



Key microstructural mechanisms of the 2-aminopurine mutagenicity: Results of extensive quantum-chemical research

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ABSTRACT

As of today, a great amount of experimental and theoretical phenomenological data have been collected in the literature according to the mutagenic action of the classical mutagen – 2-aminopurine (2AP). However, so far they have not received proper explanation and substantiation. In this Opinion Piece, we provide an overview of recent progress in computational design and modeling of the physico-chemical mechanisms of the mutagenic action of 2AP. Results of quantum-chemical studies, aimed at the elucidation of the key microstructural mechanisms of the mutagenicity of 2AP, have been summarized here. In this context, for the first time it was outlined the most important surveys: Why 2AP is incorporated into DNA in trace concentrations? Whether classical mechanisms presented in the literature according to the formation of the rare tautomers of canonical DNA bases work also for base analogue – 2AP? In what way 2AP induces replication and incorporation errors? Whether the amino-imino tautomerisation of 2AP is related to its mutagenicity, that is whether the 2AP* rare tautomer is mutagenic? It is emphasized that the applied approach has a proper theoretical substantiation, since it is based on our microstructural theory of the spontaneous point mutagenesis in DNA, and at the same time it accumulates scenarios of the origin of the induced point errors – transitions and transversions, which the classical Watson–Crick tautomeric hypothesis permits. Moreover, using author's methodology, the profiles of the main physico-chemical characteristics for the tautomerisation reactions involving 2AP, which are integral parts of the biologically important tautomerically-conformational transformations, have been presented. Obtained results open new perspectives for prediction and design of the mutagenic derivatives of the nucleotide bases of any structure and origin before their synthesis and also for planning of new experiments and interpretation of the existing data.

Abbreviations: 2AP: 2-Aminopurine; A: adenine; C: cytosine; DPT: double proton transfer; G: guanine; IRC: intrinsic reaction coordinate; KP: key point; T: thymine; w: wobble; WC: Watson–Crick; vdW: van der Waals

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Introduction

2-aminopurine (2AP) is the high-energetic structural isomer of adenine (A) nucleotide base (Hovorun, 1997) resembling it by coding properties (Freese, 1968; Wacker, Lodemann, Gauri, & Chandra, 1966). It is one of the most well known and most powerful classical mutagen, that has been intensively investigated both by the experimental (Bessman, Muzyczka, Goodman, & Schnaar, 1974; Glickman, 1985; Goodman, Hopkins, & Gore, 1977; Guest, Hochstrasser, Sowers, & Millar, 1991; Fagan et al., 1996; Freese, 1968; Osborn, Person, Phillips, & Funk, 1967; Pitsikas, Patapas, & Cupples, 2004; Ramaekers, Adamowicz, & Maes, 2002; Reha-Krantz et al., 2011; Rein & Garduno, 1976; Sowers, Boulard, & Fazakerley, 2000; Sowers, Fazakerley, Eritja, Kaplan, & Goodman, 1986; Sowers et al., 1989; Wacker, Lodemann, Gauri, & Chandra, 1966; Watanabe & Goodman, 1981, 1982) and theoretical (Broo & Holmén, 1996; Reha-Krantz et al., 2011; Ronen, 1980; Sherer & Cramer, 2001) methodologies.

Besides this, 2AP is actively used as a fluorescent probe (Alemán et al., 2014; Bonnist, Liebert, Dryden, Jeltsch, & Jones, 2012; Dallmann et al., 2010; Elvin, Alemán, & Rueda, 2011; Guest et al., 1991; Holz, Weinhold, Klimasauskas, & Serva, 1998; Jones & Neely, 2015; Lenz et al., 2007; Millar, 1996; Neely et al., 2009; Rachofsky, Osman, & Ross, 2011; Reha-Krantz, 2009; Rist & Marino, 2002; Tleugabulova & Reha-Krantz, 2007; Ward, Reich, & Stryer, 1969) to monitor the DNA and RNA conformational properties (Dallmann et al., 2010; Guest et al., 1991; Jones & Neely, 2015; Millar, 1996; Rachofsky, Osman, & Ross, 2011; Rist & Marino, 2002), the nature of the DNA-protein interactions (Jones & Neely, 2015), in particular with DNA-methyltransferases (Bonnist et al., 2012; Holz, Weinhold, Klimasauskas, & Serva, 1998; Lenz et al., 2007) and restriction enzymes (Neely et al., 2009), the function of DNA-polymerase (Reha-Krantz, 2009; Tleugabulova & Reha-Krantz, 2007) and the DNA biomolecular properties (Alemán et al., 2014; Elvin, Alemán, & Rueda, 2011).

It was experimentally established, that although 2AP enzymatically incorporates into the DNA double helix in trace concentrations (Goodman, Hopkins, & Gore, 1977), it intensively induces point mutations – base substitutions, the lion's share (~95%) of which makes up transitions (Osborn, Person, Phillips, & Funk, 1967). At this, the frequency of the origin of the A-T→G-C transitions exceeds the frequency of the occurrence of the G-C→A-T transitions (Goodman, Hopkins, & Gore, 1977; Ronen, 1980), while the experimental data according possible transversions induced by 2AP in DNA are absent in the literature at all.

To put it briefly, the phenomenon of the key microstructural mechanisms of the mutagenic action of 2AP, that has been persistently studied for decades beginning from the classical work of Freese published in 1959 year (Freese, 1959a), still remain now obscure.

Moreover, the standpoint according the origin of the 2AP mutagenic activity has not been crystallized up to date due to the fact that a proper theoretical background on the nature of the spontaneous point mutations – transitions and transversions – has not yet been clearly formulated (Fersht, Knill-Jones, & Tsui, 1982; Freese, 1959b; Diederichsen, 1998; Kordium, 2007; Mejía-Mazariegos & Hernández-Trujillo, 2009; Morgan, 1993; Umar & Kunkel, 1996). At this, the concrete geometrical architecture of the nucleobase mispairs causing transitions and transversions, as well as the precise physico-chemical mechanisms of their formation have not been clearly established (Goodman, 1997; Kochina, Yurenko, & Hovorun, 2007; Kramer, Kramer, & Fritz, 1984; Poltev & Bruskov, 1977; Sinha & Haimes, 1981; Sintim & Kool, 2006; Von Borstel, 1994).

Reported in the literature experimental data obtained by UV absorbance, fluorescence, NMR, X-ray techniques, as well as theoretical findings, evidence that 2AP is able to form not only the classical 2AP-T Watson-Crick (WC) DNA base pair (Sowers et al., 1986), but also to a smaller extent the 2AP-C mispair in WC-like or wobble (w) configuration containing protonated bases or bases in the rare tautomeric form (Fagan et al., 1996; Ramaekers, Adamowicz, & Maes, 2002; Reha-Krantz et al., 2011; Sherer & Cramer, 2001; Sowers et al., 1989; Sowers, Boulard, & Fazakerley, 2000), 2AP-A mispair (Fazakerley et al., 1987) and 2AP-G Hoogsteen mispair (Neely et al., 2009), which relative stabilities are defined by the row: 2AP-T > 2AP-C > 2AP-A > 2AP-G (Law, Eritja, Goodman, & Breslauer, 1996) (T – thymine, C – cytosine, G – guanine).

Thus, it is still unclear the formation of which of the most irregular nucleobase pairs determines the A-T→G-C transitions, while the G-C→A-T transitions are usually associated with the formation of the wrong 2AP-C mispair (Fagan et al., 1996; Glickman, 1985; Reha-Krantz et al., 2011; Sherer & Cramer, 2001; Sowers et al., 1986, 1989; Sowers, Boulard, & Fazakerley, 2000; Watanabe & Goodman, 1981, 1982), which architecture is close to the characteristic dimensions of the canonical Watson-Crick A-T(WC) and G-C(WC) DNA base pairs. This structural similarity, ensuring the successful enzymatic incorporation of the formed 2AP-C mispair into the synthesized DNA double helix, could be reached through the formation of the rare tautomers of the 2AP or C bases. It

is generally accepted in the literature that 2AP-C*(WC) pair is preferred over the 2AP*.C(WC) pair, which has a much higher energy than the first structure (Danilov, Krugliak, Kuprievich, & Shramko, 1967; Pullman & Pullman, 1962) (here and below bases in the rare tautomeric form (Kondratyuk, Samijlenko, Kolomiets', & Hovorun, 2000; Platonov et al., 2005; Samijlenko, Krechkivska, Kosach, & Hovorun, 2004) are marked with an asterisk). However, the precise mechanisms of the formation of the rare tautomers of the 2AP and C bases have not been established to date, that could be explained by the absence in the literature comprehensive analysis of possible approaches and model representations according the mutagenicity of 2AP.

In our recent papers we have performed an extensive quantum-chemical study of the key structural mechanisms of the 2AP mutagenicity (Brovarets' & Hovorun, 2010a, 2010b; Brovarets' & Pérez-Sánchez, 2016, 2017; Brovarets', Pérez-Sánchez, & Hovorun, 2016) and also thorough analysis of the evolution of the main physico-chemical parameters along the entire internal reaction coordinate (IRC) of the tautomerisations of the base pairs by the participation of 2AP involved in the origin of the induced by 2AP transitions and transversions (Brovarets', Voiteshenko, & Hovorun, 2018; Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2017, 2018). At this, we primarily relied on the microstructural theory of the spontaneous point mutations – transitions and transversions – based on the insightful ideas according the w↔WC tautomeric bistability of the nucleobase pairs, that is realized *via* the intrapair proton transfer (PT) between the bases, which is accompanied by a significant change in the geometry of the base pair – from the Watson-Crick-like to the wobble and *vice versa* (Brovarets', 2015; Brovarets' & Hovorun, 2015b, 2015d, 2015e, 2015f, 2015g, 2015j, 2016). Notably, the results obtained by using our approach are in agreement with literature experimental data (Bebenek, Pedersen, & Kunkel, 2011; Kimsey, Petzold, Sathyamoorthy, Stein, & Al-Hashimi, 2015; Maximoff, Kamerlin, & Florian, 2017; Szymanski, Kimsey, & Al-Hashimi, 2017; Wang, Hellinga, & Beese, 2011), while the predicted probabilities of the w↔WC transformations satisfactorily coincide with the experimentally measured frequencies (Huang, Arnheim, & Goodman, 1992).

