Relative Thermodynamic Stacking Constants of Deoxyribo Dinucleotides

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Abstract: - Consistent relative stacking interaction energies have been calculated for the sixteen triply ionized deoxyribose dinucleotide structures in the g-g- conformation. These values are often lower than for the corresponding ribose dinucleotides. The Watson-Crick hydrogen bonding interactions are entirely comparable for the deoxyribose and ribose nucleotide interactions. The DNA of dinucleotides may be favourably transcribed or translated to duplexes with Watson-Crick base pairing with a possible 100% accuracy. The hybridization of the RNA anticodon with transfer RNA may also be accomplished with 100 % accuracy to provide a disperse range to specify unique amino acids. The stability of the DNA codons and anticodons has enhanced their preservation. However, they could not be fully translated without a chance of error.
The stacking interactions were calculated for the overall enthalpy changes in the ZKE approximation at the HF and MP2 /6-31G* level.

Key-Words: - Thermodynamic data, base stacking, deoxyribo dinucleotides, duplexes, genetic code.

1 Introduction

The nucleic acid bases, uracil, thymine, cytosine, adenine and guanine are known to stack in aqueous solution as free bases [1], nucleosides and nucleotides [2]. The stacking also occurs in single strand polymers [3], and in double and triple helices [4]. Extensive studies of nucleic acid base stacking have been undertaken, both experimental [5-7] and theoretical (8-9) to determine the factors stabilising DNA, to determine the flexibility, curvature, thermal stability [7], or to simulate melting curves [8]. The theoretical studies have included pure ab initio [10], and semi-empirical calculations [11].
This study is to accurately determine the relative stacking energies of the bases to test the hypothesis that the original genes were formed by stacking that preceded a slow polymerization reaction that proceeded down the chain. If correct, this hypothesis predicts that the stability of the stacks formed and subsequently polymerized were assembled according to their thermodynamic stability, and these stable sequences were trapped for all time in the genes of living organisms. One suggestion for nucleotides that could have polymerized were the amino acyl derivatives of cyclic-3',5'-nucleotides [12]. In this project the sixteen base-base interactions of triply ionised dinucleotides are determined by a pure ab initio method [13]. The total energy of the polymer may then be calculated according to the one-dimensional Ising model [14].

2 Problem Formulation

The computations tabulated in this paper used the GAUSSIAN98 [15] commercial package. The standard calculations at the HF and MP2 levels including zero-point energy corrections [13], together with scaling [16], using the same basis set, 6-31G*. are as previously published [17]. Enthalpy changes at the MP2 level not including scaled zero point energies are designated as $\Delta H_{(MP2)}$. The complexes are less stable when calculated at the Hartree Fock level [13].
This paper uses the atomic unit of energy, the hartree [15].
1h = 627.5095 kcal.mol^{-1}. 1h = 4.3597482 x 10^{-18} J
Charges are in units of the electronic charge.
The method of calculating the relative stacking interactions in the gas phase was to optimize the dinucleotide structure and determine the enthalpy change for the formation of the glycosidic bond and the stacking interaction as shown in Fig.1. The enthalpy change for the formation of the glycosidic bond was then determined separately as shown in Fig.2. This enabled the stacking interaction to be isolated. In these reactions the coordinates of

bonding functional groups were not fixed, and allowed to vary during the optimization.

