Formation of Bifunctional Cross-Linked Products Due To Reaction of NAMI-A With DNA Bases – A DFT Study

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Research Article

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Abstract

It is reported that NAMI-A and other Ru-anticancer complexes preferably bind with the N7 site of guanine and can also form DNA inter-strand cross-links. Therefore, in order to understand the DNA cross-link formation capability of NAMI-A, we have investigated here the structure and energetics of the reactions of the G_{N7} -NAMI-A (a monofunctional adduct of NAMI-A with the N7 site of guanine) with the N3, N7 and O6 sites of guanine, the N1, N3 and N7 sites adenine, the O2 and N3 sites of cytosine and the O2 and O4 sites of thymine, using the M06-2X functional of density functional theory. It is found that the G_{N7} -NAMI-A can form stable cross-linked products at all the sites studied here except at the N3 site of cytosine and O2 site of thymine. The calculated reaction free energies and reaction enthalpies indicate that the N3 site of adenine (A_{N3}) and N7 site of guanine (G_{N7}) are most exothermic among all the studied reactions. This study shows that NAMI-A would favorably form the cross-linked products involving the N7 site of guanine at one side and the N7 site of guanine or the N3 site of adenine at the other side.

1. Introduction

The initiation and development of cancer which is known to be a leading cause of death worldwide is linked with DNA damage caused by a myriad of endogenous and exogenous agents including free radicals, reactive oxygen species (ROS), reactive nitrogen oxide species (RNOS), alkylating agents and ionizing radiation [1–5]. It is reported that these agents react with DNA bases and yield a plethora of modified DNA bases which can result the formation of mispairing of bases, abasic sites, strand-breaks, inter- and intra-strand DNA cross-links and DNA-protein cross-links [1, 4, 6, 5]. In addition to cancer, these DNA lesions, if not repaired, can cause several other lethal consequences including mutation, aging and neurodegenerative diseases [5, 2–4, 6]. Remarkably, anticancer drugs such as cisplatin, temozolamide, mechlorethamine etc. which are used to treat cancer also act by interacting with DNA bases [7–10].

Metal-based anticancer drugs and their interaction and reaction with DNA have gathered a lot of attention of researchers over the past few decades [10, 9, 11–14]. Ruthenium (Ru)-complexes are considered to be one of the most promising alternatives to the prevalent platinum (Pt)-based drugs due to their favourable toxicity, drug resistance and various remarkable properties [12, 15–17]. Therefore, a large number of Ru-complexes have been synthesised and tested for their anticancer activity to date [12, 18]. It is reported that two Ru-complexes, namely, NAMI-A [$\text{ImH}[\text{trans-RuCl}_4(\text{DMSO-S})\text{imidazole(Im)}]$] and KP1019 [$\text{InH}[\text{trans-RuCl}_4(\text{Indazole})_2]$] have entered the clinical trials [18, 16, 19]. Ru-based drugs are, in general, believed to act via two mechanisms: activation-by-reduction and hydrolysis. Previous studies [20–28] on the hydrolysis of NAMI-A show that the first hydrolysis where one chloride (Cl^-) ligand of NAMI-A is replaced by a water molecule is faster than its second hydrolysis. The hydrolyzed products of Ru-based drugs such as NAMI-A, ICR and KP1019 are reported to be more reactive towards biological molecules than their parent complexes [20–24, 29–31].

DNA is considered to be the prime pharmacological target for ruthenium and other metal-based anticancer drugs [9–12, 15, 16, 19, 20, 29, 31, 30]. The Ru-based drugs bind favorably to the N7 site of

guanine [30, 29, 16, 19, 20]. Moreover, Ru-based drugs can also bind to other DNA bases such as adenine and cytosine [16, 17, 19, 29, 32]. Novakova et al. [33] showed that Ru-complex, mer-[Ru(III)(terpy)Cl₃], formed DNA inter-strand cross-linking by coordinating specially with isolated guanine residues. P.M. van Vliet et al. [34] showed that the mer-[Ru(III)(terpy)Cl₃] complex formed DNA inter-strand cross-linking in vitro via binding with two guanine bases in trans-configuration. Malina et al. [35] demonstrated that Ru-based drugs such as NAMI, KP1019 and ICR yielded bifunctional inter-strand crosslinks on double helical DNA with guanine as the prevalent binding site. It is noted that NAMI can produce bifunctional adducts affecting the conformation of DNA more efficiently as compared to KP1019 and ICR [35]. Extensive researches have been carried out both experimentally and theoretically to understand the mechanisms of action of Ru-based drugs but its therapeutic effect and specific pharmacological targets are not completely understood [36, 32, 30, 31, 29, 20, 19, 12].