From the other hand, we considered scenarios of the emergence of the substitution mutations – transitions and transversions, which permits classical Watson-Crick tautomeric hypothesis (Topal & Fresco, 1976; Watson & Crick, 1953) and which are presented in the literature data, namely intramolecular mutagenic tautomerisation of the nucleotide bases (see works (Brovarets' & Hovorun, 2010c; Brovarets', Kolomiets' & Hovorun, 2012; Shukla & Leszczynski, 2013; Tolosa, Sánchez, Sansón, & Hidalgo, 2017) and refs. therein), their tautomerisation by endogenous water molecules (see works (Shukla & Leszczynski, 2013; Tolosa, Sánchez, Sansón, & Hidalgo, 2017) and refs. therein) and classical Löwdin's mechanism (Brovarets', 2010, 2015; Brovarets' & Hovorun, 2013a, 2013b, 2013c, 2014a, 2014b, 2014c, 2014d, 2014e, 2015a, 2015c, 2015h, 2018; Brovarets', Zhurakivsky, & Hovorun, 2013a, 2013b, 2013c, 2014a, 2014b, 2014c, 2014d,

2015; Gorb, Podolyan, Dziekonski, Sokalski, & Leszczynski, 2004; Löwdin, 1963, 1966; Turaeva & Brown-Kennerly, 2015). Saying in other words, we aimed to combine organically two models, one of which is hydrophobic, when one of the bases within the base pair transfer into the mutagenic tautomeric form in the context of the H-bonded base pair without the direct participation of water molecules in this process, and the other one, which at the first glance is an alternative, namely, the mutagenic tautomerization by the endogenous water molecule of the isolated base belonging to the template strand of the DNA or to the incoming nucleotide. By doing so, we pursued the goal to introduce into the scientific circulation new conception that the frequency of the spontaneous point mutations – transitions and transversions – is determined by the tiny interplay between two simultaneously acting mechanisms of tautomerization – a mechanism that does not require water molecules as a chemical agent for the mutagenic tautomerization and a mechanism of the mutagenic tautomerization of the isolated nucleotide bases by the endogenous water molecule. It could be thought that the relationship between the results of action of these two mechanisms depends on the type of the cell, and more specifically – on the structure of the replisome (Bayley, 1951; Dewar & Storch, 1985; García-Moreno et al., 1997; Mertz & Krishtalik, 2000; Petrushka, Sowers, & Goodman, 1986), in which these processes are realized.

Moreover, we tried to solve the task of the finding of the physico-chemical mechanisms of the 2AP mutagenicity in the most possible format. This will be discussed in more details below.

In particular, in this Perspective we aimed to answer on the key questions:

- Why 2AP is incorporated into DNA in trace concentrations?
- Whether classical mechanisms presented in the literature according the formation of the rare tautomers of canonical DNA bases work also for base analogue – 2AP?
- In what way 2AP induces replication and incorporation errors?
- Whether the amino-imino tautomerisation of 2AP is related to its mutagenicity, that is whether the 2AP* rare tautomer is mutagenic?

We would consider the answers to these questions one by one.

Computation ideology

All calculations have been performed using Gaussian'09 package (Frisch et al., 2010). Geometries and harmonic vibrational frequencies of the considered base pairs/mispairs and transition states (TSs) of their mutual tautomeric and conformational interconversions have been obtained at the B3LYP/6-311++G(d,p) level of theory followed by the single point energy calculations at the MP2/aug-cc-pVDZ level of theory (Brovarets' & Pérez-Sánchez, 2016, 2017; Brovarets', Pérez-Sánchez, & Hovorun, 2016; Brovarets', Voiteshenko,

Pérez-Sánchez, & Hovorun, 2017, 2018; Brovarets', Voiteshenko, & Hovorun, 2018), that showed itself good for the investigation of the similar systems (Brovarets' & Hovorun, 2010c, 2011; El-Sayed, Molina, Alvarez-Ros, Palafox, 2015; Palafox, 2014).

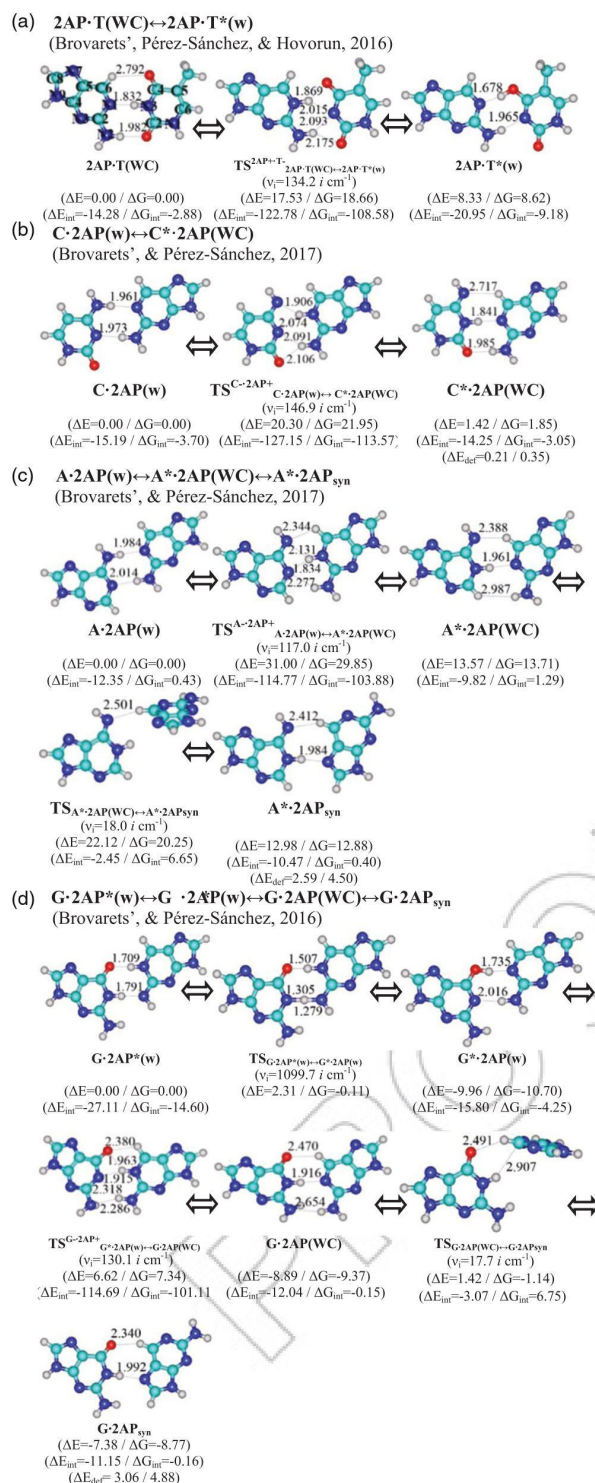
Reaction pathways of the tautomeric and conformational transitions have been obtained by following intrinsic reaction coordinate (IRC) in the forward and reverse directions from the TS using Hessian-based predictor-corrector integration algorithm (Hratchian & Schlegel, 2005) with tight convergence criteria. We have obtained the profiles of the energetic, geometric, polar, electron-topological and NBO characteristics of the H-bonds and complexes by calculating them at each point along the IRC using elaborated by Hovorun *et al.* methodology (Brovarets' & Hovorun, 2013a, 2013b, 2013c, 2014a, 2014b, 2014c, 2014d, 2014e, 2015a, 2015c, 2015h, 2016, 2018a, 2018b; Brovarets', Zhurakivsky, & Hovorun, 2013a, 2013b, 2013c, 2014a, 2014b, 2014c, 2014d, 2015). Profiles of the NBO charges along the IRC have been obtained using NBO program (Glendening, Reed, Carpenter, & Weinhold, 1994) implemented in the Gaussian'09 program package (Frisch et al., 2010).

Bader's quantum theory of Atoms in Molecules was applied to analyse the electron density distribution (Bader, 1990) using program package AIMAll (Keith, 2010) with all default options. The presence of a bond critical point (BCP), namely the so-called (3,-1) BCP, and a bond path between the donor and acceptor atoms involved in the non-covalent bonding, as well as the positive value of the Laplacian at this (3,-1) BCP ($\Delta\rho > 0$), were considered as criteria for the formation of the hydrogen, dihydrogen bond or attractive van der Waals contact (Matta, 2006, 2014; Matta & Hernández-Trujillo, 2005; Matta, Castillo, & Boyd, 2006a). Wave functions were obtained at the level of theory used for geometry optimisation.

The energies of the H-bonds E_{HB} were calculated by the empirical logansen's (logansen, 1999), Espinosa-Molins-Lecomte (EML) (Espinosa, Molins, & Lecomte, 1998; Mata, Alkorta, Espinosa, & Molins, 2011) or Nikolaienko-Bulavin-Hovorun (Nikolaienko, Bulavin, & Hovorun, 2012) formulas. The partial deuteration was applied to exclude the effect of vibrational resonances (Brovarets' & Hovorun, 2014a, 2014b, 2014c, 2014d, 2014e).

The atomic numbering scheme for the DNA bases is conventional (Saenger, 1984).

We have chosen for investigation basic level of theory, but sufficient for the calculation of the H-bonded base pairs in free state for the adequate reflection of the processes occurring in real systems (Kimsey, Petzold, Sathyamoorthy, Stein, & Al-Hashimi, 2015; Maximoff, Kamerlin, & Florian, 2017; Szymanski, Kimsey, & Al-Hashimi, 2017), in particular in the base pair recognition pocket of the high-fidelity DNA-polymerase (Mertz & Krishtalik, 2000; Petrushka, Sowers, & Goodman, 1986). At this, we have relied on the recommendations obtained in the work (Zoete & Meuwly, 2004), in which the negligibly small impact of the stacking and sugar-phosphate backbone on the tautomerisation processes has been demonstrated in the first approximation. Applied



model can be useful for the estimation of the changes at its sequential complication.

Why 2AP is incorporated into DNA in trace amounts?

It is well known from the literature that interaction of 2AP with T by the Watson–Crick scheme of the binding, realized

Figure 1. Reaction pathways of the biologically important tautomeric and conformational transitions of the structures containing canonical DNA bases and 2-aminopurine (2AP) in the main and rare tautomeric forms leading to the replication (a) and incorporation (b, c, d) errors – transitions and transversions. Relative electronic ΔE and Gibbs free ΔG energies, electronic ΔE_{int} and Gibbs free ΔG_{int} energies of interaction, the deformation energies $\Delta E_{def}(A \cdot T) / \Delta E_{def}(G \cdot C)$ necessary to apply to the mismatch to acquire the sizes of the A·T(WC)/G·C(WC) Watson–Crick DNA base pairs (in kcal·mol⁻¹), imaginary frequencies ν_i (cm⁻¹) at the TSs of the interconversions are presented below them in brackets (MP2/aug-cc-pVDZ//B3LYP/6-311++G(d,p) level of theory in vacuum at T=298.15 K). Dotted lines indicate AH...B H-bonds and continuous – loosened A-H-B covalent bridges (their lengths H...B are presented in angstroms); carbon atoms are in light-blue, nitrogen – in dark-blue, hydrogen – in grey and oxygen – in red. The base, belonging to the template strand of DNA, is situated on the left, while the base of the incoming nucleotide – on the right.

