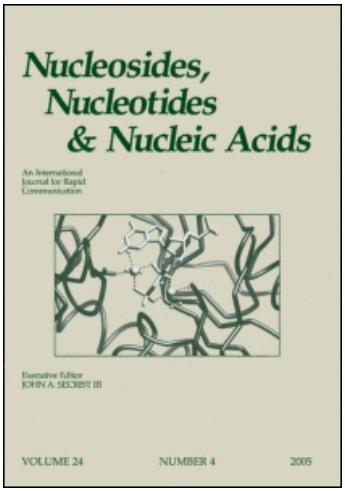

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The Influence of Nearest Neighbours on the Efficiency of Coaxial Stacking at Contiguous Stacking Hybridization of Oligodeoxyribonucleotides

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ABSTRACT

Contiguous stacking hybridization of oligodeoxyribonucleotides with a stem of preformed minihairpin structure of a DNA template was studied with the use of UV-melting technique. It was shown that the free-energy of the coaxial stacking interaction (ΔG°_{ST} at 37°C, 1 M NaCl, pH 7.4) at the complementary interface XA*pTY/ZATV (an asterisk stands for a nick) strongly depends on the type of nearest neighbor bases X and Y flanking the nicked dinucleotide step. The maximum efficiency of the coaxial stacking was observed for the PuA*pTPy/PuATPy interface, whereas the minimum efficiency was obtained for the PyA*pTPu/PyATPu interface. A 5′-phosphate residue in the nick enhances the coaxial stacking. In dependence on duplex structure the observed efficiency of A*T/AT coaxial stacking varied from (− 0.97 kcal/mol) for unphosphorylated TA*TA/TATA interface to three-fold higher value (− 2.78 kcal/mol) for GA*pTT/AATC interface.

Key Words: Coaxial stacking; Contiguous hybridization; Thermodynamic; DNA duplex; Tandem complex.

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INTRODUCTION

Contiguous stacking hybridization of oligonucleotides with the DNA template has become an increasingly promising approach used in DNA analysis by the method of molecular hybridization or template dependent ligation.[1,2] The main criteria for choice of oligonucleotides as probes is their hybridization properties. Thermodynamic stability of the tandem complexes can be estimated from the thermodynamic parameters of the individual DNA/DNA duplexes[3] and the parameters describing cooprative contacts between adjoining duplexes.[4] Two different sets of terms for the stacking interaction at the helix–helix interface were published recently, which were derived from the hybridization properties of free oligonucleotides in solution[5] or oligonucleotides immobilized in the gel.[6] There is no good correlation between the values of free-energy increments for the corresponding stacks in these two sets. The difference can result from the differences in the experimental techniques used for estimation of the parameters as well as the differences in the structural features of the analyzed nicked duplexes. Therefore the first step towards the unified thermodynamic description of coaxial stacking interaction in DNA should be the revealing of structural elements in the nicked duplexes which affect the base pairs stacks at the helix–helix interface.

In this work, we determined thermodynamic parameters of coaxial stacking at complementary XA*pTY/ZATV interfaces created in nicked DNA duplexes differing in their structures. The data obtained allow us to conclude that the efficiency of interaction between base pairs stacked at the helix–helix interface strongly depends on the type of nearest neighbors flanking the nicked dinucleotide step. The role of 5′-phosphate residue in the nick was examined too.

RESULTS AND DISCUSSION

Free-energy increments from the coaxial stacking interaction (ΔG°_{ST} at 37°C) in the nicked A*pT/AT step of the DNA duplexes at the XA*pTY/ZATV interfaces were

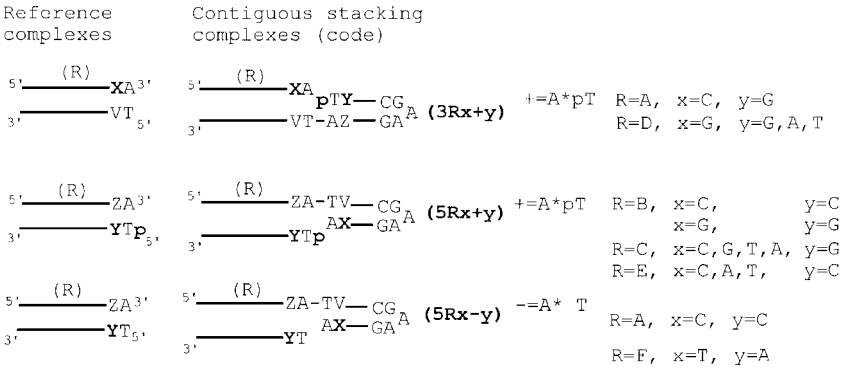

Figure 1. The schematic image of reference complexes of heptanucleotides **R** and their contiguous stacking complexes.

determined with the use of 15 model tandem complexes and 6 blunt-ended ordinary duplexes as references (see Fig. 1 and Table 1).