In order to understand the DNA inter-strand cross-link formation capability of NAMI-A, we herein perform the density functional theoretic (DFT) calculations to investigate theoretically the structure and energetics of the formation of cross-linked DNA bases due to the reactions of mono-functional adduct of NAMI-A formed at the N7 site of guanine (hereafter denoted as G_{N7}-NAMI-A) and each of the four DNA bases (as shown in Scheme 1). In view of the fact that Ru-based drugs bind predominantly with the N7 site of guanine and are hydrolyzed before reacting with any target, the G_{N7}-NAMI-A (Fig. 1a) has been chosen as a reactant for the present study. The mechanisms of the formation of mono-functional adduct of NAMI-A at the N7 site of guanine can be found in our previous work [36].

2. Computational Details

The DFT calculations were performed by modelling the Ru(III)-anticancer NAMI-A drug as the [*trans*-RuCl₄(DMSO-S)(Imidazole)]⁻ anion. The geometry optimization followed by frequency calculations for all the species involved in Scheme 1 were carried out using the hybrid meta-GGA functional M06-2X [37] and the hybrid basis set (LanL2DZ+6-31G**) in gas and aqueous phases. The effective core potential basis set (LanL2DZ) was used for Ru-atom whereas the Pople's standard basis set (6-31G**) was used for all other atoms. Each gas (aqueous) phase geometry optimization calculation was further followed by the single-point energy calculation at the M06-2X/(LanL2DZ+6-311+G**) level of theory in gas (aqueous) phase. The solvent effect was estimated using the conductor-like polarisable continuum model (CPCM) [38, 39]. The genuineness of the optimized species being total energy minima was confirmed by all positive vibrational frequencies. The gas (aqueous) phase thermal energy corrections determined at 298.15 K were also applied to the total energies obtained by single-point energy calculations to obtain the Gibbs free energies at the M06-2X/(LanL2DZ+6-311+G**) level of theory in gas (aqueous) phase. All DFT calculations and visualizations of structure and vibrational modes were done using the Gaussian09 quantum chemistry package [40] and the GaussView [41] program, respectively.

3. Results And Discussion

The optimized geometries of G_{N7} -NAMI-A and DNA bases (adenine (A), guanine (G), cytosine (C) and thymine (T)) along with the reaction sites considered for the present study are displayed in Fig. 1. The optimized geometries of the cross-linked products formed by the reaction of G_{N7} -NAMI-A at the N1, N3 and N7 sites of adenine as well as the N3, O6 and N7 sites of guanine are displayed in Fig. 2 whereas those formed at the O2 and N3 sites of cytosine and the O2 and O4 sites of thymine are displayed in Fig. 3. The certain optimized bond distances are also displayed in Figs. 2 and 3. The reaction free energies (ΔG_f) and reaction enthalpies (ΔH_f) of reactions of Scheme 1 obtained at the M06-2X/(LanL2DZ+6-311+G**) level of theory in gas phase (aqueous media) are presented in Table 1. The ΔG_f (ΔH_f) of a reaction was calculated as the sum of the Gibbs free energies (enthalpies) of the products minus the sum of the Gibbs free energies (enthalpies) of the reactants. The net NPA charges [42, 43] (in the unit of magnitude of electronic charge, $|e|$) on important atoms of cross-linked products calculated at the M06-2X/(LanL2DZ+6-311+G**) level of theory in gas phase (aqueous media) are presented in Table 2. For reactions of G_{N7} -NAMI-A with adenine, we note that Ru-Cl bond distances are appreciably larger (by ~ 0.20 – 0.33 Å) than the Ru- $A_{N1/N3/N7}$ bond distances, the Ru- A_{N1} , Ru- A_{N3} and A_{N7} bond distances in gas phase (aqueous media) being 2.12 (2.13), 2.09 (2.09) and 2.17 (2.16) Å, respectively (Fig. 2). This indicates that as compared with the chloride ligands, the adenine base is more strongly bonded with the Ru-atom in the cross-linked products. Similarly, it is found that all other Ru-DNA base bonds like Ru- $G_{N3/N7/O6}$, Ru- $C_{O2/O6}$ or Ru- $T_{O2/O4}$ are also stronger than the Ru-Cl bonds as their bond distances are shorter than those of Ru-Cl bonds (Figs. 2 and 3). In order to understand why the Ru- $A_{N1/N3/N7}$ bonds are stronger than the Ru-Cl bonds in the products, we examined the net NPA charges on the atoms concerned. It is found that the charges on the reacting sites of DNA bases are significantly more negative than those of Cl atoms in all products (Table 2). For instance, the NPA charges on N1/N3/N7 atoms of adenine are more negative than those on Cl1/Cl2 atoms by ~ 0.15 – 0.2 $|e|$ (Table 2). This shows that the Ru-DNA base bonds are stronger due to the greater negative charge on the reactive sites of DNA bases. Based on above discussion, we can conclude that Ru-DNA base bonds formed in the cross-linked products are relatively stable.