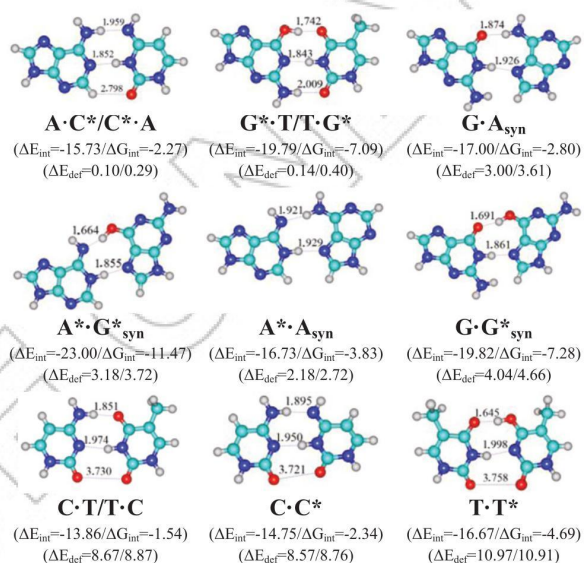


Figure 2. Geometrical structures of the 12 incorrect DNA base mispairs causing spontaneous point incorporation and replication point errors and their energetic characteristics (MP2/6-311++G(2df,pd)//B3LYP/6-311++G(d,p) level of theory, $\epsilon=1$) (Brovarets', 2015; Brovarets' & Hovorun, 2018a, 2018b). For designations see Figure 1.

by the participation of three intermolecular C6H...O4, N3H...N1 and N2H...O2 H-bonds, is the main channel determining the involvement of 2AP into the synthesized DNA double helix (Figures 1 and 2). At this, the 2AP·T(WC) base pair possesses very close energetic and geometric characteristics to the canonical A·T(WC) Watson–Crick DNA base pair (Brovarets' & Hovorun, 2010a, 2010b; Brovarets', Pérez-Sánchez & Hovorun, 2016) (Tables 1 and 2).

In the work (Brovarets', Pérez-Sánchez, & Hovorun, 2016) we have firstly expressed the assumption that significant difference of the high amplitude mobility of the 2AP·T(WC) nucleobase mispair, related to the anisotropic rotational mobility of the amino group (Hovorun, 1997; Hovorun, Mishchuk, & Kondratyuk, 1996) of the 2AP, from the analogous mobility of the amino group of the A·T(WC) base pair could be the reason for the low-probable incorporation of 2AP within the context of the 2AP·T(WC) nucleobase mispair into the double-stranded DNA during its replication in a living cell. In addition to this proper thought, we would like to

Table 1. Energetic and kinetic characteristics of the biologically important tautomeric and conformational transitions of the structures containing canonical DNA bases and 2-aminopurine in the main or rare tautomeric forms leading to replication and incorporation errors, transitions and transversions (MP2/aug-cc-pVDZ//B3LYP/6-311++G(d,p) level of theory, $\epsilon = 1$) (see Figure 1).

Tautomerisation/Conformational transition	ν_i^a	ΔE^b	ΔG^c	$\Delta \Delta E_{TS}^d$	$\Delta \Delta G_{TS}^e$	$\Delta \Delta E^f$	$\Delta \Delta G^g$	$\tau_{99.9\%}^h$	τ^i
a) 2AP·T(WC) ↔ 2AP·T*(w)	134.2	8.33	8.62	17.53	18.66	9.21	10.04	$2.53 \cdot 10^{-5}$	$3.66 \cdot 10^{-6}$
A·T(WC) ↔ A·T*(w)	99.7	14.84	13.08	20.41	20.28	5.57	7.20	$2.09 \cdot 10^{-7}$	$3.03 \cdot 10^{-8}$
b) C·2AP(w) ↔ C*:2AP(WC)	146.9	1.42	1.85	20.30	21.95	18.88	20.11	$5.87 \cdot 10^{-2}$	$8.88 \cdot 10^{-1}$
C·A(w) ↔ C*:A(WC)	588.5	-7.20	-6.07	17.61	19.51	24.81	25.58	$1.74 \cdot 10^{-2}$	$7.13 \cdot 10^{-5}$
c) A·2AP(w) ↔ A*:2AP(WC)	117.0	13.57	13.71	31.00	29.85	17.43	16.14	0.76	0.11
A*:2AP(WC) ↔ A*:2AP _{syn}	18.0	-0.59	-0.83	8.55	6.54	9.14	7.37	$5.59 \cdot 10^{-8}$	$4.07 \cdot 10^{-8}$
A·A(w) ↔ A·A*(WC)	152.4	1.09	3.63	22.56	25.86	21.47	22.24	$2.22 \cdot 10^{-4}$	$3.22 \cdot 10^{-3}$
A·A*(WC) ↔ A*:A(WC)	497.5	0.00	0.00	9.71	6.39	9.71	6.39	$2.28 \cdot 10^{-8}$	$6.42 \cdot 10^{-9}$
A*:A(WC) ↔ A*:A _{syn} (TF)	15.8	1.23	0.56	8.09	8.09	6.85	7.53	$2.66 \cdot 10^{-7}$	$5.33 \cdot 10^{-8}$
d) G·2AP*(w) ↔ G*:2AP(WC)	1099.7	-9.96	-10.70	2.31	-0.11	12.26	10.59	$4.39 \cdot 10^{-13}$	$4.52 \cdot 10^{-6}$
G*:2AP(w) ↔ G·2AP(WC)	130.1	1.07	1.33	16.58	18.04	15.51	16.70	1.77	0.28
G·2AP(WC) ↔ G·2AP _{syn}	17.7	1.51	0.60	10.31	8.23	8.80	7.63	$3.22 \cdot 10^{-7}$	$6.35 \cdot 10^{-8}$
G·A*(w) ↔ G·A(WC)	126.8	-6.73	-6.93	16.32	16.98	23.05	23.92	3.14	$5.54 \cdot 10^{-4}$
G·A(WC) ↔ G·A _{syn}	20.7	0.58	0.76	8.89	8.39	8.31	7.64	$3.47 \cdot 10^{-7}$	$6.42 \cdot 10^{-8}$

Note: ^aImaginary frequency at the TSs of the interconversions, cm^{-1} .

^bThe electronic energy of the product relatively the reactant of the tautomeric and conformational transition, $\text{kcal} \cdot \text{mol}^{-1}$.

^cThe Gibbs free energy of the product relatively the reactant of the tautomeric and conformational transition ($T = 298.15 \text{ K}$), $\text{kcal} \cdot \text{mol}^{-1}$.

^dThe electronic energy barrier for the forward reaction of the tautomeric and conformational transition, $\text{kcal} \cdot \text{mol}^{-1}$.

^eThe Gibbs free energy barrier for the forward reaction of the tautomeric and conformational transition, $\text{kcal} \cdot \text{mol}^{-1}$.

^fThe electronic energy barrier for the reverse reaction of the tautomeric and conformational transition, $\text{kcal} \cdot \text{mol}^{-1}$.

^gThe Gibbs free energy barrier for the reverse reaction of the tautomeric and conformational transition, $\text{kcal} \cdot \text{mol}^{-1}$.

^hThe time necessary to reach 99.9% of the equilibrium concentration between the reactant and the product of the tautomeric and conformational transition, s.

ⁱThe lifetime of the product of the tautomeric and conformational transition, s. All values are presented with the precision up to the last meaningful number.

give some additional structural arguments obtained by the authors at the level of the observation.

It draws the attention, that the characteristic structural feature of both the Watson–Crick DNA base pairs, as well as 12 irregular base pairs (Figure 2), which are active players in the field of the spontaneous point mutagenesis, is the presence of an exocyclic amphoteric atomic group (NH_2 or OH (Brovarets', 2015)) exposed into the major groove of DNA, able to act simultaneously as a donor and an acceptor of the H-bonding. It is quite possible that such structure of the base pairs is the guarantee of their effective recognition by the proteins of the replicative complex in the replisome (Bayley, 1951; Dewar & Storch, 1985; García-Moreno et al., 1997; Mertz & Krishtalik, 2000; Petrushka, Sowers, & Goodman, 1986).

In other words, the presence in the base pair of the non-canonical intermolecular $\text{CH} \cdots \text{O/N}$ H-bonds (Brovarets', Yurenko, & Hovorun, 2013, 2015) by the participation of the CH group (Rösel et al., 2017; Wang et al., 2015), exposed into the major groove of DNA (exactly such situation takes place in the case of 2AP·T(WC) and 2AP·C*(WC) nucleobase mispairs (Table 2)), greatly weakens the chances of such a structure to be recognized by the proteins of the replicative complex. The presence of the non-canonical H-bond or attractive van der Waals contact exposed into the minor groove of DNA does not interfere with the recognition of the pairs with such properties by the proteins of the replicative complex from the side of the minor groove, since in this case the recognition is realized by another principle – via the interaction of the amino acid residues through the H-bonds with invariant N3/O2 atoms belonging to these pairs (Brovarets', 2010, 2015). Notably, the 2AP·C*(WC) base mispair is obviously more similar to the G·C(WC) Watson–Crick base pair by its dynamical characteristics related to the rotational mobility of the amino group, since 2AP and G bases possess the amino group in the one and the same position

(Hovorun, 1997; Hovorun, Mishchuk, & Kondratyuk, 1996). Therefore, it is logical to think that 2AP would be better incorporated into the DNA double helix in the composition of the 2AP·C*(WC) nucleobase mispair instead of the 2AP·T(WC) nucleobase mispair, since its large-amplitude behavior significantly differs from the A·T(WC) Watson–Crick DNA base pair (Brovarets' & Pérez-Sánchez, 2017). This assumption requires further experimental verification.

We would also like to emphasize that the common structural feature of all 12 irregular DNA base pairs, depicted in Figure 2, is that all of them in the enzymatically-competent conformation do not create steric obstacles for their recognition in the active center of the high-fidelity DNA-polymerase from the side of the DNA minor groove. We have arrived to this conclusion by the careful comparison of the van der Waals boundaries of all incorrect base mispairs in their enzymatically competent conformations from the side of the DNA minor groove with similar joint boundaries of all four canonical A·T(WC), T·A(WC), G·C(WC), and C·G(WC) Watson–Crick DNA base pairs (Brovarets' & Hovorun, 2015i).