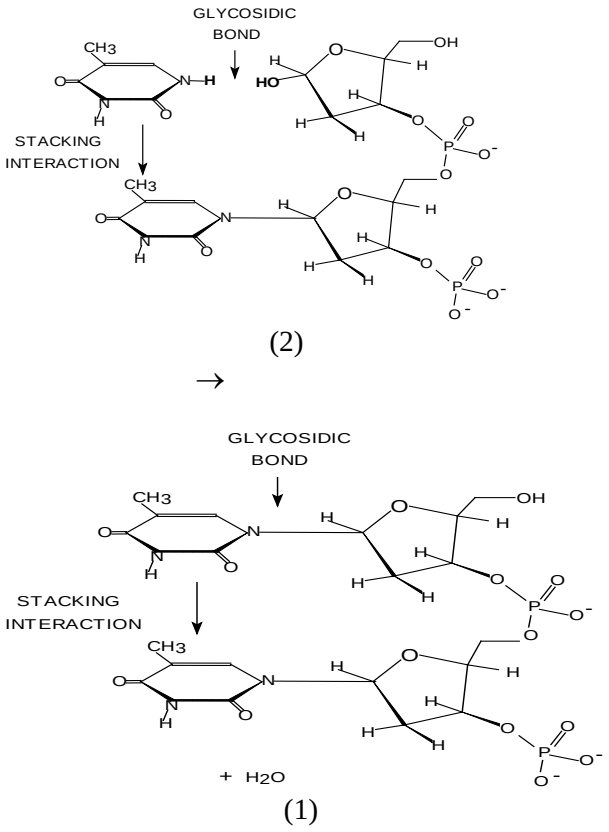

Fig.1 The formation of the glycosidic bond and stacking interactions where the enthalpy change is ΔH_3

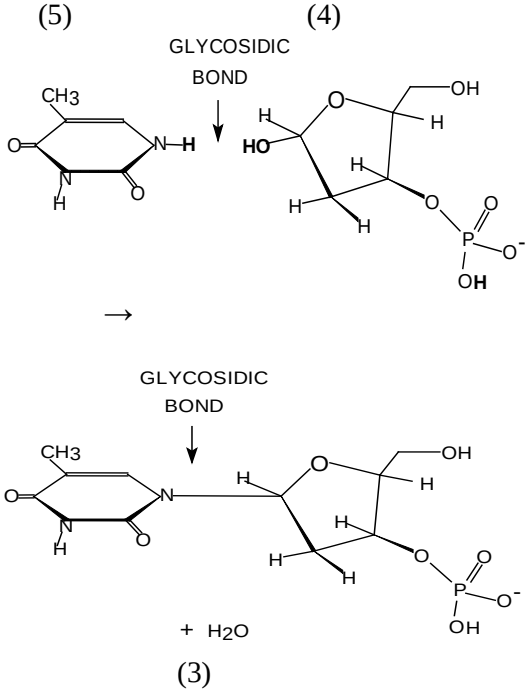

Fig.2 The formation of the glycosidic bond where the enthalpy change is ΔH_2

$$\Delta H1 + \Delta H2 = \Delta H3 \quad (1)$$

where ΔH_1 is defined as the stacking interaction.

3 Problem Solution

3.1 Conformations and Stacking Energies of the Stacked Dinucleotides

The geometry of the optimized dinucleotides is characterised by the dihedral angles shown in Fig.3. [18]

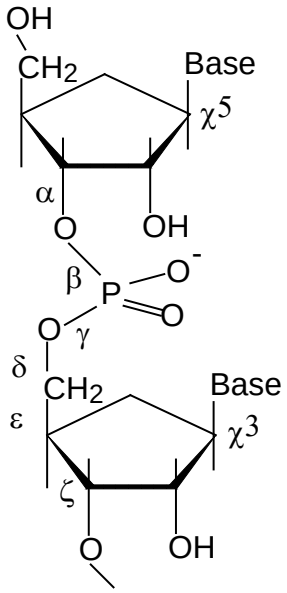

Fig.3. The dihedral angles used to define the structure of the dinucleotides.

A represenative stacked conformation of GpTp is shown in Fig.4.

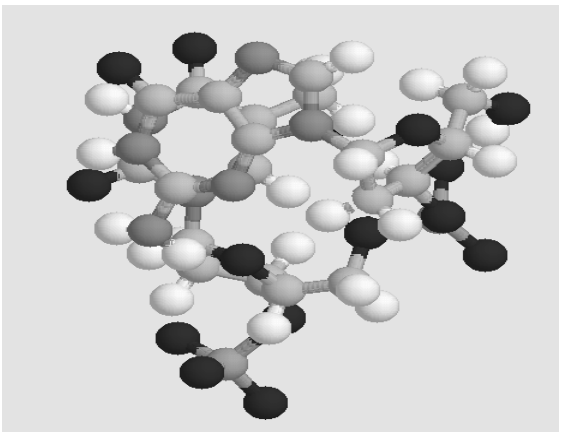

Fig.4. An optimized structure of stacked GpTp.

Table 1 The dihedral angle (degrees) of pyrimidine and purine dinucleotides. Enthalpy(h) changes for stacking. T=298.15 K.

