

Theoretical Description of the Coding Potential of Diamino-5-formamidopyrimidines*

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The results of geometry optimisation of possible Watson-Crick-like pairs of 2,6-diamino-4-oxy-5-formamidopyrimidine (fapy-adenine) or 4,6-diamino-5-formamidopyrimidine (fapy-guanine) were presented. In the absence of the external field the fapy-adenine is able to form pairs with all four canonical nucleic acid bases. However, pairs with guanine, cytosine and thymine the most stable are. Thus, the potential miscoding abilities may be observed. In contrast, in the presence of the external field the mispairing abilities of fapy-adenine become insignificant since the most stable dimers are formed with thymine.

The pairing properties of fapy-guanine are complex and depend on its tautomeric form. In the absence of an external field the 4-enol-6-keto-diamino tautomer of fapyG is able to form stable dimers with thymine and cytosine, while the 4,6-diketo-diamino tautomer forms the most stable pairs with cytosine and guanine. The presence of the water solvent does not significantly alter the pairing abilities of fapy-guanine. However, pairs with thymine are at least as stable as the Watson-Crick GC pair. Thus, in polar conditions the mispairing potential of fapyG will be extended and may be enriched by potential GC → AT transition.

Introduction

Reactive oxygen-derived free radicals are produced by ionising radiation, various chemical reagents and also during normal metabolic processes inside living cells (Sonntag, 1987; Dizdaroglu and Aruoma, 1993). Such radicals can react with genomic DNA causing modifications of purines, pyrimidines and the deoxyribose (Oliński *et al.*, 1992). Among the variety of free radical-mediated DNA lesions the most important seem to be products of the oxidation of 2'-deoxynucleosides at the C₈ position (Grollmann, 1992). The oxidation of C₈ carbon atom may result in the ring opening and formation of 2,6-diamino-4-oxy-5-formamidopyrimidine or 4,6-diamino-5-formamidopyrimidine (O'Connor *et al.*, 1988; Neto *et al.*, 1992). The names of these products are usually abbreviated as fapy-guanine (fapy-G) and fapy-adenine (fapy-A) (Lutgerink *et al.*, 1992; Muller *et al.*, 1995). The 5-formamidopyrimidines were identified in mammalian tissues such as cancerous female breast,

lung brain, and other human tissues (Malins, 1993; Oliński *et al.*, 1992). Thus, such DNA lesions are broadly present in the cancerous and normal tissues of a variety of eukaryotic organisms and are considered to be one of the causes of mutagenesis and carcinogenesis (Boiteux *et al.*, 1989). Sambamurti *et al.* (1991) observed G → T transversions and G → A transitions during transfection of modified DNA into *Escherichia coli* cells. They suggested that the presence of fapy-guanine in the DNA may be a cause of such mutations. Besides, it is generally assumed that the presence of fapy-G strongly blocks DNA synthesis *in vitro* (Tudek *et al.*, 1992; O'Connor *et al.*, 1988; Laval J., 1996). Additionally, oxidative guanine and adenine damage has been implicated in the pathology of different diseases such as Parkinson's disease (Alam *et al.*, 1997) and neuronal loss in Alzheimer's disease (Lyras *et al.*, 1997). However, there is no experimental evidence concerning mutagenic properties of both diamino-5-formamidopyrimidines.

Apparent from experimental studies on the hydroxyl radical modified DNA bases there are efforts to describe the fundamental properties of such derivatives in a theoretical way. The tautomeric properties of fapy-adenine and fapy-guanine were the subjects of theoretical investigations (Cysewski