Whether classical mechanisms presented in the literature according the formation of the rare tautomers of canonical DNA bases work also for base analogue – 2AP?

First of all, in our work (Brovarets', Pérez-Sánchez, & Hovorun, 2016) we have thoroughly checked, whether the classical physico-chemical mechanisms of the generation of the rare, mutagenic tautomers causing mutations in DNA could be applied to the 2AP. It should be noted, that these investigations, obtained by the substitution of the canonical A DNA base with 2AP, have not been yet applied and presented in the literature before, despite a long history of their existence.

Here they are:

Table 2. Electron-topological, structural, vibrational and energetic characteristics of the intermolecular H-bonds and attractive van der Waals (vdW) contacts in the investigated complexes and TSs of their tautomerisation involving canonical DNA bases and 2AP (see Figure 1) and their polar characteristics obtained at the B3LYP/6-311++G(d,p) level of theory ($\epsilon = 1$).

Base pair / TS	AH...B H-bond / A...B vdW contact	ρ^a	$\Delta\rho^b$	$100\cdot\epsilon^c$	$d_{A...B}^d$	$d_{H...B}^e$	Δd_{AH}^f	$\angle AH...B^g$	$\Delta\nu^h$	$E_{AH...B}^i / E_{A...B}^j$	μ^j
a) 2AP-T(WC) $\leftrightarrow$ 2AP-T*(w) (Brovarets', Pérez-Sánchez, & Hovorun, 2016)											
2AP-T(WC)	C6H...O4	0.005	0.016	2.18	3.879	2.792	0.0004	132.0	-3.1	0.90*	3.15
	N3H...N1	0.040	0.095	6.42	2.876	1.832	0.032	179.3	567.1	7.58	
	N2H...O2	0.023	0.082	5.68	2.998	1.982	0.010	174.9	171.4	3.78	
TS ^{2AP+T-}	N1 ⁺ H...O4 ⁻	0.033	0.099	7.55	2.918	1.869	0.036	143.2	567.7	4.48	6.45
	N1 ⁺ H...N3 ⁻	0.025	0.081	26.93	3.064	2.015	0.036	146.1	567.7	3.10	
	N2 ⁺ H...N3 ⁻	0.022	0.070	3.88	3.119	2.093	0.013	143.7	296.8	3.09	
	N2 ⁺ H...O2 ⁻	0.016	0.053	34.47	3.201	2.175	0.019	151.8	296.8	2.20	
2AP-T*(w)	O4H...N1	0.057	0.097	5.05	2.698	1.678	0.050	169.2	954.1	9.98	1.52
	N2H...N3	0.029	0.085	7.39	2.987	1.965	0.016	168.9	295.0	5.27	
A-T(WC) $\leftrightarrow$ A-T*(w) (Brovarets' & Hovorun, 2015j)											
A-T(WC)	N6H...O4	0.026	0.093	4.39	2.946	1.926	0.014	173.5	238.7	4.65	1.88
	N3H...N1	0.040	0.093	6.49	2.886	1.841	0.032	178.8	567.5	7.58	
	C2H...O2	0.004	0.014	3.40	3.975	2.890	0.0002	132.3	-4.8	0.74*	
TS ^{A+T-}	N6 ⁺ H...O4 ⁻	0.023	0.097	6.47	2.974	1.953	0.012	140.6	188.0	4.01	7.77
	N1 ⁺ H...O4 ⁻	0.055	0.128	0.19	2.723	1.656	0.053	153.6	1668.9	13.32	
	C2 ⁺ H...N3 ⁻	0.009	0.030	57.50	3.751	2.665	0.002	113.5	-28.1	1.60*	
A-T*(w)	N6H...N3	0.017	0.056	10.90	3.018	2.240	0.013	132.0	194.5	4.10	4.16
	O4H...N6	0.028	0.078	6.20	2.930	1.963	0.016	166.1	322.7	5.55	
b) C-2AP(w) $\leftrightarrow$ C*-2AP(WC) (Brovarets' & Pérez-Sánchez, 2017)											
C-2AP(w)	N4H...N1	0.030	0.082	7.41	2.991	1.961	0.022	179.6	388.7	6.16	3.48
	N2H...N3	0.028	0.083	8.07	2.996	1.973	0.017	177.4	303.1	5.35	
TS ^{C-2AP+}	N1 ⁺ H...N4 ⁻	0.033	0.087	0.80	2.845	1.905	0.038	147.0	583.8	4.67*	5.88
	N1 ⁺ H...N3 ⁻	0.022	0.072	24.99	2.985	2.074	0.038	143.4	583.8	3.03*	
	N2 ⁺ H...N3 ⁻	0.022	0.071	6.95	2.984	2.090	0.020	144.1	317.2	2.95*	
	N2 ⁺ H...O2 ⁻	0.018	0.062	21.72	3.041	2.107	0.020	150.2	317.2	2.54*	
C*-2AP(WC)	C6H...N4	0.007	0.020	3.62	3.804	2.717	0.0006	133.6	-0.5	1.10*	2.67
	N3H...N1	0.039	0.094	6.50	2.883	1.841	0.030	178.9	543.4	7.40	
	N2H...O2	0.022	0.082	5.65	3.001	1.985	0.010	174.6	170.3	3.77	
C-A(w) $\leftrightarrow$ C*-A(WC) (Brovarets' & Hovorun, 2015d)											
C-A(w)	N4H...N1	0.025	0.074	7.72	3.052	2.030	0.016	175.6	285.2	5.17	5.20
	C2H...N3	0.010	0.030	5.53	3.452	2.513	0.0005	144.2	-5.8	1.60*	
TS ^{C-A+}	N6 ⁺ H...N4 ⁻	0.014	0.045	6.68	3.151	2.317	0.006	138.8	87.4	2.27	7.77
	N1 ⁺ H...N4 ⁻	0.055	0.091	4.92	2.749	1.688	0.064	166.8	1023.3	10.35	
	C2 ⁺ H...N3 ⁻	0.015	0.048	8.72	3.068	2.375	0.003	120.1	24.0	2.62*	
C*-A(WC)	N6H...N4	0.029	0.082	7.63	2.983	1.959	0.021	173.8	371.7	6.01	3.10
	N3H...N1	0.040	0.093	6.59	2.895	1.852	0.031	178.9	551.6	7.46	
	C2H...O2	0.005	0.017	1.48	3.628	2.798	0.0001	133.1	-5.9	0.96*	
c) A-2AP(w) $\leftrightarrow$ A*-2AP(WC) $\leftrightarrow$ A*-2AP _{syn} (Brovarets' & Pérez-Sánchez, 2017)											
A-2AP(w)	N6H...N1	0.029	0.081	7.56	3.002	1.976	0.019	179.4	1228.3	11.38	1.09
	N2H...N1	0.026	0.076	7.89	3.043	2.021	0.016	179.7	1191.9	11.20	
TS ^{A-2AP+}	C6 ⁺ H... N6 ⁻	0.016	0.052	9.19	3.023	2.344	0.003	119.0	34.8	2.82*	9.98
	N1 ⁺ H... N6 ⁻	0.020	0.071	47.64	2.887	2.131	0.045	126.4	696.6	2.86	
	N1 ⁺ H... N1 ⁻	0.039	0.089	1.05	2.860	1.834	0.045	162.3	696.6	6.44	
	N2 ⁺ H... N1 ⁻	0.015	0.048	4.15	3.154	2.277	0.007	144.0	107.6	2.71	
A*-2AP(WC)	C6H...N6	0.013	0.039	5.58	3.332	2.388	0.002	144.2	22.3	2.04*	4.57
	N1H...N1	0.029	0.082	7.01	2.991	1.961	0.019	175.2	346.1	5.77	
	C2H...HN2	0.001	0.005	75.42	3.668	2.987	0.0008	125.7	14.4	0.17*	
TS ^{A*-2AP(WC)}	C6H...O6	0.010	0.029	2.16	3.554	2.501	0.0025	162.6	26.8	1.52*	4.78
A*-2AP _{syn}	C6H...N6	0.012	0.036	5.36	3.413	2.412	0.001	152.4	9.7	1.89*	4.93
	N1H...N7	0.027	0.080	6.02	3.009	1.984	0.017	174.3	295.2	5.27	
A-A(w) $\leftrightarrow$ A-A*(WC) $\leftrightarrow$ A*-A(WC) $\leftrightarrow$ A*-A _{syn} (Brovarets' & Hovorun, 2013b, 2014b, 2015g, 2015i)											
A-A(w)	N6H...N1	0.023	0.071	7.97	3.077	2.058	0.015	175.8	259.1	4.88	2.26
	C2H...N1	0.010	0.028	5.61	3.503	2.538	0.0005	147.5	-2.2	1.52*	
TS ^{A-A+}	N6 ⁺ H...N6 ⁻	0.026	0.079	4.53	2.927	2.005	0.019	147.9	298.2	5.30	9.22
	N1 ⁺ H...N6 ⁻	0.043	0.095	3.74	2.790	1.788	0.045	156.3	697.0	6.41	
	N1 ⁺ H...N1 ⁻	0.017	0.063	120.80	3.009	2.189	0.045	132.7	697.0	2.05	
	C2 ⁺ H...N1 ⁻	0.009	0.029	3.44	3.278	2.668	-0.0008	115.1	-12.7	1.58*	
TS _{A-A*(WC)}	N6 ⁺ H...N6 ⁻	0.099	0.034	5.16	2.614	1.464	0.142	176.0	1820.2	13.92	4.72
	N1 ⁺ H...N1 ⁻	0.098	0.029	4.70	2.637	1.479	0.144	179.5	1713.1	13.50	
	C2 ⁺ H...HC2 ⁻	0.003	0.009	60.46	3.467	2.702	0.001	127.2	22.6	0.42*	
A*-A(WC)	N6H...N6	0.035	0.091	7.19	2.918	1.885	0.028	176.3	491.5	7.01	1.75
	N1H...N1	0.034	0.087	6.76	2.943	1.904	0.030	179.0	474.6	6.88	
TS _{A*-A(WC)}	N6H...N6	0.028	0.082	3.34	2.960	1.985	0.021	157.5	362.0	5.92	5.46
	N1H...N6	0.011	0.033	40.58	3.317	2.447	0.004	143.3	63.7	1.61	
	N1...N6	0.002	0.008	67.50	3.790	-	-	-	-	0.39*	
A*-A _{syn} (TF)	N6H...N6	0.032	0.098	7.17	2.937	1.921	0.023	168.9	410.4	6.35	7.96

1. intramolecular tautomerization (Brovarets' & Hovorun, 2010c; Brovarets', Kolomiets', & Hovorun, 2012; Shukla & Leszczynski, 2013; Tolosa, Sánchez, Sansón, & Hidalgo, 2017), which in case of 2AP consists in the single proton transfer from the N2 nitrogen atom of the N2H2 amino group to the neighboring N1 nitrogen atom leading to the 2AP* rare tautomer;
2. intermolecular tautomerisation of the base by the molecule-partner, e.g. by the single water molecule (Brovarets', Kolomiets', & Hovorun, 2012; Shukla & Leszczynski, 2013; Tolosa, Sánchez, Sansón, & Hidalgo, 2017) or "complementary" T DNA base, bounded from the side of the Watson-Crick edge, – the Löwdin's mechanism (Brovarets', 2010, 2015; Brovarets' & Hovorun, 2013a, 2013b, 2013c, 2014a, 2014b, 2014c, 2014d, 2014e, 2015a, 2015c, 2015h, 2018a, 2018b; Brovarets', Zhurakivsky, & Hovorun, 2013a, 2013b, 2013c, 2014a, 2014b, 2014c, 2014d, 2015; Gorb, Podolyan, Dziekonski, Sokalski, & Leszczynski, 2004; Löwdin, 1963, 1966; Turaeva & Brown-Kennerly, 2015) through the double proton transfer (DPT) along the intermolecular H-bonds.