Dinucleotide	α	β	γ	δ	ε
TpTp	113	67	64	153	71
CpTp	109	80	71	151	68
TpCp	107	77	57	138	70
CpCp	107	72	62	153	67
GpGp	107	77	63	156	61
GpAp	118	72	73	162	68
ApAp	113	72	72	156	68
ApGp	110	78	71	144	69

Table1 (cont)

Dinucleotide	ξ	χ (5')	χ (3')	$\Delta H_{298.15}$ (stacked)
TpTp	81	0	30	-0.03956
CpTp	81	43	38	-0.03314
TpCp	86	8	62	-0.02894
CpCp	81	31	31	-0.03683
GpGp	82	19	33	-0.05653
GpAp	89	13	32	-0.02929
ApAp	81	1	24	-0.03018
ApGp	80	39	49	-0.03767

Table 1 (cont.)

Dinucleotide	α	β	γ	δ	ε
TpAp	107	68	64	148	69
ApTp	112	75	69	153	68
CpAp	110	70	70	157	67
ApCp	111	69	65	154	70
CpGp	117	70	75	162	63
GpCp	108	79	65	164	63
TpGp	107	69	65	148	69
GpTp	114	69	71	175	61

Table1 (cont)

Dinucleotide	ξ	χ (5')	χ (3')	$\Delta H_{298.15}$ (stacked)
TpAp	82	17	42	-0.04069
ApTp	78	10	28	-0.02852
CpAp	82	48	36	-0.03681
ApCp	80	33	32	-0.03780
CpGp	67	74	100	-0.04560
GpCp	83	11	36	-0.06213
TpGp	83	17	40	-0.03365
GpTp	80	15	40	-0.04108

The ratio, GpCp/CpGp > 1, is also found for many DNA sequences [19].
The total energies and zero point energies (hartrees) for the respective equilibrium geometries.
are shown in Table 2

Table 2
MP2 /6-31G* total energies and zero point energies (hartrees) for the respective equilibrium geometries.

Molecule	MP2 hartree	ZPE (HF) hartree
TpTp	-2800.38521	0.52958
TpCp	-2741.33272	0.51215
TpAp	-2813.51549	0.52705
TpGp	-2888.57068	0.53177
CpTp	-2741.34479	0.51276
CpCp	-2676.43897	0.49605
CpAp	-2754.48487	0.50946
CpGp	-2829.54296	0.51577

Molecule	MP2 hartree	ZPE (HF) hartree
ApTp	-2813.52710	0.52693
ApCp	-2754.48275	0.50962
ApAp	-2826.66541	0.52375
ApGp	-2901.71595	0.52899
GpTp	-2888.58418	0.53263
GpCp	-2829.55248	0.51580
GpAp	-2901.70752	0.52818
GpGp	-2976.77098	0.53471

Also recorded are the enthalpy changes where the model is MP2, basis set 6-31G* and the zero point energies (HF) have been scaled and included. These values are given in Table.3-6

3.2 The Thermodynamic Data for Stacked Pyrimidine Dinucleotides at 298.15 K, HF Model, Basis Set 6-31G*.

The Gaussian program also produces the following thermodynamic data in which the zero-point energy is not scaled.

Table 3. The thermodynamic data for the stacking and glycosidic bond formation in the pyrimidine dinucleotides. Energies are in hartree (1 h = 627.5095 kcal.mol^{-1}) [15].

Di-nucleotide	E (HF) Total Electronic Energy	ZPE Zero-Point Energy.	H Electr. + Therm Enthalpy.
TpTp(1)	-2794.21631	0.52958	-2793.65371
dpTp(2)	-2418.72135	0.43171	-2418.26203
Tp (3)	-870.04279	0.26881	-869.75983
dp (4)	-494.56821	0.17175	-494.38804
T (5)	-451.49466	0.12318	-451.36399
TpCp (1)	-2735.31568	0.51215	-2734.77156
dpCp (2)	-2359.82751	0.41458	-2359.38635
Tp (3)	-870.04184	0.26892	-869.75873
dp (4)	-494.56929	0.17182	-494.38703
T (5)	-451.49466	0.12317	-451.36395
CpTp (1)	-2735.33174	0.51276	-2734.78697
dpTp (2)	-2418.72753	0.43148	-2418.26820
Cp (3)	-811.15031	0.25192	-810.88536
dp (4)	-494.56958	0.17169	-494.38942
C (5)	-39260509	0.10616	-392.49254
CpCp (1)	-2676.43894	0.49605	-2675.91216
dpCp (2)	-2359.83037	0.41456	-2359.38920
Cp (3)	-811.15175	0.25201	-810.88670
dp (4)	-494.56872	0.17174	-494.38852
C (5)	-392.60395	0.10632	-392.49124
H_2O	-76.01075	0.02148	-75.98777

Table.3 (cont).