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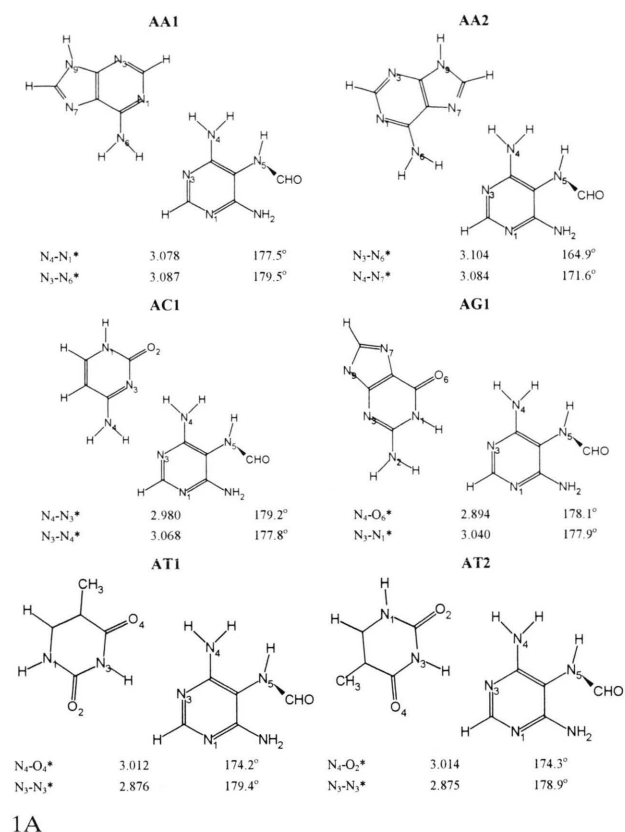
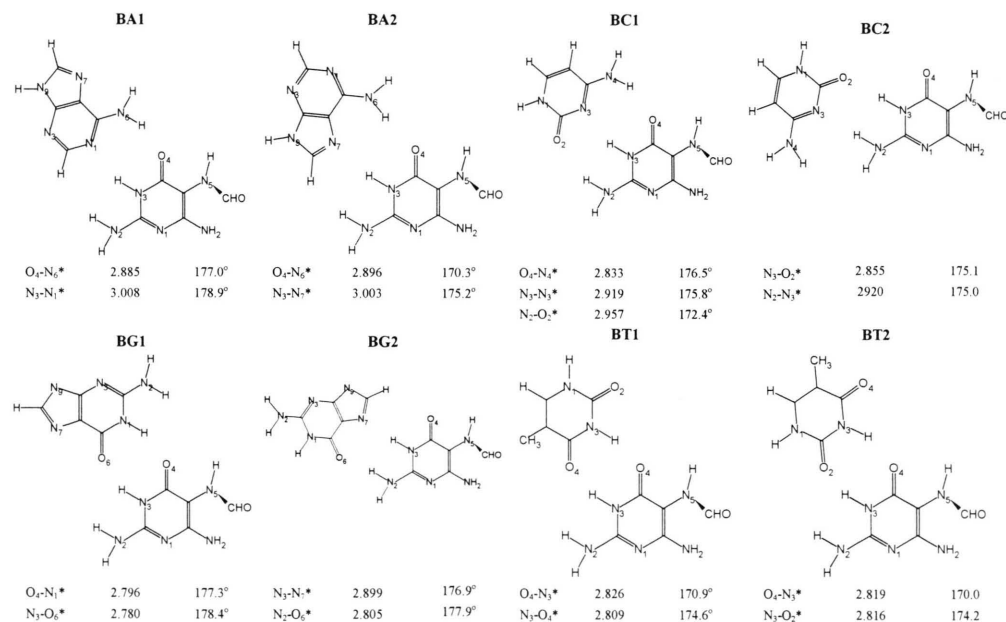
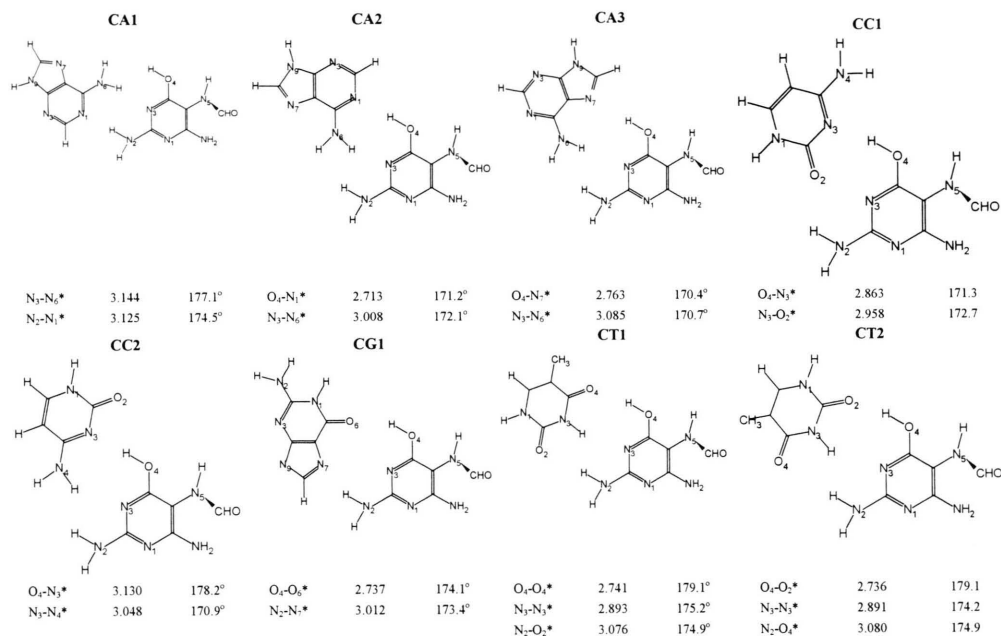