The coaxial stack was created when heptanucleotide **R** formed the duplex adjacent to a preexisting minihairpin of a template. The structures of studied duplexes were chosen to reveal the influence of the nearest neighbors flanking the 5′-phosphorylated A*pT or unphosphorylated A*T nicked dinucleotide.

We studied two main types of the complexes (Fig. 1). The first type complexes **3Rx+y** are formed when the 3′-OH terminal nucleotide of hybridizing heptanucleotides **R** and 5′-phosphorylated chain of preexisting duplexes involve to **X**A*pT**Y**/ZATV interface. In the complexes **5Rx + y** of the second type the **X**A*pT**Y**/ZATV the interface is formed by the 5′-phosphorylated nucleotide of heptanucleotides **R** and 3′-OH terminal nucleotide of the preexisting duplexes. Furthermore we examined the complexes of second type without phosphate at the nick. In these complexes **5Rx − y** the 5′-OH nucleoside of the heptanucleotide **R** and 3′-OH terminal nucleotide of preexisting duplex formed dephosphorylated **X**A*T**Y**/ZATV interface. So the studied complexes **3Rx+y**, **5Rx + y**, and **5Rx − y** differ from each other by 1) the end of of heptanucleotide **R** involved to stack formation (**5′** or **3′**), 2) the type of nearest neighbor nucleotides (**x**,**y**) flanking nicked dinucleotide, 3) the presence of 5′-phosphate in the nick (+ or −), and 4) the nucleotide sequence of heptanucleotide **R**.

Thermodynamic parameters of formation of oligonucleotide complexes were obtained with the use of UV-melting technique in accordance with our earlier studies.[4] Because the hairpins were designed to be stable below 75°C[7] the transitions involving coaxially stacked heptanucleotide duplexes could be followed separately and described using a two-state model assumption.[4,5,8,9] It was shown previously that the free-energy increments from the coaxial stacking interaction (ΔG°_{ST} at 37°C) in this case can be determined as the difference between the free-energy of formation of heptanucleotide duplex with hairpin DNA template and free-energy of formation of corresponding ordinary duplex.[4,5,8] Melting temperatures (T_m) and the thermodynamic parameters (ΔS°, ΔH°, and ΔG°_{37}) of formation for all studied duplexes listed in the Table. Free-energy increments from the different type of coaxial stacks formed at the same helix–helix interface XA*(p)TY/ZATV and their codes are represented ibidem.

As a result (Table) contiguous stacking hybridization of the heptanucleotides enhances the stability of their duplexes in all cases studied. However the stabilizing effect (ΔG°_{ST}) of coaxial stack is significantly varied. The minimum efficiency of the coaxial stacking ($\Delta G^\circ_{ST} = -0.97$ kcal/mol) was observed for TA*TA/TATA interface (complex **5Ft-a**). At the same time for the GA*pTT/AATC interface (complex **3Dg + t**) the ΔG°_{ST} value is increased threefold up to maximum value observed (− 2.78 kcal/mol).

There is a correlation between efficiency of coaxial stacks and structural features of the region close to the helix–helix interface in the nicked duplexes (Fig. 2).

1. The efficiency of the coaxial stacking (ΔG°_{ST}) at the phosphorylated helix–helix interface is higher than at the unphosphorylated one. Presence of the phosphate at the interface CA*pTC/GATG (complex **5Bc + c**) contributes −0.2 kcal/mol to the ΔG°_{ST} value of the interaction at the interface CA*TC/GATG (complex **5Ac − c**) (Fig. 2).
2. In the different complexes with the same interfaces the efficiences of the coaxial stacking are similar, i.e. the sequence of heptanucleotides **R** does not affect the values

Table 1. Thermodynamic parameters (ΔS°, ΔH°, and ΔG°_{37}) of ordinary and contiguous stacking hybridization of heptadeoxyribonucleotides and free-energy increments (ΔG°_{ST}) of coaxial stacking interaction at the DNA helix-helix interfaces.[a]