Di-nucleotide	G (HF) Electronic + Thermal Free Energy	S Entropy (cal K^{-1} mol^{-1})	ΔH stacking and ΔH glycosidic bond
TpTp (1)	-2793.74574	193.701	ΔH stacking
dpTp (2)	-2418.34432	173.208	= -0.040
Tp (3)	-869.81364	113.241	
dp (4)	-494.42874	85.680	ΔH glycosidic
T (5)	-451.40248	81.002	= - 0.007
TpCp (1)	-2734.86174	189.807	ΔH stacking
dpCp (2)	-2359.46664	168.971	= -0.029
Tp (3)	-869.81264	113.476	
dp (4)	-494.42985	85.918	ΔH glycosidic
T (5)	-451.40251	81.165	= -0.005
CpTp (1)	-2734.87743	190.390	ΔH stacking
dpTp (2)	-2418.35115	174.584	= -0.033
Cp (3)	-810.93676	108.183	
dp (4)	-494.43025	85.920	ΔH glycosidic
C (5)	-392.52859	78.878	=-0.003
CpCp (1)	-2676.00010	185.093	ΔH stacking
dpCp (2)	-2359.46970	169.429	= -0.037
Cp (3)	-810.93822	108.440	
dp (4)	-494.42935	85.940	ΔH glycosidic
C (5)	-392.52730	75.877	= -0.006
H_2O	-76.00537	44.987	

3.3 The Thermodynamic Data for Stacked Purine Dinucleotides at 298.15 K, HF Model, Basis Set 6-31G*.

The corresponding thermodynamic data for the purine dinucleotides is given in Table 4..

Table 4. The thermodynamic data for the stacking and glycosidic bond formation in the deoxyribose dinucleotides. Energies are in hartree (1 h =627.5095 kcal.mol^{-1})

Di-nucleotide	E (HF) Total Electronic Energy	ZPE Zero-Point Energy.	H Electr. + Therm Enthalpy.
ApAp (1)	-2820.22623	0.52375	-2819.67048
dpAp (2)	-2431.73200	0.42856	-2431.27622
Ap (3)	-883.06012	0.26618	-882.78027
dp (4)	-494.57006	0.17185	-494.38778
A (5)	-464.50818	0.12042	-464.38086
ApGp (1)	-2895.10201	0.52899	-2894.54016
dpGp (2)	-2506.59992	0.43370	-2506.13801
Ap (3)	-883.05750	0.26579	-882.77800
dp (4)	-494.56940	0.17176	-494.38917
A (5)	-464.50939	0.12019	-464.38232
GpAp (1)	-2895.09646	0.52818	-2894.53520
dpAp (2)	-2431.73101	0.42828	-2431.27548
Gp (3)	-957.92830	0.27107	-957.64261
dp (4)	-494.56988	0.17174	-494.38973
G (5)	-539.38085	0.12560	-539.24747
GpGp (1)	-2909.98060	0.53471	-2969.41218
dpGp (2)	-2506.59777	0.43370	-2506.13596
Gp (3)	-957.92246	0.27115	-957.63670
dp (4)	-494.56909	0.17184	-494.38884
G (5)	-539.37306	0.12552	-539.23976

Table 4 (cont.)

Di-nucleotide	G (HF) Electronic + Thermal Free Energy	S Entropy (cal K^{-1} mol^{-1})	ΔG stacking and ΔG glycosidic bond
ApAp (1)	-2819.76103	190.592	ΔH stacking
dpAp (2)	-2431.35810	172.312	= -0.030
Ap (3)	-882.83345	111.929	
dp (4)	-494.43051	85.713	ΔH glycosidic
A (5)	-464.41844	79.099	= -0.007
ApGp (1)	-2894.63229	193.904	ΔH stacking
dpGp (2)	-2506.22195	176.674	= -0.038
Ap (3)	-882.83119	111.946	
dp (4)	-494.43001	85.943	ΔH glycosidic
A (5)	-464.41986	79.019	= -0.004
GpAp (1)	-2894.62799	195.302	ΔH stacking
dpAp (2)	-2431.35743	172.466	= -0.029
Gp (3)	-957.69774	116.033	
dp (4)	-494.43040	85.603	ΔH glycosidic
G (5)	-539.28705	83.318	= -0.003
GpGp (1)	-2969.50591	197.259	ΔH stacking
dpGp (2)	-2506.21966	176.166	= -0.057
Gp (3)	-957.69185	116.066	
dp (4)	-494.42954	85.673	ΔH glycosidic
G (5)	-539.27934	83.304	= -0.006

3.4 The Thermodynamic Data for Stacked Pyrimidine Purine Dinucleotides at 298.15 K, HF Model, Basis Set 6-31G*.

The corresponding thermodynamic data for the pyrimidine purine dinucleotides is given in Table 5.