Fig. 1. Structures of the analysed pairs of fapy-adenine and fapy-guanine and standard nucleic acid bases. Part A presents possible dimers formed by the most stable tautomer of fapy-A. Parts B and C contain the pairs of two most stable tautomers of fapy-G with canonical DNA bases. Part D presents pairs of these tautomers of fapy-G which are able to form three hydrogen bonds with DNA bases. Apart of the structure of the analysed pair the optimised geometrical parameters are presented. Below each dimer the hydrogen bond lengths and hydrogen bond angles are noted followed the column specifying the donor centres. A star denotes the atom at the standard DNA base. The hydrogen bond lengths are expressed in angstroms and were measured as the distance between donor centres. Hydrogen bond angles (in degrees) were estimated as the angle between first donor centre – hydrogen atom and second donor.





1C

et al., 1995, 1996; Cysewski, 1999). However, no results have been presented until now describing the pairing abilities of diamino-5-formamidopyrimidines. This paper is the continuation of a project describing the properties of hydroxyl radical modified nucleic acid bases. The aim of this work is to characterise the pairing potential of fapy-guanine and fapy-adenine by means of gradient geometry optimisations. The intermolecular hydrogen bond formations is considered as one of driving forces toward pairs formation between DNA bases. This work analyses the stability of pairs consisting of the fapy-A or fapy-G and one of the canonical nucleic acid bases. The analogues of Watson-Crick-like were build and optimised by means of non-empirical quantum chemistry technique.

Results and Discussion

The diamino-5-formamidopyrimidines may have intricate structures since they are able to form a non-planar part coming from C₈-N₉ bond breaking and they are able to form a broad range of tautomers. The tautomeric form is crucial for the description of the electrostatic and pairing properties of fapy-adenine and fapy-guanine. In the pre-

vious paper (Cysewski, 1998b) the results of theoretical geometry prediction of fapy-adenine (fapy-A) and fapy-guanine (fapy-G) tautomers were presented. Although the fapy-adenine may potentially exist in 54 tautomeric forms the most probable structure corresponds to the diamino-keto isomer. The fapy-guanine has 172 potential tautomeric structures. Two of them are most stable and may change their order depending on the polarity of the environment. In vapour the most probable is the 4-enol-6-keto-diamino tautomer, while in a water environment the 4,6-diketo-diamino isomer is dominant. A more polar solvent stabilises more polar fapy-guanine tautomers.