No.	Complex	Code	ΔS°[b]	ΔH°	ΔG°_{37}	T_m,°C[c]	ΔG°_{ST}
A	5′TGTTTGA 3′ACAAACT	**A**	−119.1[e] ± 3.9	−42.0[e] ± 1.2	−5.04[e] ± 0.06	19.7[e] ± 0.3	–
A.1	5'TGTTTGA--TGCG$_A$ 3'ACAAA**C**T A**C**GA	**5Ac-c**	−157.8 ± 4.6	−55.7 ± 1.4	−6.74 ± 0.06	32.7 ± 0.3	−1.70[e] ± 0.08
A.2	$_A$AGCA--TGTTTGA 3' GC**G**T**p** A**C**AAACT 5'	**3Ac + g**	−151.5 ± 5.0	−54.0 ± 1.5	−7.01 ± 0.02	34.1 ± 0.2	−1.97 ± 0.06
B	5′TGTTT**G**A 3′ACAAA**C**T**p**	**B**	−120.7 ± 5.4	−42.7 ± 1.6	−5.25 ± 0.16	21.3 ± 0.9	–
B.1	5'TGTTTGA--TGCG$_A$ 3'ACAAA**C**T**p** A**C**GA	**5Bc + c**	−149.7 ± 4.8	−53.6 ± 1.6	−7.14 ± 0.10	34.8 ± 0.5	−1.89 ± 0.19
B.2	5'TGTTTGA--TCCG$_A$ 3'ACAAA**C**T**p** A**G**GA	**5Bg + c**	−146.9 ± 2.0	−53.1 ± 0.6	−7.52 ± 0.04	37.0 ± 0.2	−2.27 ± 0.17
C	5′TGTTT**C**A 3′ACAAA**G**T**p**	**C**	−107.1 ± 3.8	−38.3 ± 1.1	−5.09 ± 0.04	18.5 ± 0.3	–
C.1	5'TGTTTCA--TGCG$_A$ 3'ACAAA**G**T**p** A**C**GA	**5Cc + g**	−139.3 ± 7.0	−49.8 ± 2.2	−6.61 ± 0.09	31.4 ± 0.7	−1.52 ± 0.10
C.2	5'TGTTTCA--TCGCG$_A$ 3'ACAAA**G**T**p** A**G**CGA	**5Cg + g**	−127.6 ± 13.0	−46.7 ± 4.0	−7.09 ± 0.07	34.2 ± 0.6	−2.00 ± 0.08
C.3	5'TGTTTCA--TAGCG$_A$ 3'ACAAA**G**T**p** A**T**CGA	**5Ct + g**	−145.7 ± 12.6	−51.6 ± 3.8	−6.42 ± 0.11	30.5 ± 0.5	−1.33 ± 0.12
C.4	5'TGTTTCA--TTGCG$_A$ 3'ACAAA**G**T**p** A**A**CGA	**5Ca + g**	−133.3 ± 10.5	−48.0 ± 3.2	−6.62 ± 0.08	31.3 ± 0.5	−1.53 ± 0.09

D	5′TGATAGA 3′ACTATCT	**D**	−114.1 ± 1.4	−39.8 ± 0.4	−4.44 ± 0.05	14.7 ± 0.3	–
D.1	5′TGATA**G**A **p**T**G**CG_A 3′ACTATCT--ACGA	**3Dg + g**	−163.4 ± 13.7	−57.4 ± 4.2	−6.73 ± 0.04	32.8 ± 0.2	−2.29 ± 0.07
D.2	5′TGATA**G**A **p**T**A**GCG_A 3′ACTATCT--ATCGA	**3Dg + a**	−149.6 ± 1.9	−53.0 ± 0.6	−6.59 ± 0.05	31.6 ± 0.3	−2.15 ± 0.09
D.3	5′TGATA**G**A **p**T**T**GCG_A 3′ACTATCT--AACGA	**3Dg + t**	−159.2 ± 14.9	−56.6 ± 4.7	−7.22 ± 0.14	35.4 ± 0.7	−2.78[d] ± 0.14
E	5′TGATAGA 3′ACTATCT**p**	**E**	−109.8 ± 3.8	−38.6 ± 1.1	−4.58 ± 0.09	15.0 ± 0.3	–
E.1	5′TGATAGA--TGCG_A 3′ACTAT**C**T**p** A**C**GA	**5Ec + c**	−150.5 ± 4.0	−53.2 ± 1.2	−6.50 ± 0.06	31.1 ± 0.4	−1.92 ± 0.10
E.2	5′TGATAGA--TTGCG_A 3′ACTAT**C**T**p** A**A**CGA	**5Ea + c**	−148.5 ± 2.9	−52.8 ± 0.9	−6.72 ± 0.03	32.4 ± 0.2	−2.15 ± 0.07
E.3	5′TGATAGA--TAGCG_A 3′ACAAA**C**T**p** A**T**CGA	**5Et + c**	−153.4 ± 5.8	−53.9 ± 1.8	−6.29 ± 0.06	30.1 ± 0.4	−1.71 ± 0.10
F	5′TGATATA 3′ACTATAT	**F**	−99.0 ± 5.62	−34.5 ± 1.66	−3.77 ± 0.09	6.6 ± 0.4	–
F.1	5′TGATATA--TAGCG_A 3′ACTAT**A**T A**T**CGA	**5Ft-a**	−145.5 ± 8.79	−49.9 ± 2.62	−4.75 ± 0.14	20.6 ± 0.5	−0.97[d] ± 0.10

[a]The data were obtained by UV-melting technique. Experiments were carried out in buffer containing 10 mM sodium phosphate (pH 7.4), 1 M NaCl, 0.1 mM EDTA.

