioorg. Khim.*, 1995, **21**, 709 [*Russ. J. Bioorg. Chem.*, 1995, **21** (Engl. Transl.)].
16. M. J. Lane, T. Paner, I. Kashin, B. D. Faldasz, B. Li, F. J. Gallo, and A. S. Benight, *Nucleic Acids Res.*, 1997, **25**, 611.
17. D. H. Mathews, J. Sabina, M. Zuker, and D. H. Turner, *J. Mol. Biol.*, 1999, **288**, 911.
18. A. E. Walter, D. H. Turner, J. Kim, M. H. Lyttle, P. Muller, D. H. Mathews, and M. Zuker, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 9218.
19. A. E. Walter and D. H. Turner, *Biochemistry*, 1994, **33**, 12715.
20. L. A. Marky and K. J. Breslauer, *Biopolymers*, 1987, 26, 1601.
21. M. Zhong and N. R. Kallenbach, *J. Mol. Biol.*, 1993, **230**, 766.
22. S. G. Lokhov, A. A. Koshkin, I. V. Kutyavin, M. P. Mityakin, M. A. Podyminogin, and A. V. Lebedev, *Bioorg. Khim.*, 1995, **21**, 197 [*Russ. J. Bioorg. Chem.*, 1995, **21** (Engl. Transl.)].
23. V. V. Koval, N. A. Lokteva, S. L. Karnaukhova, and O. S. Fedorova, *J. Biomol. Struct. Dyn.*, 1999, **17**, 259.
24. P. V. Riccelli, F. Merante, K. T. Leung, S. Bortolin, R. L. Zastawny, R. Janeczko, and A. S. Benight, *Nucleic Acids Res.*, 2001, **29**, 996.
25. V. A. Vasiliskov, D. V. Prokopenko, and A. D. Mirzabekov, *Nucleic Acids Res.*, 2001, **29**, 2303.
26. D. V. Pyshnyi, I. A. Pyshnaya, A. S. Levina, E. L. Goldberg, V. F. Zarytova, D. G. Knorre, and E. M. Ivanova, *J. Biomol. Struct. Dyn.*, 2001, **19**.
27. *Handbook of Biochemistry and Molecular Biology: Nucleic Acids*, Ed. G. D. Fasman, CRC Press, Cleveland, 1975, **1**, 589.
28. D. V. Pyshnyi, S. G. Lokhov, V. N. Sil´nikov, G. V. Shishkin, E. M. Ivanova, and V. F. Zarytova, *Bioorg. Khim.*, 1999, **25**, 40 [*Russ. J. Bioorg. Chem.*, 1999, **25** (Engl. Transl.)].
29. S. G. Lokhov and D. V. Pyshnyi, *FEBS Lett.*, 1997, **420**, 134.
30. M. Petersheim and D. H. Turner, *Biochemistry*, 1983, **22**, 256.
31. J. Norberg and L. Nilsson, *Biophysical J.*, 1995, **69**, 2277.
32. I. Hirao, G. Kawai, S. Yoshizawa, Y. Nishimura, H. Ishido, K. Watanabe, and K. Miura, *Nucleic Acids Res.*, 1994, **22**, 576.
33. A. V. Fotin, A. L. Drobyshev, D. Y. Proudnikov, A. N. Perov, and A. D. Mirzabekov, *Nucleic Acids Res.*, 1998, **26**, 1515.
34. T. Xia, J. SantaLucia, Jr., M. E. Burkard, R. Kierzek, S. J. Schroeder, X. Jiao, C. Cox, and D. H. Turner, *Biochemistry*, 1998, **37**, 14719.
35. K. J. Breslauer, R. Frank, H. Blocker, and L. A. Marky, *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 3746.
36. J. SantaLucia, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 1460.
37. D. J. Patel and C. W. Hilbers, *Biochemistry*, 1975, **14**, 2651.
38. A. Yu. Denisov, T. V. Maltseva, A. Sandstrom, D. V. Pyshnyi, E. M. Ivanova, V. F. Zarytova, and J. Chattopadhyaya, *J. Biomol. Struct. Dyn.*, 1997, **14**, 809.
39. K. M. Guckian, B. A. Schweitzer, R. X. -F. Ren, C. J. Sheils, D. C. Tahmassebi, and E. T. Kool, *J. Am. Chem. Soc.*, 2000, **122**, 2213.
40. W. Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag, New York—Berlin—Heidelberg—Tokyo, 1984.
41. A. Aymami, M. Coll, G. A. van der Marel, J. H. van Boom, A. H.-J. Wang, and A. Rich, *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 2526.
42. J. M. Pieters, R. M. Mans, H. van der Elst, G. A. van der Marel, J. H. van Boom, and C. Altona, *Nucleic Acids Res.*, 1989, **17**, 4551.
43. C. Roll, C. Ketterle, V. Faibis, G. V. Fazakerley, and Y. Boulard, *Biochemistry*, 1998, **37**, 4059.
44. S. Bommarito, N. Peyret, and J. SantaLucia, Jr., *Nucleic Acids Res.*, 2000, **28**, 1929.

Received November, 23, 2001