These tautomeric forms were used to describe the pairing potential of the diamino-5-formamidopyrimidines. The Watson-Crick-like pairs were constructed and their geometry was optimised by *ab initio* quantum chemistry gradient minimisation. The 6-31G basis set was applied to find the optimal geometry without any restriction on the coordinates. Single point energy estimation was performed in the more refined basis set (6-31G**). The pair stability energy was calculated as the difference between pair energy and isolated monomers with correction for basis superposition error

<p>DC1</p> <p> $O_4-N_4^*$ 2.686 179.5° $N_7-N_3^*$ 2.849 179.6° $N_2-N_6^*$ 2.882 176.4° </p>	<p>DC2</p> <p> $O_4-O_2^*$ 2.704 177.9° $N_7-N_3^*$ 2.906 177.8° $N_2-N_6^*$ 2.963 175.3° </p>	<p>DC3</p> <p> $O_4-N_4^*$ 3.032 174.0° $N_7-N_3^*$ 3.041 176.9° $N_2-O_2^*$ 3.102 169.0° </p>	<p>DC4</p> <p> $O_4-N_4^*$ 2.982 172.5° $N_7-N_3^*$ 3.093 172.4° $N_2-O_2^*$ 3.195 171.9° </p>
<p>DC5</p> <p> $O_4-N_4^*$ 2.869 175.7° $N_7-N_3^*$ 2.957 174.7° $N_2-O_2^*$ 3.159 166.8° </p>	<p>DC6</p> <p> $O_4-N_4^*$ 3.018 175.2° $N_7-N_3^*$ 3.023 177.5° $N_2-O_2^*$ 2.947 174.5° </p>	<p>DG1</p> <p> $O_4-O_2^*$ 2.592 179.3° $N_7-N_1^*$ 2.901 170.7° $N_2-N_6^*$ 3.024 173.5° </p>	<p>DG2</p> <p> $O_4-N_2^*$ 3.033 179.0° $N_7-N_1^*$ 3.070 179.7° $N_2-O_6^*$ 3.050 173.2° </p>
<p>DG3</p> <p> $O_4-N_2^*$ 3.028 177.5° $N_7-N_1^*$ 3.058 177.5° $N_2-O_6^*$ 2.887 177.2° </p>	<p>DG4</p> <p> $O_4-N_2^*$ 3.039 175.9° $N_7-N_1^*$ 3.028 175.2° $N_2-N_6^*$ 2.797 177.9° </p>	<p>DT1</p> <p> $O_4-O_2^*$ 2.798 177.4° $N_7-N_3^*$ 2.901 176.4° $N_2-O_4^*$ 2.967 177.0° </p>	<p>DT2</p> <p> $O_4-O_2^*$ 2.684 178.8° $N_7-N_3^*$ 2.917 171.4° $N_2-O_4^*$ 3.325 171.9° </p>
<p>DT3</p> <p> $O_4-O_2^*$ 2.794 178.2° $N_7-N_3^*$ 2.895 177.3° $N_2-O_4^*$ 2.976 176.1° </p>	<p>DT4</p> <p> $O_4-O_4^*$ 2.683 178.9° $N_7-N_3^*$ 2.913 172.3° $N_2-O_4^*$ 3.317 171.9° </p>		

1D

(BSSE) (Frisch *et al.*, 1986). The energy of each pair was also calculated in the presence of the water electrostatic field (Schmidt *et al.*, 1993). More details about the calculations may be found in a previous paper (Cysewski P., 1998).

The pairing potential of fapy-adenine is presented in Figs 1A and 2. The geometric parameters of the studied pairs of fapy-A are collected in the first figure. The second presents the values of dimer energy. In the absence of the external field the fapy-adenine is able to form pairs with all four canonical nucleic acid bases. However, the most

stable pairs are those with guanine, cytosine and thymine.

