DENSITY FUNCTIONAL BASED REACTIVITY STUDIES ON AZIRIDINIUM ION INTERMEDIATES

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Abstract

Reactivities of the aziridinium ion intermediates of six anticancer drugs belonging to the nitrogen mustard family are analysed using conceptual density functional theory based reactivity descriptors. Reactivity of the species is found to depend on di-electric of the solvent. Enthalpy, Gibbs energy and entropy of formation of aziridinium ions are analysed at different temperatures and solvents at B3LYP/6-31+G(d) level of theory. Further, the natural bond orbital (NBO) analysis is performed which seemed to be quite informative.

Key words

DNA cross-linking agents, aziridinium ion, DFT based reactivity descriptors, solvent medium.

1. Introduction

Nitrogen mustards represent the earliest and perhaps the most extensively studied DNA inter-strand cross-linking agents and is being used in cancer chemotherapy for more than five decades [1,2]. This class of drug molecules form a positively charged reactive aziridinium ion (Az^+) intermediate which is unstable and reacts easily with cellular biomolecules such as DNA, RNA, proteins etc. [3]. Cytotoxicity effect of these drug molecules is attributed by their alkylation potential with the most nucleophilic centres of the DNA bases and N7 position of guanine is the most preferred site, forming mono- and cross-linked adducts [4]. It was also reported that the alkylation occurs preferentially at the endocyclic nitrogen and exocyclic oxygen atoms of the DNA bases [5,6]. In an important work, Shukla et al. used quantum mechanical calculations to observe the interaction of mustine molecule with different DNA bases [7]. Mustine, the most primitive and extensively employed nitrogen mustard is the most reactive one. Because of its high reactivity, more stable analogues were sought and substitution of its methyl group by electron withdrawing groups make the nitrogen atom less nucleophilic which slows down the rate of Az^+ ion formation, in turn reducing reactivity [8]. Chlorambucil, melphalan, spiromustine, uracil mustard, bendamustine are some example of such analogues, (Figure I). Despite being a potent anti-tumour agent, these drug molecules suffer a major drawback that limit their clinical application is that these drugs are not cell specific ie., apart from cancerous cells, they attack other normal cells of the body and hence obstruct DNA replication and transcription [1,3,4]. Thus stability/reactivity of the Az^+ ion is important during alkylation process. Earlier, it was shown that reactivity of Az^+ ion is effected by different factors such as solvent, external electric field, configuration of the species etc. [9-10].

DFT based reactivity descriptors such as global hardness, chemical potential, global electrophilicity etc. are efficient tools to describe the reactivity pattern of chemical species [11]. These descriptors have been tested and studied by several research groups and are found to be very useful in rationalizing the reactivity patterns of the molecular systems and reviewed well [12].

In the present work, we considered six nitrogen mustards (Figure I) and studied reactivity of their corresponding Az^+ ions using DFT based reactivity descriptors. Global reactivity descriptors specifically global hardness (η) and global electrophilicity (ω) are calculated in gas phase as well as in three different solvent phases (ranging from polar to non-polar). Apart from that, interaction energy between Az^+ ion intermediates and GC base pairs have been observed. Further, NBO analysis is performed with the same level of theory.

2. Theoretical details of reactivity descriptors and computational details

In DFT, chemical potential (μ) is defined as the first derivative of energy with respect to the number of electrons [13]:

$$\mu = \left(\frac{\partial E}{\partial N}\right)_{v(\vec{r})} \quad \text{(I)}$$

and global hardness (η) [14] as:

$$\eta = \frac{1}{2}\left(\frac{\partial^2 E}{\partial N^2}\right)_{v(\vec{r})} = \frac{1}{2}\left(\frac{\partial \mu}{\partial N}\right)_{v(\vec{r})} \quad \text{(II)}$$

Where, E is the energy and N is the number of electrons of an electronic system at constant external potential, $v(\vec{r})$.

In most of the applications, chemical potential (μ) and chemical hardness (η) are calculated using finite difference approximation in terms of IP and EA which leads to the working formulae

$$\mu = \frac{-(IP + EA)}{2} \quad \text{(III)}$$

$$\eta = \frac{IP - EA}{2} \quad \text{(IV)}$$

Use of Koopmans' theorem defines the IP and EA in terms of the energies of highest occupied molecular orbital (ε_{HOMO}) and lowest unoccupied molecular orbital (ε_{LUMO}) as:

$$IP = -\varepsilon_{HOMO} \quad \text{(V)}$$

$$EA = -\varepsilon_{LUMO} \quad \text{(VI)}$$

and therefore, μ and η can be expressed as:

$$\eta = \frac{\varepsilon_{LUMO} - \varepsilon_{HOMO}}{2} \quad \text{(VII)}$$

and $$\mu = \frac{\varepsilon_{LUMO} + \varepsilon_{HOMO}}{2} \quad \text{(VIII)}$$

Parr and co-workers proposed global electrophilicity (ω) as a measure of electrophilicity of a ligand [16] as:

$$\omega = \frac{\mu^2}{2\eta} \quad \text{(IX)}$$

It is the measure of capacity of a species to accept an arbitrary number of electrons.