In the case of 2AP tautomerisation by the intramolecular proton transfer and single water molecule it was established by us for the first time (Brovarets', Pérez-Sánchez, & Hovorun, 2016) that the populations of the 2AP* mutagenic tautomer (1.2×10^{-14}) and tautomerised 2AP*·H₂O complex (1.7×10^{-13}) are by orders (~5!) less than the population of the similar structures by the participation of the canonical A DNA base (1.1×10^{-10} and 6.8×10^{-8} , respectively) (Brovarets', Pérez-Sánchez, & Hovorun, 2016). This means that these mechanisms of 2AP tautomerisation could not be considered as the sources of the 2AP rare tautomers formation in comparison with the A DNA base.

Although the data according the presence in the active center of the DNA-polymerase of the water molecules (Bayley, 1951; Dewar & Storch, 1985; García-Moreno et al., 1997; Mertz & Krishtalik, 2000; Petrushka, Sowers, & Goodman, 1986) able to induce 2AP* tautomerization is not still available in the literature, it could be assumed that tautomerisation by single water molecule could occur under the specific conditions inside the replisome, when it becomes permeable to water in virtue of certain circumstances, or in the cytoplasm of the cell in the water environment.

In the case of the tautomerization by Löwdin's mechanism we have not detected at all the local minimum corresponding to the 2AP*·T*_{O2}(WC) base pair tautomerised *via* the DPT on the hypersurface of the potential energy of the 2AP·T(WC) pair. It means, that Löwdin's mechanism also does not work for the generation of the rare tautomer of 2AP.

In what way 2AP induces point replication errors?

Analysing this question, which for a long time has remained unanswered, we focused our attention on the study of the mutagenic tautomerization of the 2AP·T(WC) nucleobase mispair

with Watson-Crick-like geometry – 2AP·T(WC) ↔ 2AP·T*(w) – by comparison it with analogical process for the Watson-Crick A·T(WC) DNA base pair – A·T(WC) ↔ A·T*(w). As a result of a painstaking investigation, it was found out that the microstructural mechanism of the mutagenic action of 2AP in the induction of the *replication errors*, that occur at the stage of the strand separation just before the act of the replication, consists in the generation with the highest probability of the mutagenic tautomer T* due to the 2AP·T(WC) → 2AP·T*(w) tautomerisation reaction (4.8×10^{-7}) rather than it takes place for the Watson-Crick A·T(WC) DNA base pair – A·T(WC) → A·T*(w) tautomerisation reaction (2.5×10^{-10}). At this, the mutagenic effect is achieved due to the greater stability of the 2AP·T*(w) nucleobase pair ($\Delta E_{\text{int}} = -20.95$ and $\Delta G_{\text{int}} = -9.18 \text{ kcal} \cdot \text{mol}^{-1}$) in comparison with the analogical A·T*(w) DNA base pair ($\Delta E_{\text{int}} = -13.44$ and $\Delta G_{\text{int}} = -1.61 \text{ kcal} \cdot \text{mol}^{-1}$) (Brovarets', Pérez-Sánchez, & Hovorun, 2016) (Figure 1).

In contrast to this, the 2AP·T(WC) → 2AP*·T(w) tautomerization process causing the formation of the 2AP* mutagenic tautomer has been revealed to be the non-mutagenic due to two reasons. First, the population of the dynamically non-stable 2AP*·T(w) nucleobase mispair (8.6×10^{-13}) (Brovarets', Pérez-Sánchez, & Hovorun, 2016) is much less than the population of the A*·T(w) DNA base mispair (5.4×10^{-8}) (Brovarets' & Hovorun, 2015j). Moreover, this base pair is dynamically unstable and has extremely small lifetime ($\sim 2 \times 10^{-14} \text{ s}$), which is by orders lower than the time spent by the DNA-polymerase machinery on the dissociation of the base pair into the monomers ($\sim 10^{-9} \text{ s}$) (Brovarets', 2015). Secondly, the irregular base pairs, formed by the participation of 2AP* and all canonical DNA bases, acquire enzymatically-competent conformation with a much lower probability than similar pairs by the participation of the canonical A DNA base. Thus, we came to the conclusion that the amino-imino tautomerisation of 2AP has no relation to the emergence of spontaneous replication errors induced by this mutagen.

It is important to follow how the mutagenic tautomer T*, formed according to the 2AP·T(WC) ↔ 2AP·T*(w) pathway, would further cause mutations in DNA. Let's consider all possible variants of the binding of the generated T* mutagenic tautomer with all four canonical DNA bases of the incoming nucleotide during the further acts of the DNA replication:

- the $T^* + A \rightarrow T^* \cdot A(w) \rightarrow T \cdot A(WC)$ tautomeric transformation (Brovarets' & Hovorun, 2015j) could partially "treat" the damage and the T*·A(w) mispair would resume its original canonical T·A(WC) status;
- the $T^* + G \rightarrow T^* \cdot G(WC) \rightarrow T \cdot G^*(WC)$ pathways would lead to the A·T → G·C transitions (Brovarets' & Hovorun, 2015b, 2015c);
- formation of the short Watson-Crick $T^* + T \rightarrow T^* \cdot T(WC)$ mispair would cause transversions, when purine base is substituted by the pyrimidine (Brovarets' & Hovorun, 2015e; Brovarets', Zhurakivsky, & Hovorun, 2014a);
- the $T^* + C \rightarrow T^* \cdot C(w) \rightarrow T \cdot C(WC)$ tautomerization would also cause transversions (Brovarets' & Hovorun, 2013a, 2015f).

Notably, considered transversions would arise with significantly lower probability, that is in a good agreement with experimental data, according to which 2AP produces point mutations, among which ~95% are A·T→G·C transitions (Osborn, Person, Phillips, & Funk, 1967). One of the reasons of this is the fact that the deformation of the short Watson–Crick-like T*·T(WC) and T·C(WC) DNA base mispairs to the classical Watson–Crick sizes requires significant energy during the thermal fluctuations (Brovarets' & Hovorun, 2013a, 2015e, 2015f; Brovarets', Zhurakivsky, & Hovorun, 2014a).

This negative result could have the simple physico-chemical explanation – energy of the 2AP tautomerisation significantly exceeds (by $\Delta G_{2AP} - \Delta G_A = 21.69 - 13.60 = 8.09 \text{ kcal} \cdot \text{mol}^{-1}$ under normal conditions) the analogical value for the canonical A DNA base.

Structural mechanisms of the incorporation errors induced by 2AP

In order to study the mechanisms of the *incorporation errors* induced by 2AP occurring at the stage of the formation of the pair through the binding of the template base with the base of the incoming nucleotide in the active center of the high-fidelity DNA-polymerase during the DNA replication, we have considered and analyzed the energetic and kinetic characteristics of all possible combinations of the X·2AP(w,Wc)/2AP·X(w,Wc) mispairs involving 2AP in the main tautomeric form and also X*·2AP(w,Wc)/2AP*·X(w,Wc) mispairs involving 2AP* in the rare tautomeric form. Furthermore, we investigated the ability of these mispairs involving 2AP (Brovarets' & Hovorun, 2018a, 2018b; Brovarets' & Pérez-Sánchez 2016, 2017; Brovarets', Pérez-Sánchez, & Hovorun, 2016; Brovarets', Voiteshenko, & Hovorun, 2018; Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2017, 2018) to acquire the enzymatically competent sizes according to the proposed by us w↔WC tautomeric pathways (Brovarets', 2015; Brovarets' & Hovorun, 2015b, 2015d, 2015e, 2015f, 2015g, 2015j, 2016) or to perform *anti*↔*syn* conformational transitions in the purine-purine base pairs by the participation of 2AP, realized through the rotation of the base belonging to the incoming nucleotide (Brovarets' & Hovorun, 2015i), by substituting canonical A DNA base with 2AP.

At this, the total probability of the enzymatic incorporation of the mispairs involving 2AP into the DNA structure would be defined as the multiplication of the probabilities of the independent events affecting on this:

- probability of the formation of the base pair due to the binding of the bases with 2AP;
- probability of the w↔WC tautomeric transformations during the time that spend high-fidelity replication DNA-polymerase on the incorporation of one nucleotide into the DNA double helix ($8.33 \times 10^{-4} \text{ s}$ (Kirmizialtin, Nguyen, Johnson, & Elber, 2012)), that could be estimated by the formula presented in the work (Podolyan, Gorb, & Leszczynski, 2003);

- probability of the acquisition of the Watson–Crick geometry via the obligatory deformation of the formed mispairs to the Watson–Crick sizes;
- probability of the *anti*↔*syn* conformations, that could be defined as the population of the final complexes with base in the *syn*-orientation, since these processes are under thermodynamic control.

Then the possibility of the origin of the induced by 2AP mutations have been defined as the ratio of the aforementioned total probabilities obtained for the 2AP and A bases.