In the absence of the external field the standard adenine–thymine pair is characterised by a stability energy equal to -55.86 kJ/mol (in 6-31G basis set) and -46.11 kJ/mol (in 6-31G**/6-31G basis), respectively. Comparison of these values to the stabilisation energies of fapy-adenine dimer lead to the conclusion that the pairs with guanine and cytosine are more stable than the standard AT pair. The stability of the fapy-adenine complex with thymine is almost the same as that of the AT pair. The ener-

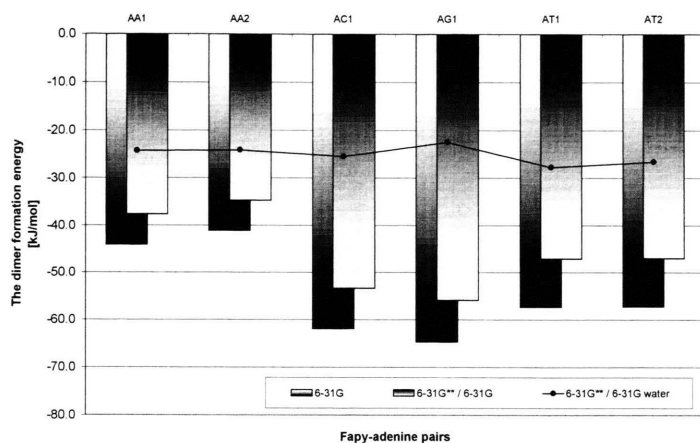


Fig. 2. Stabilisation energies of fapy-adenine pairs. The plotted energies were calculated as difference between energies of the pair and isolated DNA base and fapy-adenine. The two bars represent energies related to different approximations (6-31G and 6-31G**/6-31G). The energies were corrected for the basis superposition error (BSSE) (Frisch *et al.*, 1986). The solid line stands for results estimated in the presence of the electrostatic water field in the 6-31G**/6-31G approximation (Schmidt *et al.*, 1993).

gies of fapy-adenine and adenine pairs are lower than standard the Watson-Crick AT pair. Thus, one can expect that there is a driving force toward formation of alternative pairs of fapy-adenine with guanine and cytosine. The higher stability of these pairs may be responsible for AT → CG transversions and AT → GC transitions.

However, the presence of the external field may change the pairing abilities of fapy-adenine. The significant impact of water electrostatic potential on the relative pairs' stabilities may be observed. In this case all pairs are characterised by almost the same energy. However, pairs with thymine are more probable than others. In the same conditions the standard adenine–thymine pair is characterised by the energy equal to -30.79 kcal/mol. Thus, in the presence of the external field the mispairing abilities of fapy-adenine become insignificant.

The fapy-guanine pairing potential was analysed using two different methods. The pairs of the two most stable tautomers were used for building Watson-Crick-like pairs with standard nucleic acid bases. Additionally the pairs of other tautomers of fapy-G were also analysed if they were able to form three hydrogen bonds. The results of geometry optimisation are presented in Figs 1 B and 1 C. The energy of studied fapy-guanine pairs is plotted in Fig. 3. The 4,6-diketo-diamino tautomer of fapy-G forms very stable pairs with cytosine and guanine. The standard guanine–cytosine pairs is characterised by the following stabilisation energies: -129.41 kJ/mol (in 6-31G basis set) and -114.18 kJ/mol (in 6-31G**/6-31G basis), respectively. The 4-enol-6-keto-diamino tautomer has a different pairing ability. In the absence of an external field the most stable dimers are formed with

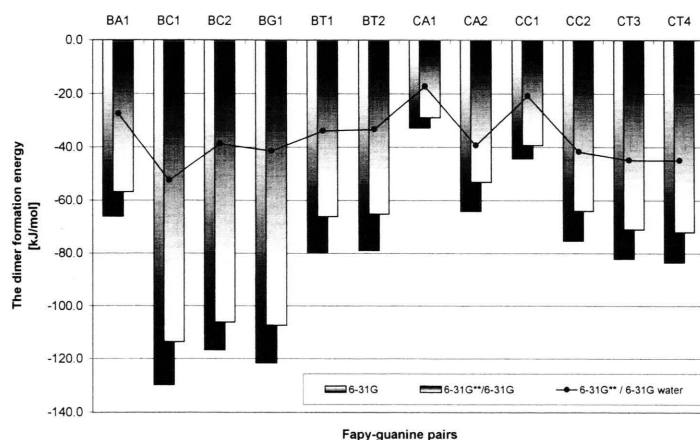


Fig. 3. Stabilisation energies of 4,6-diketo-diamino tautomer and 4-enol-6-keto-diamino fapy-guanine pairs. Notation as in Fig. 2.

thymine and cytosine. However, the stabilisation energy is less compared to GC pair.

