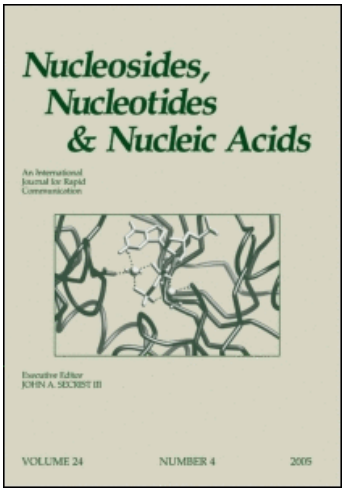

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The 5'-Purine-Pyrimidine-3' Stacks are More Stabilizing in a Self-3'-Pyrinudine-Purine-5' complementary DNA Duplex than the 5'-Purine-Purine-3' 3'-Pyrimidine-Pyrimidine-5' Stack

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The $\begin{matrix}\text{5'-Purine–Pyrimidine-3'}\\ \text{3'-Pyrimidine–Purine-5'}\end{matrix}$ Stacks are More Stabilizing in a Self-complementary DNA Duplex than the $\begin{matrix}\text{5'-Purine–Purine-3'}\\ \text{3'-Pyrimidine–Pyrimidine-5'}\end{matrix}$ Stack

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Abstract: First experimental evidence is herein reported supporting the earlier quantum chemical calculations that $\begin{matrix}\text{5'-Purine–pyrimiidine-3'}\\ \text{3'-Pyrimidine–Purine-5'}\end{matrix}$ stack is more stable than $\begin{matrix}\text{5'-pyrimiidine–Purine-3'}\\ \text{3'-Purine–Pyrimidine-5'}\end{matrix}$.

Both the NMR data as well as solubility experiments show that the purine(3'→5')purine dinucleotides are better stacked than the corresponding pyrimidine(3'→5')pyrimidine and pyrimidine(3'→5')purine dinucleotides[4]. On the other hand, the strength of the 2'→5' stacking[1] follows the opposite order, which increases in the following manner: A2'→5'G < A2'→5'A < 2'→5' < A2'→5'U ≤ A2'→5'C.

On the other hand, quantum chemical calculations by Ornstein et al[3] for the ten possible dimers in B-type DNA arrangement as early as 1978 showed that the total stacking energies depend both on the composition and sequence. Thus they dissected the stabilizing energies of the base-paired dimers into horizontal (*i.e.* basepairing) and vertical (*i.e.* base stacking) components. Thus a $\begin{matrix}\text{5'-pyrimiidine–Purine-3'}\\ \text{3'-Purine–Pyrimidine-5'}\end{matrix}$ base-paired dimer is more stable than a $\begin{matrix}\text{5'-Purine–pyrimiidine-3'}\\ \text{3'-Pyrimidine–Purine-5'}\end{matrix}$.

The authors[3] argued that the origin of this energy difference basing on the DNA sequence comes from the geometries of stacking overlaps, since stacking interaction between adjacent bases produces stable double-stranded, helical structures.

No experimental evidence at the oligomeric level has been however so far provided that backs-up the above quantum chemical calculations.

Table 1 : Concentration-dependent T_m of different oligonucleotides[#]

Oligo #	DNA duplex	Tm(C°)				
		6 mM	5 mM	4 mM	3 mM	2 mM
1	5'-d CATGTTTGGC -3' 3'-d TACAAACCG$_{Pnz}$-5'	46.026	45.434	44.488	43.697	42.394
2	5'-d CATGTTTGGC -3' 3'-d TACAAACCG-5'	37.361	36.804	35.918	35.410	33.648
3	5'-d CATGTTTGGG -3' 3'-d TACAAACCC$_{Pnz}$-5'	44.167	43.929	43.738	43.473	41.075
4	5'-d CATGTTTGGG- 3' 3'-d TACAAACCC-5'	34.254	34.109	33.413	32.819	30.875
5	5'-d CATGTTTGGT -3' 3'-d TACAAACCA$_{Pnz}$-5'	41.171	40.683	39.023	38.072	37.998
6	5'-d CATGTTTGGT -3' 3'-d TACAAACCA-5'	31.909	31.795	29.661	28.982	28.742
7	5'-d CATGTTTGGA -3' 3'-d TACAAACCT$_{Pnz}$-5'	-	-	-	-	37.700
8	5'-d CATGTTTGGA -3' 3'-d TACAAACCT-5'	-	-	-	-	26.200