As a result of the painstaking analysis, it was revealed that among all possible combinations of the X·2AP(w,Wc)/2AP·X(w,Wc) mispairs involving 2AP in the main tautomeric form only two of them can produce *incorporation errors* through the $C \cdot 2AP(w) \rightarrow C^* \cdot 2AP(WC) \rightarrow C^* \cdot 2AP(WC)$ and $A \cdot 2AP(w) \rightarrow A^* \cdot 2AP(WC) \rightarrow A^* \cdot 2AP_{syn} \rightarrow A^* \cdot 2AP_{syn}(WC)$ tautomeric transformations (here and below in bold are marked the pairs, which characteristic glycosidic sizes coincide with the analogical parameters of the Watson–Crick DNA base pairs; the base, belonging to the template strand, is situated on the left) (Figure 1; Tables 1 and 2).

These observations have been received in view of the facts that the probabilities of the enzymatic incorporation of the C·2AP(w) and A·2AP(WC) mispairs involving 2AP into the DNA structure are by orders of magnitude higher than the similar probabilities for the C·A(w) and A·A(WC) mispairs by the participation of the canonical A DNA bases: $P_{C \cdot 2AP} / P_{C \cdot A} = P(C + 2AP \rightarrow C \cdot 2AP(w)) \cdot P(C \cdot 2AP(w) \rightarrow C^* \cdot 2AP(WC)) \cdot P(C^* \cdot 2AP(WC) \rightarrow C^* \cdot 2AP(WC)) / (P(C + A \rightarrow C \cdot A(w)) \cdot P(C \cdot A(w) \rightarrow C^* \cdot A(WC)) \cdot P(C^* \cdot A(WC) \rightarrow C^* \cdot A(WC))) = 1.9 \cdot 10^4$ and $P_{A \cdot 2AP} / P_{A \cdot A} = P(A + 2AP \rightarrow A \cdot 2AP(w)) \cdot P(A \cdot 2AP(w) \rightarrow A^* \cdot 2AP(WC)) \cdot P(A^* \cdot 2AP(WC) \rightarrow A^* \cdot 2AP_{syn}) \cdot P(A^* \cdot 2AP_{syn} \rightarrow A^* \cdot 2AP_{syn}(WC)) / (P(A + A \rightarrow A \cdot A(w)) \cdot P(A \cdot A(w) \rightarrow A^* \cdot A(WC)) \cdot P(A^* \cdot A(WC) \rightarrow A^* \cdot A(WC)) \cdot P(A^* \cdot A(WC) \rightarrow A^* \cdot A_{syn}(TF)) \cdot P(A^* \cdot A_{syn}(TF) \rightarrow A^* \cdot A_{syn}(WC))) = 40.5$ (Brovarets' & Pérez-Sánchez, 2017) (TF – Topal-Fresco base mispair (Topal & Fresco, 1976; Brovarets', Zhurakivsky, & Hovorun, 2014b)) (Figure 1; Tables 1 and 2).

Notably, established $C \cdot 2AP(w) \leftrightarrow C^* \cdot 2AP(WC)$ w↔WC tautomerization could be realized in both cases, when 2AP is the base of the incoming nucleotide, as well as when 2AP belongs to the template, producing at this C·G→T·A transitions and with significantly less probability A·T→G·C transitions, when C DNA base is incorporated into DNA opposite the template 2AP nucleobase. However, the latter process is less probable, since in this case the C base is shifted towards the minor groove of the DNA, where it is known to occur steric obstacles with amino acid residues that recognize the pairs with Watson–Crick architecture by the invariant N3/O2 atoms (Watson & Crick, 1953; Topal & Fresco, 1976), whereas these complications are absent, when 2AP as the base of incoming nucleotide is shifted relatively the C base towards the major groove of DNA.

In the case of the formation of the A·2AP(w) base, the transversions occur, when pyrimidine base (in this case T) is substituted by the purine, in particular – A. At this, it is only possible the variant, when 2AP is the base of the incoming nucleotide, but not *vice versa*, because in the latter case the

2AP·A*_{syn} base mispair, which is stabilized by the participation of the N6H...N1 and N2H...N7 H-bonds, as it is evidenced by the quantum-chemical calculations, is not able to acquire enzymatically-competent conformation 2AP·A*_{syn}(WC) through the significant steric clashes and probability ratio $P_{2AP·A}/P_{A·A}=3.6 \times 10^{-4}$ shows that this process is not mutagenic (Brovarets' & Pérez-Sánchez, 2017).

We have also established that *incorporation errors* could occur only in the case, when 2AP* in the rare tautomeric form as a base of the incoming nucleotide binds with the G base by the two N1H...O6 and N1H...N2 H-bonds and the formed G·2AP*(w) base pair transforms *via* the pathway of the tautomeric-conformational conversions $G·2AP^*(w) \rightarrow G^*·2AP(w) \rightarrow G·2AP(WC) \rightarrow G·2AP_{syn} \rightarrow G·2AP_{syn}(WC)$ (Figure 1). Estimated ratio of the probabilities $P_{G·2AP^*/P_{G·A^*}} = [P(G + 2AP^* \rightarrow G·2AP^*(w)) \cdot P(G·2AP^*(w) \rightarrow G^*·2AP(w)) \cdot P(G^*·2AP(w) \rightarrow G·2AP(WC)) \cdot P(G·2AP(WC) \rightarrow G·2AP_{syn}) \cdot P(G·2AP_{syn} \rightarrow G·2AP_{syn}(WC))] / [P(G + A^* \rightarrow G·A^*(w)) \cdot P(G·A^*(w) \rightarrow G·A(WC)) \cdot P(G·A(WC) \rightarrow G·A_{syn}) \cdot P(G·A_{syn} \rightarrow G·A_{syn}(WC))] = 1.9 \cdot 10^7$ obtained on the basis of the numerical data points that this route of structural transformation is mutagenic, generating appropriate transversions, when pyrimidine base (in this case C) is replaced by the analogue of the purine base – 2AP. This also causes low-probable transitions and transversions, since in the next rounds of the DNA replication 2AP pairs not only with T, but also with the C and A bases, as we have shown earlier (Figure 1; Tables 1 and 2).

It draws the attention that in the course of the aforementioned reactions the heterocycles of the nucleotide bases hold their planarity, despite their ability to bend quite easily (Govorun et al., 1992; Hovorun, Gorb, & Leszczynski, 1999; Nikolaienko, Bulavin, & Hovorun, 2011), and the methyl group of the T DNA base does not change its orientation.

Profiles of the physico-chemical characteristics of the nucleobase mispairs involving 2AP along the intrinsic reaction coordinate of the tautomerisation

By applying elaborated by our research group original methodology (Brovarets' & Hovorun, 2013a, 2013b, 2013c, 2014a, 2014b, 2014c, 2014d, 2014e, 2015a, 2015c, 2015h, 2016, 2018a, 2018b; Brovarets', Zhurakivsky & Hovorun, 2013a, 2013b, 2013c, 2014a, 2014b, 2014c, 2014d, 2015), we have followed in details the changes of the physico-chemical characteristics of the base mispairs involving 2AP and of the H-bonds stabilizing them at each point of the IRC of the tautomerisation reactions *via* the DPT or PT that are the integral parts of the biologically important tautomeric-conformational transformations. In such a way, we received the profiles along the IRC of the electronic energy E , the first derivative of the electronic energy with respect to the IRC – $dE/dIRC$, the dipole moment of the base pair μ , the NBO charges q_{NBO} of the hydrogen atoms involved in the tautomerisations, the distance $R(H_9-H_9)$ between the H_9 glycosidic hydrogens, the glycosidic angles α_1/α_2 , the electron density ρ , the Laplacian of the electron density $\Delta\rho$ and the ellipticity ε at the (3,-1) BCPs of the intrapair covalent and hydrogen bonds, the distances $d_{A...Br}$, d_{AH/HB_r} , the angles $\angle AH...B$ and

the energy of the intermolecular H-bonds E_{HB} (Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2017, 2018; Brovarets', Voiteshenko, & Hovorun, 2018) (Figure 3).

In order to simplify analysis, the DPT or PT tautomerisation reactions in the mispairs containing 2AP were pairwise combined and considered in the one and the same direction, taking into account their high similarity between the courses of these processes (Figure 3):

1. the $T·2AP^*(w) \leftrightarrow T^*·2AP(w)$ and $G·2AP^*(w) \leftrightarrow G^*·2AP(w)$ DPT tautomerization reactions in the wobble nucleobase pairs, that could be considered as a preparatory stage before the further $w \leftrightarrow WC$ tautomerisations as an integral part of the non-mutagenic $T·2AP^*(w) \leftrightarrow T^*·2AP(w) \leftrightarrow T·2AP(WC)$ and $2AP^*·G(w) \leftrightarrow 2AP·G^*(w) \leftrightarrow 2AP·G(WC) \leftrightarrow 2AP·G_{syn}$ or mutagenic $G·2AP^*(w) \leftrightarrow G^*·2AP(w) \leftrightarrow G·2AP(WC) \leftrightarrow G·2AP_{syn}$ tautomeric-conformational pathways (Brovarets' & Pérez-Sánchez, 2016), have been established to proceed through the *asynchronous concerted mechanism* through the sequential proton transfer along the intermolecular H-bonds (Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2017) (Figure 3a, 3b; Table 2);
2. the $2AP·T(WC) \leftrightarrow 2AP·T^*(w)$ and $2AP·C^*(WC) \leftrightarrow 2AP·C(w)$ Watson-Crick $\leftrightarrow$ wobble tautomerisation pathways, determining the origin of the induced by 2AP replication and incorporation errors, respectively, were established to proceed according to the stepwise concerted mechanism *via* the sequential intrapair proton transfer between the bases followed by the shifting of the 2AP according the pyrimidine T/C bases (Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2018) (Figure 3c, 3d; Table 2);
3. the $G^*·2AP(w) \leftrightarrow G·2AP(WC)$ and $A·2AP(w) \leftrightarrow A^*·2AP(WC)$ $w \leftrightarrow WC$ tautomerisations, which are involved into the mutagenic $G·2AP^*(w) \leftrightarrow G^*·2AP(w) \leftrightarrow G·2AP(WC) \leftrightarrow G·2AP_{syn} \leftrightarrow G·2AP_{syn}(WC)$ and $A·2AP(w) \leftrightarrow A^*·2AP(WC) \leftrightarrow A^*·2AP_{syn} \leftrightarrow A^*·2AP_{syn}(WC)$ tautomeric-conformational pathways of the origin of the induced by 2AP incorporation errors, have been established to occur through the stepwise concerted mechanism *via* the sequential intrapair proton transfer followed by the shifting of the G*/A DNA bases according the 2AP nucleobase (Brovarets', Voiteshenko, & Hovorun, 2018) (Figure 3e, 3f; Table 2).