[b]ΔS°- cal/(mol ± K), ΔH°-kcal/mol, ΔG°_{37}-free-energy at 37°C in kcal/mol.

[c]Melting temperatures calculated for total concentration of oligonucleotide strands $2 \cdot 10^{-5}$ M.

[d]The maximum and minimum values of ΔG°_{ST} are underlined.

[e]The error is estimated in accordance with [9].

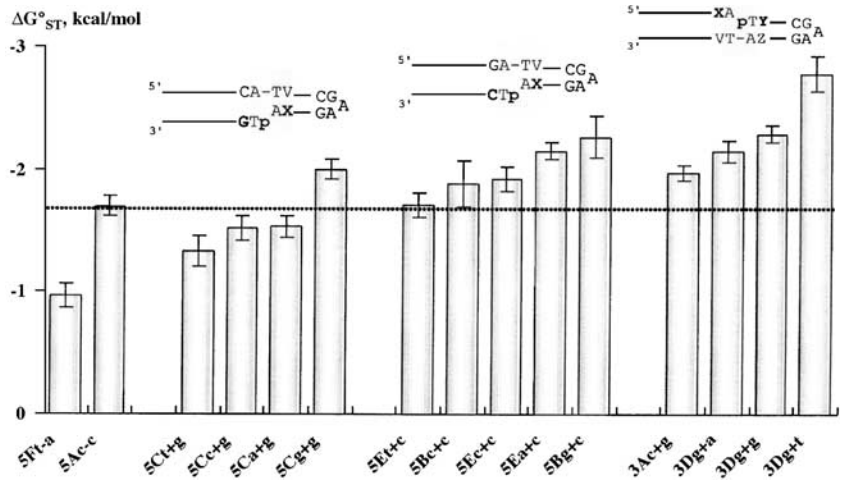

Figure 2. The efficiency of coaxial stacking at the XA*pTY/ZATV interfaces depending on the features of complex structure and nearest neighbors X and Y; dotted line is the level of the averaged value of ΔG°_{ST}.

ΔG°_{ST}. For example, in the complexes **5Bc + c** and **5Ec + c** formed by various heptamers **B** and **E** the free-energy of interaction at the interface CA*pTC/GATG is identical (-1.89 and -1.92 kcal/mol respectively).

3. A more interesting fact is that the phosphorylated coaxial stacks formed with participation of 5′-phosphorylated terminal nucleotide of heptamer (complex **5Rx + y**) are lower than the corresponding coaxial stacks formed by the 3′OH-terminal nucleotide (complex **3Rx + y**) (Fig. 2). For example, the difference between the values of ΔG°_{ST} for complexes **3Ac + g** and **5Cc + g** is -0.45 kcal/mol and for complexes **3Dg + g** and **5Cg + g** is -0.29 kcal/mol.
4. The most important result obtained here concerns the influence of nearest neighbors on the efficiency of coaxial stacking interaction in the nick with the effect of the same base being different in dependence on its position (X or Y) (Fig. 2). For the series of XA*pT**G**/CATV (Y = G) interfaces in the complexes **5Cx + g** the efficiency of A*pT/AT stacks increases from -1.33 to -2.0 kcal/mol in the following order of the 5′-X nearest neighbor: T < C ≈ A < G. The same order is observed also for series of XA*pTC/GATV (Y = C) interfaces in the **5Rx + c** complexes. Furthermore the comparison of these two series allows one to find that the free-energy increment from each coaxial stack XA*pT**C**/GATV is higher than the increment from corresponding XA*pT**G**/CATV stack, X being the same base. The similar conclusion follows from the analysis of ΔG°_{ST} values obtained for interfaces **GA***pTY/ZATC (complexes **3Dg + y**): a pyrimidine placed at Y-position instead of purine enhances coaxial stacking interaction.

So the type of nucleotides has the opposite effect to coaxial stacking for nearest neighbor position upstream and downstream from nicked dinucleotide: the purines

favor the base stacking in the nick flanking interrupted dinucleotide from 5′-side as well as pyrimidines do at the 3′-flank.

Thus the evidence that the structural features of nicked duplex influence the efficiency of the coaxial stacking at DNA helix–helix interface is represented. To establish the unified thermodynamic description of the coaxial stacking interaction attention should be paid to characterization of the effects of at least the nearest neighbors of the nick. In distinction from the uninterrupted dinucleotide within double helix where effects of nearest neighbors are averaged, the analysis of coaxial stacking at the helix–helix interface allows one to determine the effects of nearest neighbors due to asymmetry of interrupted dinucleotide base pairs.

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