Thus, in the non-polar environment the fapy-guanine will have a potential mispairing character corresponding to the formation of alternative dimers with guanine apart from the normal pairing with cytosine. The high stability of the fapy-G–G pair may be responsible for GC → GC transversions.

In the presence of the water electrostatic potential the standard guanine–cytosine pair is characterised by energy equal to -43.01 kJ/mol. The comparison of this value to data presented in Fig. 3 leads to the conclusion that presence of the water solvent does not change significantly the pairing abilities of fapy-guanine. However, this time the pairs with thymine are at least as stable as the Watson-Crick GC pair. Thus, in polar conditions the mispairing potential of fapy-G will be extended and may also include GC → AT transition.

The last group of pairs studied in this paper consisted of those tautomers of fapy-guanine, which are potentially able to form three hydrogen bonds. Despite the lower stability of such tautomers they are able to form very stable pairs with standard nucleic acid bases. The results of calculations are presented in Figs 1 D and 4. As one can see the formation of three hydrogen bonds may compensate for the lower stability of tautomers and may lead to highly favourable dimers. For example DC1, CD2 and DG1 pairs are more stable not only than the standard GC pair but also than BC1 and BG1 dimers. This fact makes the problem of the miscoding properties of fapy-guanine (and perhaps other DNA base derivatives) more complex. This feature has been neglected until now (Morgan, 1993). The

calculations of Hroudá *et al.* (1994) have shown that, concerning A–T pair formation, any modification of the tautomer form of adenine or thymine leads to the significant destabilisation of the resulting pair. This is not the case for fapy-G dimers. A similar conclusion was drawn in the case of pairing of 8-oxo-guanine (Cysewski, 1998c). However, despite the very high stability of pairs formed by other fapy-G tautomers the overall mispairing potential remains unchanged. Both in polar and non-polar environments the most stable pairs are formed with cytosine, guanine and thymine.

Unfortunately there are no experimental data to compare our results with. However, several groups of investigators have observed that oxidatively modified guanine moiety may give rise to G → C transversions (Tudek *et al.*, 1992; Sambamurti *et al.*, 1991). Since 8-oxo-guanine can pair with cytosine and adenine during replication but not with guanine, another guanine derivative could be responsible for G → C substitution. Our results suggest that the presence of fapy-guanine may be involved in G:C mispairing, which may yield G → C substitution. Our results also imply that in hydrophobic environment fapy-adenine may have miscoding properties too. Since, the interior of DNA is much less polar than the exterior, one can anticipate that fapy-adenine may also contribute to mispairing.

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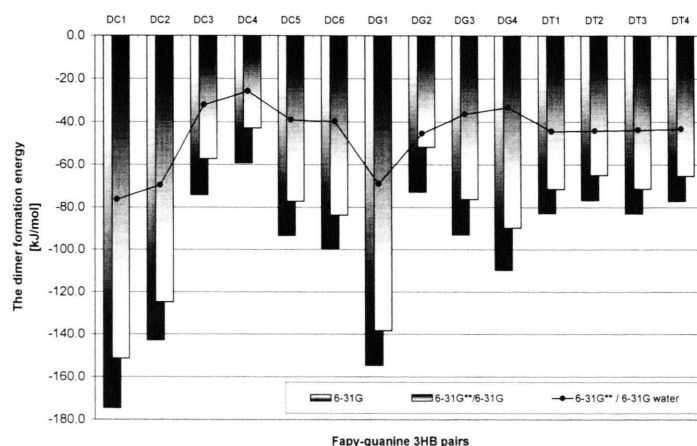


Fig. 4. Stabilisation energies of pairs consisting of fapy-guanine tautomers that are able to form three hydrogen bonds with canonical DNA bases. Notation as in Fig. 2.

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