All of these tautomerisation reactions are dipole-active processes with maximum of the dipole moment lying in the range from 4.03 D for the $T·2AP^*(w) \leftrightarrow T^*·2AP(w)$ DPT tautomerisation reaction to 10.62 D for the $G^*·2AP(w) \leftrightarrow G·2AP(WC)$ $w \leftrightarrow WC$ tautomerisation reaction attained in the vicinity of the TS, occur non-dissociatively and are accompanied by the consequent replacement along the IRC of the unique patterns of the specific intermolecular interactions, including H-bonds and loosened covalent bridges – altogether ten patterns with simultaneously maximum five H-bonds for the $w \leftrightarrow WC/WC \leftrightarrow w$ tautomerisations, pointing on the complexity of their structural rearrangement, and five patterns with

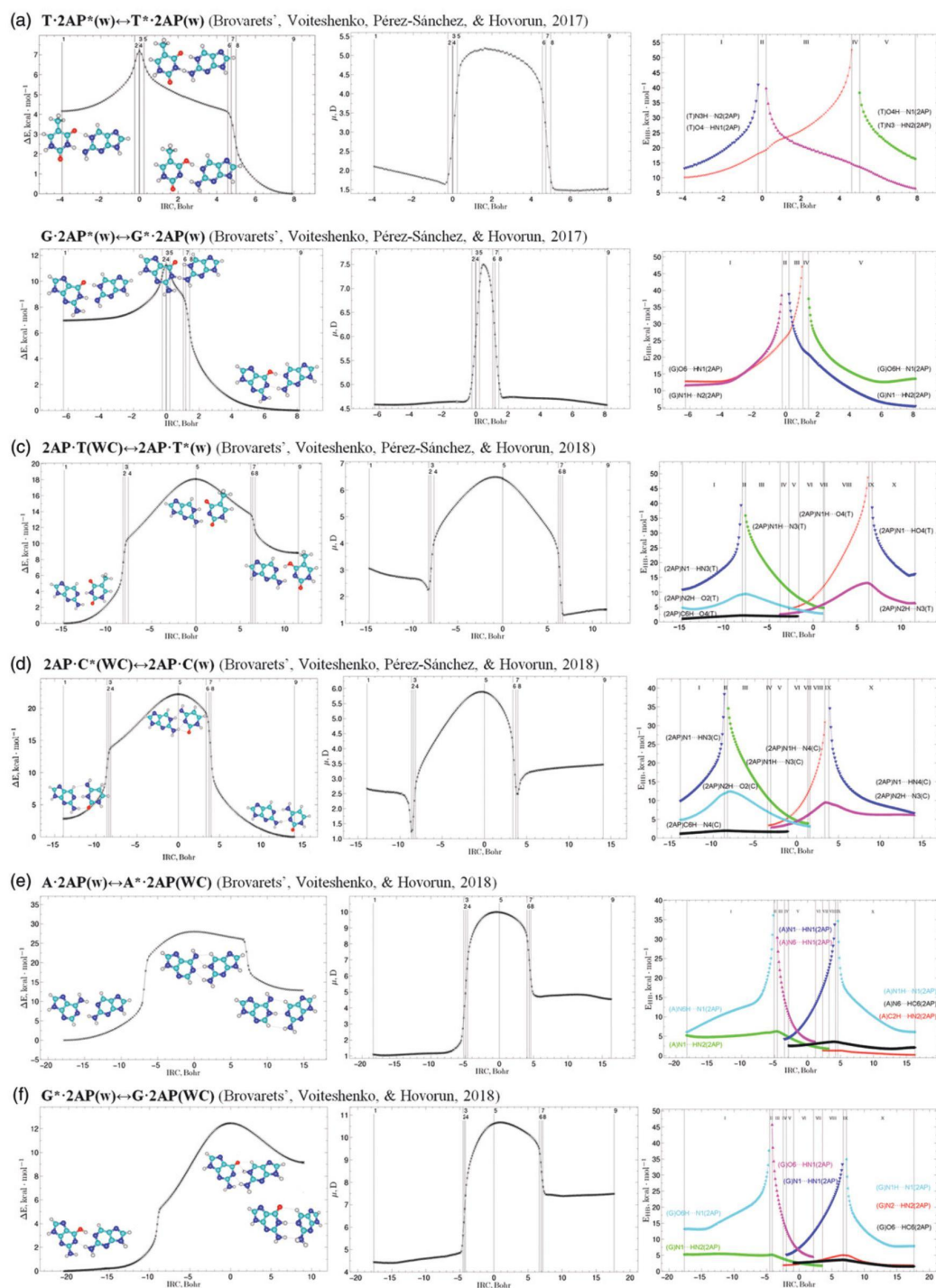


Figure 3. Profiles of the relative electronic energy ΔE , the dipole moment μ and the patterns of the intermolecular H-bonds, which energy E_{HB} has been estimated by the EML formula (Espinoso, Molins, & Lecomte, 1998; Mata, Alkorta, Espinoso, & Molins, 2011) along the IRC of the tautomerisations via the sequential DPT or PT followed by the shifting of the bases relative to each other within the nucleobase pair obtained at the B3LYP/6-311++G(d,p) level of theory ($\epsilon = 1$).

simultaneously maximum three H-bonds for the DPT tautomerization reactions (Figure 3).

Calculations of the first derivatives of the electronic energy E by the IRC enable us to establish that these $dE/dIRC$ curves possess maximum and minimum coinciding with the 2nd and 8th key points (KPs) (Figure 3). Basing on this, we proceed to divide the whole region of the reaction

pathway of these reactions to the regions of the reagent (between 1st and 2nd KPs), transition state (between 2nd and 8th KPs) and product (between 8th and 9th KPs). Notably, the *TS region*, where it directly occurs the mutual reorientation of the bases relative to each other, proton transfer and breakage or formation of the intermolecular bonds, was established to be the widest for the $2AP \cdot T(WC) \leftrightarrow 2AP \cdot T^*(w)$

(with width 14.90 Bohr consisting 56.4% from the total width of the reaction 26.44 Bohr), $2AP \cdot C^*(WC) \leftrightarrow 2AP \cdot C(w)$ (12.63 Bohr/28.00 Bohr · 100% = 45.1%) and $T \cdot 2AP^*(w) \leftrightarrow T^* \cdot 2AP(w)$ (5.27 Bohr/11.90 Bohr · 100% = 44.3%) reactions, the product region is the widest for the $G \cdot 2AP^*(w) \leftrightarrow G^* \cdot 2AP(w)$ (6.69 Bohr/14.41 Bohr · 100% = 46.4%) reaction, the reagent region is the widest for the $G^* \cdot 2AP(w) \leftrightarrow G \cdot 2AP(WC)$ (13.11 Bohr/35.30 Bohr · 100% = 37.1%) and $A \cdot 2AP(w) \leftrightarrow A^* \cdot 2AP(WC)$ (13.17 Bohr/34.69 Bohr · 100% = 38.0%) reactions (Figure 3).

Notably, the TS regions for the $w \leftrightarrow WC/WC \leftrightarrow w$ tautomerisation reactions almost entirely (between the 4th and 6th KPs) correspond to the shifting of the protonated or deprotonated bases inside the $G^- \cdot 2AP^+$, $A^- \cdot 2AP^+$, $2AP^+ \cdot T^-$, $2AP^+ \cdot C^-$ ion pairs, while their narrow regions correspond to the PT in the Watson-Crick or wobble base mispairs (Figure 3c–3f). In the case of the $T \cdot 2AP^*(w) \leftrightarrow T^* \cdot 2AP(w)$ and $G \cdot 2AP^*(w) \leftrightarrow G^* \cdot 2AP(w)$ DPT tautomerisation reactions, the proton transfer processes directly occur at the whole TS region (Figure 3a, 3b). However, all reactions are accompanied by the compression of the base pairs reflected by the decreasing of the intermolecular distances, becoming especially pronounced at the TS region (Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2017, 2018; Brovarets', Voiteshenko, & Hovorun, 2018).

It was established that all of them are characterized by the nine key points along the IRC, which are the "fingerprints" of these reactions (Table 3). For the $w \leftrightarrow WC$ tautomerisations three of the KPs are stationary structures (1st and 9th) and TSs (5th), four of them (2nd, 4th, 6th and 8th) are structures, where the appropriate $AH \cdots B$ H-bond transforms into the covalent bond and vice versa, for which the Laplacian of the electron density $\Delta\rho_{A \cdots H}/\Delta\rho_{H \cdots B}=0$, and two others (3rd and 7th) are complexes with loosened A-H-B covalent bridge with equalized geometrical and electron-topological properties $\rho_{A-H}=\rho_{H-B}$. At this, the 4th, 5th (TSs) and 6th KPs are ion pairs – $2AP^+ \cdot T^-$, $2AP^+ \cdot C^-$ (Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2017), $G^- \cdot 2AP^+$, $A^- \cdot 2AP^+$ (Brovarets', Voiteshenko, & Hovorun, 2018) for the $w \leftrightarrow WC/WC \leftrightarrow w$ tautomerisations (Figure 3c–3f; Table 3). In the case of the $T \cdot 2AP^*(w) \leftrightarrow T^* \cdot 2AP(w)$ and $G \cdot 2AP^*(w) \leftrightarrow G^* \cdot 2AP(w)$ DPT tautomerisations, the 3rd and 4th (TSs) KPs are degenerated coinciding with each other; in the 2nd, 5th, 6th and 8th KPs the Laplacian of the electron density $\Delta\rho_{A \cdots H}/\Delta\rho_{H \cdots B}=0$ (5th and 6th KPs are $T^- \cdot 2AP^+$ and $G^- \cdot 2AP^+$ wobble-like ion pairs) and in the 3rd and 7th KPs geometrical and electron-topological properties equalize $\rho_{A-H}=\rho_{H-B}$ (Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2018) (Figure 3a, 3b; Table 3).

Profiles of the electronic energies contain two kinks for the $w \leftrightarrow WC/WC \leftrightarrow w$ tautomerisation reactions, which are the boundaries of the existence of the $G^- \cdot 2AP^+$, $A^- \cdot 2AP^+$, $2AP^+ \cdot T^-$, $2AP^+ \cdot C^-$ ion pairs corresponding to the 3rd and 6th KPs, while the single kink for the DPT reactions corresponds to the terminal point of the existence of the $T^- \cdot 2AP^+$ and $G^- \cdot 2AP^+$ ion pairs (Figure 3).