Table 5. The thermodynamic data for the stacking and glycosidic bond formation in the deoxyribose dinucleotides. Energies are in hartree (1 h =627.5095 kcal.mol^{-1})

Di-nucleotide	E (HF) Total Electronic Energy	ZPE Zero-Point Energy.	H Electr. + Therm Enthalpy.
TpAp (1)	-2807.21289	0.52705	-2806.65304
dpAp (2)	-2431.70937	0.42903	-2431.25310
Tp (3)	-870.03959	0.27006	-869.75551
dp (4)	-494.56711	0.17244	-494.38633
T (5)	-451.49870	0.12320	-451.36794
ApTp (1)	-2807.22535	0.52693	-2806.66593
dpTp (2)	-2418.72789	0.43179	-2418.26836
Ap (3)	-883.05918	0.26617	-882.77936
dp (4)	-494.57023	0.17187	-494.38994
A (5)	-464.50816	0.12033	-464.38094
CpAp (1)	-2748.34029	0.50946	-2747.79921
dpAp (2)	-2431.73151	0.42832	-2431.27592
Cp (3)	-811.15131	0.25178	-810.88648
dp (4)	-494.56403	0.17153	-494.38401
C (5)	-392.60573	0.10610	-392.49324
ApCp (1)	-2748.33476	0.50962	-2747.79371
dpCp (2)	-2359.82725	0.41456	-2359.38617
Ap (3)	-883.05721	0.26611	-882.77743
dp (4)	-494.56819	0.17174	-494.38802
A (5)	-464.50779	0.12043	-464.38047
H_2O	-76.01075	0.02148	-75.98777

Table.5 (cont).

Di-nucleotide	G (HF) Electronic + Thermal Free Energy	S Entropy (cal K^{-1} mol^{-1})	ΔH stacking and ΔH glycosidic bond
TpAp (1)	-2806.74531	194.196	ΔH stacking
dpAp (2)	-2431.33452	171.352	= -0.041
Tp (3)	-869.80886	112.272	
dp (4)	-494.42664	84.837	ΔH glycosidic
T (5)	-451.40653	81.216	= -0.001
ApTp (1)	-2806.75716	192.018	ΔH stacking
dpTp (2)	-2418.35094	173.789	= -0.029
Ap (3)	-882.83244	111.725	
dp (4)	-494.43064	85.672	ΔH glycosidic
A (5)	-464.41851	79.063	= -0.006

Di-nucl eotide	G (HF) Electronic + Thermal Free Energy	S Entropy (cal K^{-1} mol^{-1})	ΔH stacking and ΔH glycosidic bond
CpAp (1)	-2747.88941	189.827	ΔH stacking
dpAp (2)	-2431.35812	173.001	= -0.037
Cp (3)	-810.93795	108.325	
dp (4)	-494.42483	85.913	ΔH glycosidic
C (5)	-392.52930	75.892	= -0.007
ApCp (1)	-2747.88309	188.123	ΔH stacking
dpCp (2)	-2359.46655	169.178	= -0.014
Ap (3)	-882.83057	111.855	
dp (4)	-494.42874	85.703	ΔH glycosidic
A (5)	-464.41804	79.080	= 0.012

Table 6. The thermodynamic data for the stacking and glycosidic bond formation in the pyrimidine / purine deoxyribose dinucleotides. Energies are in hartree (1 h =627.5095 kcal.mol^{-1})