The feasibility of formation of cross-linked products can be determined by examining the ΔH_f and ΔG_f values of the reactions. It is apparent from Table 1 that all the studied reactions are exothermic as their ΔH_f values are negative in both gas phase and aqueous media, their values lie in the range from -19.37 to -6.79 (-11.16 to -2.43) kcal/mol in gas phase (aqueous media). Further, the ΔG_f values are also found to be negative for all the reactions in both gas phase and aqueous media, excepting the reaction at the N3 site of cytosine (C_{N3}) for which ΔG_f is somewhat positive (0.12 kcal/mol) in aqueous media (Table 1). Although negative, ΔG_f is also very low (-0.31 kcal/mol) for reaction at the O2 site of thymine (T_{O2}) in aqueous media. Thus, our calculations indicate that G_{N7} -NAMI-A would not form stable products at the N3 site of cytosine and the O2 site of thymine in biological media. However, it can form stable cross-linked products at the $A_{N1/N3/N7}$, $G_{N3/N7/O6}$, C_{O2} and T_{O4} sites in both gas phase and aqueous media. Considering the aqueous media results more relevant for biological systems, both the ΔH_f and ΔG_f values show that the N3 site of adenine (A_{N3}) and N7 site of guanine (G_{N7}) are most exothermic among all the

studied reactions (Table 1). The ΔG_f (ΔH_f) for reactions at the A_{N3} and G_{N7} are -8.39 (-11.16) and -8.38 (-10.21) kcal/mol, respectively (Table 1). According to the Bell-Evans-Polanyi principle, the activation energies for reactions at the A_{N3} and G_{N7} sites would be very less as compared to the other reaction sites. This shows that NAMI-A would favorably form the cross-linked products involving the N7 site of guanine at one side and the N7 site of guanine or the N3 site of adenine at the other side. This is in agreement with experimental observation that Ru-based drugs such as NAMI, KP1019 and ICR form bifunctional inter-strand crosslinks on double helical DNA with guanine as the prevalent binding site [35]. Based on our present study, we propose the following mechanism (as shown in Fig. 4) for the formation of the cross-linked products from the reaction of NAMI-A with DNA bases.

4. Conclusions

The DNA cross-link formation capability of the Ru-anticancer drug NAMI-A has been evaluated by studying the structure and energetics of the reactions of the G_{N7} -NAMI-A (monofunctional adduct of NAMI-A at the N7 site of guanine) at the N1, N3 and N7 sites of adenine; N3, N7 and O6 sites of guanine; O2 and N3 sites of cytosine and O2 and O4 sites of thymine, using density functional theory calculations. It is found that the G_{N7} -NAMI-A can form stable cross-linked products at all the sites studied here except at the N3 site of cytosine and O2 site of thymine. The calculated reaction free energies (ΔG_f) and reaction enthalpies (ΔH_f) indicate that the N3 site of adenine (A_{N3}) and N7 site of guanine (G_{N7}) are most exothermic among all the studied reactions. In aqueous media, the ΔG_f (ΔH_f) for reactions at the A_{N3} and G_{N7} sites are -8.39 (-11.16) and -8.38 (-10.21) kcal/mol, respectively. Thus, this study shows that NAMI-A would favorably form the cross-linked products involving the N7 site of guanine at one side and the N7 site of guanine or the N3 site of adenine at the other side, which is in agreement with experimental observation that NAMI-A produce bifunctional cross-linked products with guanine as the prevalent binding site. Based on our present study, we propose that the formation of the cross-linked products from the reaction of NAMI-A with DNA bases would follow the Scheme 2.

Declarations

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Tables

Table 1: Reaction free energies (ΔG_f) and reaction enthalpies (ΔH_f) of reactions of Scheme 1 obtained at the M06-2X/(LanL2DZ+6-311+G) level of theory in Gas Phase (Aqueous Media)**