The gas phase geometrical minima of the species are optimized using 6-31+G(d) basis set with Becke three parameter exchange and Lee, Yang and Parr correlation functional, B3LYP [17] and confirmed by frequency calculation. Geometry optimization is followed by single point calculations at the same level of theory at different temperatures (ranging from a low to high value, 77K to 315K) and in three different solvents (n-octanol ε = 9.86, 1,2-ethanediol ε = 40.24, and water ε = 78.35), using polarizable continuum model (PCM)[18]. The di-electric constant of the fluids in different parts of human body are different, varying from non-polar to a polar one and hence three solvents having a range of di-electric constant are chosen. The global reactivity descriptors (chemical potential, global hardness and global electrophilicity) are calculated using Equations (VII)-(IX). All calculations are performed using Gaussian09 [19].

3. Results and discussion

3.1. Variation of reactivity descriptors

The reactivity/stability of corresponding aziridinium ion intermediates is monitored using global hardness and global electrophilicity values. The in gas phase hardness and electrophilicity were calculated at B3LYP/6-31+G(d) level of theory. Further to realize the effect of solvents on the reactivity pattern, we repeat our calculations in three different solvent media at the same level of theory, variations are shown in Figures II(a) and II(b).

In gas phase, the hardness order is: mustine > spiromustine > chlorambucil > uracil mustard > bendamustine > melphalan and changes to mustine > spiromustine > chlorambucil > uracil mustard > melphalan > bendamustine in aqueous phase. On the other hand electrophilicity is in the order uracil mustard > spiromustine > melphalan > chlorambucil > bendamustine > mustine in gas phase and uracil mustard > melphalan > bendamustine > chlorambucil > spiromustine > mustine in aqueous phase. It is important to note that, as we move from gas phase to a solvent phase, hardness of the Az^+ ions do not change much, Figure II(a). Contrarily, electrophilicity shows a dramatic drop in their values (on moving from gas phase to n-octanol), indicating low reactivity of the Az^+ ions in solvent phases, Figure II(b). With further increase in di-electric of solvents (moving from n-octanol to water), such sharp variation is not observed. Low electrophilicity of the Az^+ ions might affect the acceptance of electron density from guanine (in DNA), Scheme I. This in turn weaken the tendency (measured in terms of interaction energy between the two species) of the Az^+ ions to bind with guanine (in DNA) in solvent phases. To verify this, we calculated the interaction energies between Az^+ ion and GC-base pair. The BSSE corrected interaction energies are reported in Table I and are calculated using super-molecular approach (for the sake of

simplicity, we have considered only one GC base pair instead of the DNA chain). Interaction energy values clearly show a sharp drop as we move from gas to aqueous phase; thus supporting the above results. Trend of interaction energy in gas phase however does not tally with the aqueous phase trend indicating that the extent of solvation varies from species to species.

3.2. Thermochemical properties

Thermodynamic feasibility of the Az^+ ion formation process ($drug \rightarrow azi^+ + Cl^-$), is observed from ΔH (change in enthalpy) and ΔG (change in Gibbs free energy) involved in the process at different temperatures as well as in different solvent media using supermolecular approach (e. g. $\Delta H = (H_{azi}^{+} + H_{Cl}^{-}) - H_{drug}$).

Variations of ΔG and ΔH in different solvents at 298.15K are summarised in Figure III. It is interesting to note that, as the di-electric constant of the medium increases (moving from gas phase to aqueous phase), ΔG and ΔH values drop suddenly (still exhibiting positive values), clearly indicating the effect of solvent polarity on both the parameters, figures III(a) and III(b). It is conclusive to comment that the Az^+ ion formation is comparatively favourable in solvent phase compared to gas phase.

Our next step is to analyse the effect of temperature on the thermodynamic parameters. ΔG and ΔH for the ion formation process are calculated at five different temperatures in gas and aqueous phases and are shown in Figures IV(a – d).