Di-nucl eotide	E (HF) Total Electronic Energy	ZPE Zero-Point Energy.	H Electr. + Therm Enthalpy.
CpGp (1)	-2823.21096	0.51577	-2822.66335
dpGp (2)	-2506.59777	0.44395	-2506.13587
Cp (3)	-811.14655	0.25165	-810.88190
dp (4)	-494.56424	0.17149	-494.38426
C (5)	-392.60372	0.10600	-392.49135
GpCp (1)	-2823.22505	0.51580	-2822.67731
dpCp (2)	-2359.82588	0.41433	-2359.38492
Gp (3)	-957.92461	0.27162	-957.63851
dp (4)	-494.56963	0.17184	-494.38937
G (5)	-539.37592	0.12576	-539.24244
TpGp (1)	-2882.09718	0.53177	-2881.53187
dpGp (2)	-2506.60011	0.43376	-2506.13813
Tp (3)	-870.04400	0.26873	-869.76049
dp (4)	-494.56750	0.17176	-494.38727
T (5)	-451.49697	0.12325	-451.36610
GpTp (1)	-2882.10900	0.53263	-2881.54303
dTp (2)	-2418.72579	0.43153	-2418.26635
Gp (3)	-957.92655	0.27140	-957.64067
dp (4)	-494.56877	0.17171	-494.38860
G (5)	-539.38024	0.12574	-539.24677

Table.6 (cont).

Di-nucleotide	G (HF) Electronic + Thermal Free Energy	S Entropy (cal K^{-1} mol^{-1})	ΔH stacking and ΔH glycosidic bond
CpGp (1)	-2822.75325	189.227	ΔH stacking
dpGp (2)	-2506.21908	175.129	= -0.046
Cp (3)	-810.93337	108.333	
dp (4)	-494.42514	86.033	ΔH glycosidic
C (5)	-392.52736	75.787	=- 0.005
GpCp (1)	-2822.76738	189.564	ΔH stacking
dpCp (2)	-2359.46564	169.877	= -0.062
Gp (3)	-957.69340	115.525	
dp (4)	-494.43015	85.823	ΔH glycosidic
G (5)	-539.28194	83.142	= -0.006
TpGp (1)	-2881.62558	197.221	ΔH stacking
dpGp (2)	-2506.22214	176.807	= -0.034
Tp (3)	-869.81486	114.434	
dp (4)	-494.42819	86.116	ΔH glycosidic
T (5)	-451.40482	81.485	=-0.005
GpTp (1)	-2881.63577	195.191	ΔH stacking
dTp (2)	-2418.34940	174.787	= -0.026
Gp (3)	-957.69510	114.554	
dp (4)	-494.42940	85.862	ΔH glycosidic
G (5)	-539.28628	83.140	= 0.014
H_2O			

Also recorded are the free energy changes where the model is MP2, basis set 6-31G* and the entropy values have been taken from the low accuracy HF data recorded in Table 3-6. These free energy values also contain the zero point energies (HF) which have been scaled and included. These values are given in Table.7

Table.7. The ΔG values for stacking (h) and glycosidic bond formation (h) for pyrimidine and purine deoxyribose dinucleotides**.** T=298.15 K.

Di-nucleotide	ΔG stacking	ΔG glycosidic
TpTp	-0.03592	-0.00290
TpCp	-0.02574	-0.00048
TpAp	-0.03851	-0.00371

TpGp	-0.02989	-0.00149
CpTp	-0.03006	0.00236
CpCp	-0.03359	-0.00222
CpAp	-0.03416	-0.00338
CpGp	-0.04170	-0.00098
ApTp	-0.02480	-0.00208
ApCp	-0.03437	-0.00292
ApAp	-0.02594	-0.00285
ApGp	-0.03350	-0.00017
GpTp	-0.03832	-0.00162
GpCp	-0.05737	-0.00151
GpAp	-0.02568	0.00069
GpGp	-0.05212	-0.00176

The relative stacking free energy values, ΔG, given in Table.8, indicate that the deoxyribose dinucleotide stacking values are generally lower than, or comparable, for the ribose dinucleotide values, ensuring that most DNAs would be more stable with regard to stacking than most RNAs. The sum of the free energy values for the deoxyribose dinucleotides is -0.562 h, whereas that for the ribose dinucleotides is -0.506 h

Table.8 Comparison of the ΔG stacking Values for Ribose Dinucleotides and 2-Deoxyribose Dinucleotides

	U	C	A	G
U	-0.02347	-0.02792	-0.02752	-0.03208
C	-0.02507	-0.03205	-0.03071	-0.04256
A	-0.02491	-0.03008	-0.02714	-0.02933
G	-0.03345	-0.05684	-0.02620	-0.03704

	T	C	A	G
T	-0.03592	-0.02574	-0.03851	-0.02989
C	-0.03007	-0.03359	-0.03416	-0.04170
A	-0.02480	-0.03437	-0.02594	-0.03350
G	-0.03832	-0.05737	-0.02568	-0.05212

The values recorded are largely in-line with published stacking energies [20-21].