Reaction Site	ΔG_f	ΔH_f
G _{N7} -NAMI-A_A _{N1}	-7.64 (-4.93)	-12.33(-8.13)
G _{N7} -NAMI-A_A _{N3}	-11.55 (-8.39)	-15.28 (-11.16)
G _{N7} -NAMI-A_A _{N7}	-2.08 (-1.22)	-6.79 (-4.74)
G _{N7} -NAMI-A_G _{N3}	-5.52 (-4.64)	-9.49 (-7.19)
G _{N7} -NAMI-A_G _{O6}	-14.28 (-4.77)	-17.06 (-6.95)
G _{N7} -NAMI-A_G _{N7}	-16.52 (-8.38)	-19.37 (-10.21)
G _{N7} -NAMI-A_C _{O2}	-9.97 (-1.65)	-13.64 (-5.37)
G _{N7} -NAMI-A_C _{N3}	-9.87 (0.12)	-13.77 (-3.64)
G _{N7} -NAMI-A_T _{O2}	-4.01 (-0.31)	-7.43 (-2.43)
G _{N7} -NAMI-A_T _{O4}	-5.36 (-2.28)	-7.18 (-4.13)

Table 2: The net NPA Charges (in the unit of magnitude of electronic charge) of some important atoms calculated at M06-2X/(LanL2DZ+6-311+G) level of theory in Gas phase (Aqueous media)**

Reaction site	Net Charge						DNA bases
	Ru	Cl1	Cl2	N	S	GN7	
G _{N7} -NAMI-A_A _{N1}	0.094 (0.129)	-0.318 (-0.355)	-0.361 (-0.397)	-0.448 (-0.458)	1.456 (1.438)	-0.452 (-0.464)	-0.520 (-0.530)
G _{N7} -NAMI-A_A _{N3}	0.089 (0.123)	-0.327 (-0.358)	-0.364 (-0.401)	-0.452 (-0.462)	1.462 (1.446)	-0.456 (-0.465)	-0.498 (-0.501)
G _{N7} -NAMI-A_A _{N7}	0.107 (0.141)	-0.315 (-0.339)	-0.323 (-0.379)	-0.447 (-0.461)	1.450 (1.431)	-0.452 (-0.460)	-0.460 (-0.468)
G _{N7} -NAMI-A_G _{N3}	0.109 (0.163)	-0.265 (-0.325)	-0.402 (-0.398)	-0.443 (-0.445)	1.434 (1.407)	-0.442 (-0.453)	-0.570 (-0.572)
G _{N7} -NAMI-A_G _{O6}	0.192 (0.209)	-0.349 (-0.369)	-0.336 (-0.387)	-0.421 (-0.431)	1.415 (1.416)	-0.428 (-0.439)	-0.625 (-0.648)
G _{N7} -NAMI-A_G _{N7}	0.118 (0.172)	-0.337 (-0.386)	-0.336 (-0.378)	-0.432 (-0.427)	1.438 (1.420)	-0.439 (-0.455)	-0.440 (-0.448)
G _{N7} -NAMI-A_C _{O2}	0.236 (0.273)	-0.307 (-0.382)	-0.393 (-0.427)	-0.428 (-0.427)	1.422 (1.406)	-0.442 (-0.457)	-0.607 (-0.633)
G _{N7} -NAMI-A_C _{N3}	0.137 (0.169)	-0.277 (-0.346)	-0.419 (-0.428)	-0.440 (-0.433)	1.438 (1.425)	-0.443 (-0.462)	-0.592 (-0.594)
G _{N7} -NAMI-A_T _{O2}	0.165 (0.195)	-0.298 (-0.354)	-0.392 (-0.419)	-0.435 (-0.439)	1.432 (1.420)	-0.423 (-0.431)	-0.618 (-0.628)
G _{N7} -NAMI-A_T _{O4}	0.172 (0.195)	-0.359 (-0.365)	-0.338 (-0.394)	-0.435 (-0.440)	1.446 (1.434)	-0.426 (-0.430)	-0.613 (-0.632)

Schemes

Schemes 1 and 2 are available in the Supplemental Files section

Figures

Figure 1

The optimized geometries of G_{N7} -NAMI-A and DNA bases (adenine (A), guanine (G), cytosine (C) and thymine (T)) along with the reaction sites studied here , as obtained at M06-2X/(LanL2DZ+6-31G**)level of theory in gas phase.

Figure 2

The optimized geometries of the cross-linked products formed by the reaction of G_{N7}-NAMI-A at the N1, N3 and N7 sites of adenine as well as at the N3, O6 and N7 sites of guanine along with their certain interatomic distances (Å), as obtained at the M06-2X/(LanL2DZ+6-31G**) level of theory in gas phase (aqueous media)

Figure 3

The optimized geometries of the cross-linked products formed by the reaction of G_{N7} -NAMI-A at the O2 and N3 sites of cytosine as well as at the O2 and O4 sites of thymine along with their certain interatomic distances (Å), as obtained at M06-2X/(LanL2DZ+6-31G**) level of theory in gas phase (Aqueous media)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Scheme1.png](#)
- [Scheme2.png](#)