[#]For the preparation and properties of Pnz (phenazine)-tethered DNA see ref 2

Table 2 : The thermodynamics of duplex formation of different oligonucleotides[#]

Oligo #	DNA duplex	ΔH	ΔS	-TΔS	ΔG
1	5'-dCATGTTTGGC -3' 3' -dTACAAACCG$_{Pnz}$-5'	-278.9	-0.77	230.7	-48.2
2	5'-dCATGTTTGGC -3' 3' -dTACAAACCG -5'	-253.7	-0.72	213.7	-40.0
3	5'-dCATGTTTGGG -3' 3' -dTACAAACCC$_{Pnz}$ -5'	-283.7	-0.79	236.4	-47.3
4	5'-dCATGTTTGGG -3' 3' -dTACAAACCC -5'	-250.4	-0.71	212.7	-37.7
5	5'-dCATGTTTGGT -3' 3' -dTACAAACCA$_{Pnz}$-5'	-259.7	-0.73	216.7	-43.1
6	5'-dCATGTTTGGT -3' 3' -dTACAAACCA -5'	-238.5	-0.68	203.3	-35.2
7	5'-dCATGTTTGGA -3' 3' -dTACAAACCT$_{Pnz}$-5'	-269.1		226.2	-42.9
8	5'-dCATGTTTGGA -3' 3' -dTACAAACCT -5'	-249.2		214.9	-34.4

[#]For the preparation and properties of Pnz (phenazine)-tethered DNA see ref 2

In this work, we report the results of our test of the above assumption through the studies on the following sets of isomeric oligo-DNAs in which only the terminal basepair has been swapped between the pair of oligos #1 and 3, #2 and 4, #5 and 7 and #6 and 8 in order to change the intrastrand stacking behaviour in both strands. Thus Table 1 shows the concentration-dependent T_m of various oligo-DNAs and Table 2 shows the thermodynamics.

It shows that the sum of the terminal $\begin{matrix}5'\text{-Purine}-\text{pyrimiidine-}3'\\3'\text{-Pyrimidine}-\text{Purine-}5'\end{matrix}$ stackings in the oligo pairs # 1/2 and #5/6 are more stablizing than the sum of the analogous $\begin{matrix}5'\text{-pyrimiidine}-\text{Purine-}3'\\3'\text{-Purine}-\text{Pyrimidine-}5'\end{matrix}$ stackings in the oligos # 3/4 and 7/8.

In each pair of the oligo-DNAs, we have the same number of hydrogen bonds, hence the observed differences in their thermal stabilities can be attributed to the differences in the intrastrand vertical stacking behaviour between the two terminal nucleotides in oligo # 1 - 8.

This work thus provides first experimental evidence supporting the earlier quantum chemical calculations[3].

We believe that this observation should also be applicable for intrastrand stacking in the core part or in any other part of the duplex, which should be taken in to account for the design of oligos for the antisense and catalytic RNA research.

Methods

All oligonucleotides were synthesised in 1μM scale with 8-channel Applied Biosystems 392 DNA/RNA synthesiser, deprotected with aqueous ammonia (55°C, 16 h) and purified twice on reverse-phase HPLC using the gradient of CH_3CN from 5% to 50% in 0.1M triethylamonium acetate.

Melting measurements. UV melting profiles were obtained by scanning A_{260} absorbency versus time at heating rate of 1°C/min and temperature gradient 10-60°C/50 min. The T_ms were calculated from the maximum points of the first derivatives of the melting curves with accuracy of 0.5°C. All measurements were carried in 20 mM Na_2HPO_4/NaH_2PO_4,0.1M NaCL buffer at pH 7.3.

Thermodynamics. For the thermodynamic calculations of $\Delta H°$, $\Delta S°$ and $\Delta G°$, T_ms for five different oligonucleotide concentrations (1, 2, 3, 4, and 5μM total single strand concentration) were measured starting from the highest concentration and then stepwise decreasing the concentration by dilution at the same buffer concentration. Before each melting experiment denaturation and renaturation of the samples was carried out by heating solutions to 70°C for 3 min followed by slow cooling down to the RT and keeping at 10°C for 30 min. The resulting Tm -values were fitted to a van't Hoff plot of T_m^{-1} vs ln C_T and thermodynamic parameters were calculated using follow equations:

$1/T_m = (R/\Delta H°)LnCT+\Delta S°/\Delta H°$ $R/\Delta H°$ = slope $\Delta S°/\Delta H°$ = intercept

$\Delta G°(298K) = \Delta H°-T\Delta S°$, ($C_T$ = total single strand concentration)

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