Conclusions and perspectives

Based on our microstructural theory of the spontaneous point mutagenesis in DNA (Brovarets' & Hovorun, 2015b, 2015d, 2015e, 2015f, 2015g, 2015j, 2016, 2018a, 2018b) with

Table 3. Characteristics of the nine key points of the $w \leftrightarrow w$, $WC \leftrightarrow w/w \leftrightarrow WC$ tautomerization reactions in the mispairs by the 2AP participation occurring via the PT or DPT obtained at the B3LYP/6-311++G(d,p) level of theory in vacuum (see Figure 3).

Key point	IRC coordinates, Bohr			
	a) $T \cdot 2AP^*(w) \leftrightarrow T^* \cdot 2AP(w)$ (Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2017)	b) $G \cdot 2AP^*(w) \leftrightarrow G^* \cdot 2AP(w)$ (Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2017)	c) $2AP \cdot T(WC) \leftrightarrow 2AP \cdot T^*(w)$ (Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2018)	d) $2AP \cdot C^*(WC) \leftrightarrow 2AP \cdot C(w)$ (Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2018)
1	(-4.00): $T \cdot 2AP^*(w)$	(-6.28): $G \cdot 2AP^*(w)$	(-14.87): $2AP \cdot T(WC)$	(-13.93): $2AP \cdot C^*(WC)$
2	(-0.24): $\Delta\rho_{H \cdots N2}=0$	(-0.26): $\Delta\rho_{H \cdots N2}=0$	(-8.21): $\Delta\rho_{N1 \cdots H}=0$	(-8.66): $\Delta\rho_{N1 \cdots H}=0$
3	(-0.01): $\rho_{N3-H}=\rho_{H-N2}$	(-0.02): $\rho_{N1-H}=\rho_{H-N2}$	(-7.96): $\rho_{N1-H}=\rho_{H-N3}$	(-8.42): $\rho_{N1-H}=\rho_{H-N3}$
4	(0.00): $TS_{T \cdot 2AP^*(w) \leftrightarrow T^* \cdot 2AP(w)}$	(0.00): $TS_{G \cdot 2AP^*(w) \leftrightarrow G^* \cdot 2AP(w)}$	(-7.59): $\Delta\rho_{H \cdots N3}=0$	(-8.18): $\Delta\rho_{H \cdots N3}=0$
5	(0.21): $\Delta\rho_{N3 \cdots H}=0$	(0.21): $\Delta\rho_{N1 \cdots H}=0$	(0.00): $TS_{2AP^+ \cdot T^-}$	(0.00): $TS_{2AP^+ \cdot C^-}$
6	(4.62): $\Delta\rho_{O4 \cdots H}=0$	(1.03): $\Delta\rho_{O6 \cdots H}=0$	(6.20): $\Delta\rho_{H \cdots O4}=0$	(3.37): $\Delta\rho_{H \cdots N4}=0$
7	(4.72): $\rho_{O4-H}=\rho_{H-N1}$	(1.18): $\rho_{O6-H}=\rho_{H-N1}$	(6.44): $\rho_{N1-H}=\rho_{H-O4}$	(3.73): $\rho_{N1-H}=\rho_{H-N4}$
8	(5.03): $\Delta\rho_{H \cdots N1}=0$	(1.44): $\Delta\rho_{H \cdots N1}=0$	(6.69): $\Delta\rho_{N1 \cdots H}=0$	(3.97): $\Delta\rho_{N1 \cdots H}=0$
9	(7.90): $T^* \cdot 2AP(w)$	(8.13): $G^* \cdot 2AP(w)$	(11.57): $2AP \cdot T^*(w)$	(14.07): $2AP \cdot C(w)$
f) $G^* \cdot 2AP(w) \leftrightarrow G \cdot 2AP(WC)$ (Brovarets', Voiteshenko, & Hovorun, 2018)				
				(-17.65): $G^* \cdot 2AP(w)$
				(-4.54): $\Delta\rho_{H \cdots N1}=0$
				(-4.30): $\rho_{O6-H}=\rho_{H-N1}$
				(-4.18): $\Delta\rho_{O6 \cdots H}=0$
				(0.00): $TS_{G^* \cdot 2AP(w) \leftrightarrow G \cdot 2AP(WC)}$
				(6.63): $\Delta\rho_{N1 \cdots H}=0$
				(6.99): $\rho_{N1-H}=\rho_{H-N1}$
				(7.24): $\Delta\rho_{H \cdots N1}=0$
				(16.61): $A^* \cdot 2AP(WC)$

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simultaneous consideration of the possible scenarios of the origin of the point errors that permits the Watson–Crick tautomeric hypothesis (Watson & Crick, 1953), it was found out for the first time the key microstructural mechanisms of the mutagenicity of the 2AP mutagen.

It was established that 2AP as a mutagen is the source of the formation of both induced replication and incorporation errors.

In case of the *replication errors*, 2AP exerts the mutagenic pressure on DNA by the generation of the T* mutagenic tautomers within the 2AP·T(WC) base pair due to its transformation into the wobble 2AP·T*(w) base pair relatively to the 2AP·T(WC)→2AP·T*(w) reaction pathway with the probability in $\sim 10^3$ times higher than for the processes by the participation of the canonical A DNA base.

We have shown that 2AP effectively produces *incorporation errors* – transitions and to a lesser extent – transversions at the DNA replication. Thus, the G·C→A·T transitions are caused by the wobble C·2AP(w) mispair by its C·2AP(w)→C*·2AP(WC)→C*·2AP(WC) transformation into the enzymatically-competent C*·2AP(WC) base mispair in the process of the thermal fluctuations, occurring with the probability in $\sim 10^4$ times higher than for the processes by the participation of the canonical A DNA base.

It was established that the root causes of the incorporation errors – transversions – are the chains of the A·2AP(w)→A*·2AP(WC)→A*·2AP_{syn}→A*·2AP_{syn}(WC) ($P_{A\cdot 2AP}/P_{A\cdot A}\sim 40$) and G·2AP*(w)→G*·2AP(w)→G·2AP(WC)→G·2AP_{syn}→G·2AP_{syn}(WC) ($P_{G\cdot 2AP^*}/P_{G\cdot A}\sim 10^7$) structural transformations.

We have demonstrated that amino-imino tautomerism of 2AP is not involved into the origin of the induced by this compound replication point errors in DNA.

Moreover, using author's methodology it was received the profiles of the physico-chemical characteristics for the tautomerisation reactions *via* the DPT and PT involving 2AP, which are integral parts of the biologically important tautomeric-conformational transformations and enable us to receive reliable picture of their courses (Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2017, 2018; Brovarets', Voiteshenko, & Hovorun, 2018).

Obtained by us theoretical data are in agreement with the existing experimental results and also allow to interpret them from the unified physico-chemical positions (Glickman, 1985; Goodman, Hopkins, & Gore, 1977; Guest et al., 1991; Fagan et al., 1996; Pitsikas, Patapas, & Cupples, 2004; Reha-Krantz et al., 2011; Sowers, Boulard, & Fazakerley, 2000; Sowers et al., 1986, 1989; Watanabe & Goodman, 1981, 1982) and theoretical methodologies (Broo & Holmén, 1996; Reha-Krantz et al., 2011; Ronen, 1980; Sherer & Cramer, 2001). This, in its turn, indicates the adequacy of the applied theoretical platform and existing ideas about the mechanisms of the emergence of the point errors that occur during the replication of the DNA in a living cell (Brovarets' & Hovorun, 2015b, 2015d, 2015e, 2015f, 2015g, 2015j, 2016, 2018a, 2018b).

The small probability of the incorporation of 2AP into the replicating double-stranded DNA could be logically connected with the presence of a non-classical CH...O/N H-bond in the 2AP·T(WC)/2AP·C*(WC) base pairs by the

participation of 2AP, exposed into the major groove of DNA, which reduces the efficiency of the recognition of these irregular base pairs by the proteins of the replicative complex.

At the same time, the presence of the CH...O/N H-bonds, exposed into the major groove of DNA, in the irregular purine-purine nucleobase pairs involving 2AP is a guarantee of the acquisition of these complexes of the enzymatically-competent conformation, which is realized by the rotation of one of these bases around the CH...O/N H-bonds from the *anti*- to *syn*- conformation (Brovarets' & Hovorun, 2015i).

Moreover, the approaches proposed in our works (Brovarets' & Hovorun, 2010a, 2010b; Brovarets' & Pérez-Sánchez, 2016, 2017; Brovarets', Pérez-Sánchez, & Hovorun, 2016; Brovarets', Voiteshenko, & Hovorun, 2018; Brovarets', Voiteshenko, Pérez-Sánchez, & Hovorun, 2017, 2018) according the clarification of the 2AP mutagenicity can be extended to any modified nucleotide base as receipt. At this, the most difficult point is the searching (localization) of the transition states on the potential energy surface for the definition of the routes of the corresponding tautomeric-conformational transitions. Accumulated by us experience could be especially valuable at the localization of these transition states, which can be used by analogy.

Furthermore, careful attention should be paid to the consideration of the environment, at least at the level of the continuum models using dielectric permeability in the range from 2 to 4, characteristic for the recognition pocket of the high-fidelity replicative DNA-polymerase.

It is quite important for the future investigations in this topic to consider the possible impact of the sugar residues (Brovarets, Zhurakivsky, & Hovorun, 2010e) or surrounding environment at the macromolecular level, e.g. active center of the high-fidelity replicative DNA-polymerase (Maximoff, Kamerlin, & Florian, 2017), on the progress of the mutagenic tautomerisation of the complexes involving 2AP.

It seems an attractive idea to use both theoretical and especially experimental fluorescence properties of 2AP (Alemán et al., 2014; Elvin, Alemán, & Rueda, 2011) for the confirmation and possible refinement of the established by us intimate mechanisms of its mutagenicity. Thus, it would be interesting to calculate using time-dependent density functional theory (TD-DFT) the fluorescent properties of the 2AP·C(w), A·2AP(w), G·2AP*(w) and 2AP·T(WC) nucleobase mispairs involving 2AP, responsible for the origin of the induced by 2AP mutations due to their ability to perform tautomeric-conformational transformations, as in the isolated states, so as stacked structures inside isolated oligonucleotides or in the active center of the high-fidelity DNA-polymerase. As a result of this, it would be possible to establish their fluorescence properties, in particular, fluorescence intensity, fluorescence quenching, decay lifetimes and also to study the effect of the anisotropic rotation of the amino group (Hovorun, 1997; Hovorun, Mishchuk, & Kondratyuk, 1996), influence of the sequence of the bases in the oligonucleotides and "neighbor's effect" for explaining the existing experimental data (Abou-Zied, 2013; Jean &

Hall, 2001, 2002) or planning of the new biologically important experiments.

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