It is evident from Figure IV that as temperature increases, gas as well as aqueous phase ΔG drops significantly (Figures IV(a–b)) whereas ΔH decreases slightly (Figures IV(c–d)). It is to be worth mentioning that spiromustine exhibit exceptional behaviour in gas as well as in aqueous phase. Our observations suggest that the Az^+ ion formation is favoured at high temperature and polar solvents. As cytoplasm is a polar medium, this observation implies that the Az^+ ion formation is favoured in cytoplasm at normal body temperature.

3.3. NBO analysis

The concept of natural bond orbital (NBO) analysis is helpful to study the distribution of electrons in atomic and molecular orbitals for the one-electron density matrix for defining the shape of the atomic orbitals in the molecular environment and then derive molecular bonds from electron density between atoms [20] and hence we have performed NBO analysis at B3LYP/6-31+G(d) level of theory.

The effect of substitution of the methyl group of mustine by electron withdrawing groups is observed from the calculated NBO charges on the drug molecules (carbon and

nitrogen centers, ‘a’ in Scheme 1). The NBO charges at the N center of the drug is in the order: mustine (–0.5553) > bendamustine (–0.5545) > uracil mustard (–0.5491) > spiromustine (–0.5226) > chlorambucil (–0.5081) > melphalan (–0.4878). It is seen that all the drug molecules possess lesser negative charge on the N center compared to mustine. Thus, substitution at the N atom becomes successful in withdrawing electronic charges from N atom which is necessary to slow down the rate of Az^+ ion formation.

4. Conclusion

We have made an effort to study the reactivity, interaction energy and thermodynamics of Az^+ ions. Our study reveals that-

1. Reactivity of the Az^+ ion intermediates lowered on incorporation of solvent media which in turn reduces the capability of the species to accept electron density from guanine. Exceptional drop in electrophilicity of the Az^+ ions in polar solvent make them stable.
2. The Az^+ ion intermediates of the prototype drugs exhibit significant interaction energy with GC base pair in gas as well as in aqueous phases and is chief key for their cytotoxicity.
3. High temperature and polarity of the solvent favours the thermodynamics of the Az^+ ion formation. Thus body temperature and polarity of cytoplasm allows formation of the Az^+ ion.
4. NBO analysis shows that substitution at the N atom may slow down the rate of Az^+ ion formation.

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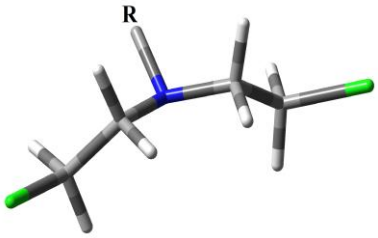
Table I

Gas and aqueous phase interaction energies (in kcal/mol) at B3LYP/6-31+G(d) level of theory.

Drug molecule	Interaction energy	
	Gas phase	Aqueous phase
Uracilmustard	-56.97	-25.20
Spiromustine	-52.71	-20.86
Chlorumbucil	-50.40	-25.03
Mustine	-48.64	-23.08
Melphalan	-46.76	-34.96
Bendamustine	-44.56	-24.26



Nitrogen mustard

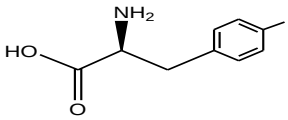
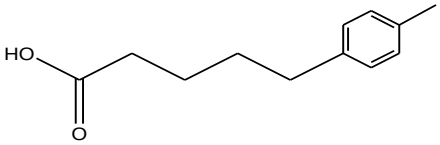
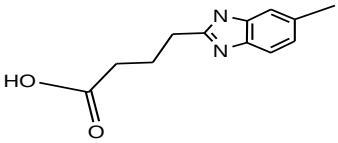
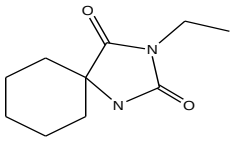
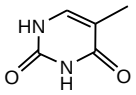
Entry	R=	
1	CH_3	Mustine
2		Melphalan
3		Chlorambucil
4		Bendamustine
5		Spiromustine
6		Uracil mustard

Figure I: Structures of few clinically used nitrogen mustards.