3.5 The Watson-Crick Free Energy Values for Horizontal Base Pairing, Table 9.

The Watson-Crick horizontal hydrogen bonding interactions in the A-T and G-C dimers were calculated in the same manner as for the stacking interactions for charges on both nucleotides being either 0, 1, or 2, giving a total dimer charge of either 0, 2 or 4. Non Watson-Crick hydrogen bonding in solution was not considered [20].
Using this data the free energy of formation of the two hydrogen bonds for an adenine-thymine (A-T) interaction and the three hydrogen bonds of a guanine-cytosine (G-C) were calculated as shown in Table 9

Table 9. Ribose dinucleotide MP2 /6-31G* total energies and zero point energies (hartrees) for the respective equilibrium geometries.

Molecule	MP2 hartree	ZPE hartree	S(HF) cal. $mol^{-1}K^{-1}$
Charge per Nucleotide = 0			
Ap-Up	-3002.45946	0.57386	215.500
Ap	-1527.37680	0.26649	132.293
Up	-1475.05250	0.27279	128.133
ΔH (h)	-0.02869		
ΔG (h)	-0.00734		
Charge per Nucleotide = 0			
CpGp	-3057.65643	0.59223	218.281
Cp	-1455.18783	0.28503	129.422
Gp	-1602.41676	0.30506	136.111
ΔH (h)	-0.04997		
ΔG (h)	-0.02752		

The value for the uncharged Watson-Crick horizontal base pairing may also be calculated from the respective ribose dinucleotides carrying charges of -2 and -4, by compensating for the electric repulsion of the formal charges using Coulomb's Law, where,

$$\Delta H(-2) = \Delta H(0) + 332.159\, Q1.Q2 \;/\; D\, R \quad (2)$$

ΔH(-2) is the enthalpy change for the formation of the Watson Crick hydrogen bonding when the nucleotides carry a total charge of -2, taken to be evenly distributed over the phosphate oxygen atoms that are only bonded to the phosphorus atom. ΔH(0) is the corresponding value where the total formal charge on the two nucleotides is zero, Q1 and Q2

are the charges on the respective nucleotides in units of the electronic charge. R is the distance in Angstrom separating the charges, and D is the dielectric constant [23]. This is largely an empirical constant as the molecule is in a charged state difficult to physically replicate.
Assuming the dielectric constant is unity [23], the values obtained from ΔH(-2) values are

ΔH(0) for A-T = -0.02693 h
ΔH(0) for C-G = -0.04559 h

The data for the corresponding deoxyribose Watson-Crick interactions were the same as for the ribose dinucleotides within the achievable accuracy. Although the A-T interaction is regarded as very weak in a double helix [24], it is expected that the bases would substantially stack before completely hydrogen bonding, so that the entropy change for free nucleotides would be less than that actually involved in the hydrogen bonding. For this reason the A-T hydrogen bonding value was taken as an adjustable parameter and increased by 10% to improve correlation with experimental results. The values used in the calculations are as shown in Table.10.

Table 10. Thermodynamic data for Watson-Crick Hydrogen Bonding Interactions in the gas phase (h). T=298.15 K.

Hydrogen Bonding Interaction	ΔH (h)	ΔG (h)	ΔG (h) adjusted value
A-U	-0.028689	-0.00734	-0.031699
G-C	-0.049973	-0.02752	-0.049973

The results should be divided by 50 to be realistic for aqueous solutions [25].

3.6 The Watson-Crick Base Pairing in the Ten Anti-parallel Dublet Duplexes

When only Watson-Crick base pairing is considered, the free energy values for stacking and hydrogen bonding may be combined to predict the stability of the ten anti-parallel dublet duplexes, with the values shown in Table 11.

Table 11. Free energy(h) changes calculated for the ten antiparallel deoxyribose dublet duplexes. T=298.15K.

Duplex 5'-3'/ 3'-5'	ΔG (h) DNA/DNA	ΔG (h) DNA/RNA	ΔG (h) RNA/RNA
AT/TA	-0.11300	-0.113106	-0.113212
TT/AA AA/TT	-0.12525	-0.126455 -0.112800	-0.114007
TC/AG GA/CT	-0.13309	-0.133613 -0.140239	-0.135795
TA/AT	-0.14042	-0.129429	-0.118435
CT/GA AG/TC	-0.14524	-0.141073 -0.140239	-0.136070
CA/GT TG/AC	-0.14572	-0.147192 -0.142273	-0.144461
AC/TG GT/CA	-0.15436	-0.149492 -0.150070	-0.145210
CG/GC	-0.18336	-0.184206	-0.185058
CC/GG GG/CC	-0.18565	-0.170568 -0.184113	-0.169034
GC/CG	-0.21469	-0.214153	-0.213617

Whilst a dinucleotide stack may try to hybridize with any other dinucleotide stack in an anti-parallel duplex, these free energy values predict that the correct Watson-Crick duplexes will be the most preferred energetically, in every case. This sequence of free energy values is in general correlation with experimental determinations [26]. In general both polynucleotide strands of the duplex may be translated with different free energy changes, with one translation being preferred.

3.7 The Watson-Crick base pairing in the ribose nucleotide / ribose nucleotide anti-parallel triplet duplexes (Hybridization)

This sort of pairing occurs in the translation of mRNA into protein mediated by the pairing of mRNA and tRNA in the ribosome [27]. This occurs in an anti-parallel hybridization where stacking may be involved [28] to some extent. The standard genetic code allows for some degeneracy of the triplets that code for a particular amino acid in a particular species [19]. In this calculation the free energy values for the formation of the antiparallel triplet stacks for a particular amino acid were averaged, as shown in Table 12.

Table 12. Free energy(h) values for RNA-RNA hybridization in anti-parallel triplet stacks. T=298.15 K.

Amino Acid	Codons	ΔG triplet anti- parall duplex
i-Leu /I	AUU AUC AUA	-0.20426
Phe /F	UUU UUC	-0.20721
Lys /K	AAA AAG	-0.20735
Asn /N	AAU AAC	-0.21154
Tyr /Y	UAU UAC	-0.21594
STOP	UAA UAG UGA	-0.21794
Met /M	AUG	-0.22598
Leu /L	UUA UUG CUU CUC CUA CUG	-0.22628
Glu /E	GAA GAG	-0.22913
Asp /D	GAU GAC	-0.23330
Gln /Q	CAA CAG	-0.23780
Val /V	GUU GUC GUA GUG	-0.24168
His /H	CAU CAC	-0.24197
Ser /S	UCU UCC UCA UCG	-0.24448
Thr /T	ACU ACC ACA ACG	-0.25388
Trp /W	UGG	-0.26352
Cys /C	UGU UGC	-0.27390
Pro /P	CCU CCC CCA CCG	-0.27772
Gly /G	GGU GGC GGA GGG	-0.28497
Arg /R	CGU CGC CGA CGG	-0.30100
Ala /A	GCU GCC GCA GCG	-0.32230

The considerable achievement of the standard genetic code is to use the whole spread of accessible free energy values whilst maintaining the high degree of specificity of the Watson-Crick base pairs as being the preferred hybridization. Clearly steric factors may greatly improve the actual pairing, but there is always the possibility of error, especially if the temperature rises.

4. Conclusion

1.The results are broadly in agreement with literature gradations [11,20-21], and experimental studies [7], except that the GpCp and CpGp interactions are large causing greater stacking for the group pyrimidine-purine dinucleotides than for the group purine-purine dinucleotides.
2.The sum of the free energy changes for the formation of the Watson-Crick hydrogen bonding interactions (A+T) is less than for (G+C) ensuring a ratio of (A+T)/(G+C) of < 1.0 in poly deoxy nucleotides.
3.The di-deoxynucleotides free energies of stacking are lower or comparable in energy to that of the corresponding ribose dinucleotides rendering DNA stacking more stable than in RNA.
9.Both the codons and anticodons may be naturally translated as the free energy change to form the complimentary strand (Watson-Crick hydrogen bonding + stacking interaction) is negative.
10.There is a potential for an error in translation of the codons if the free energy change for the stacking of an added base is more negative than for the sum of the (Watson Crick hydrogen bonding interaction + the stacking free energy) for the correct complimentary added base, unless steric effects are dominant.
The separation of values appears sufficient for them to have possibly influenced the formation of the first genes.

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