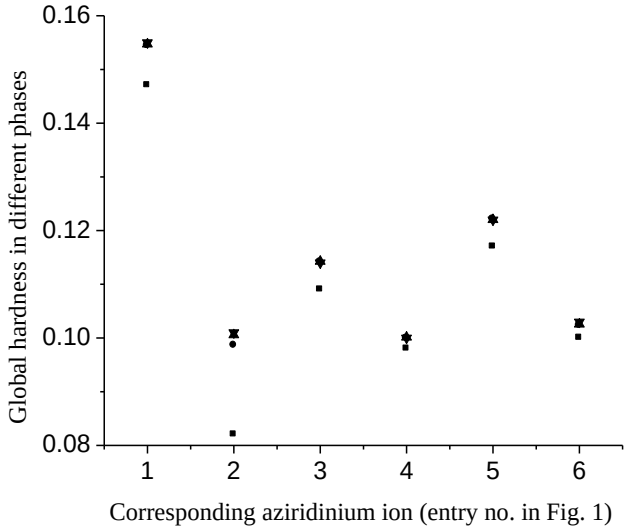


Figure II(a): Global hardness

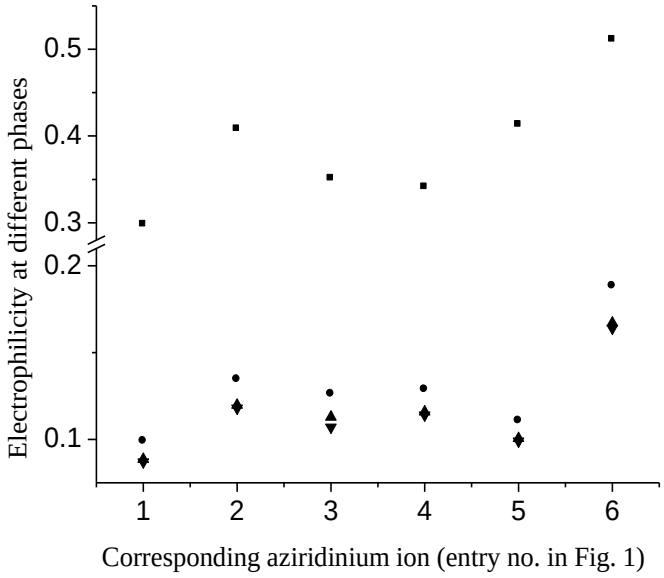


Figure II(b): Global electrophilicity

Figure II: Variation of electrophilicity (in ev) and hardness (in ev) of the aziridinium ions from gas to aqueous phase (■-gas phase, ●-n-Octanol, ▲-1,2-Ethanediol, ▼-water).

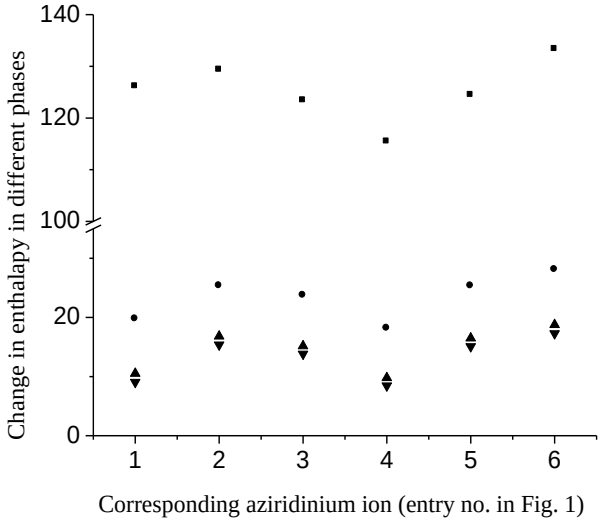


Figure III(a): Variation of ΔH

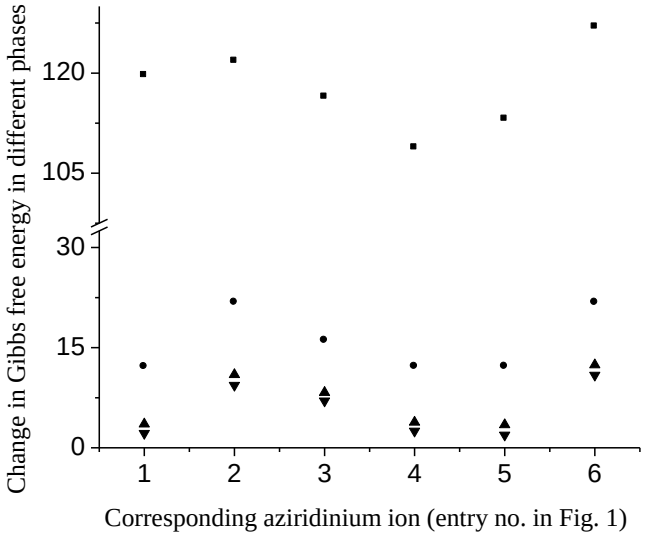


Figure III(b): Variation of ΔG

Figure III: Variation of enthalpy (ΔH) and Gibbs free energy (ΔG) involved in aziridinium ion formation process from different drug molecules (all values are in kcal/mol) (■-gas, ●-n-Octanol, ▲-1,2-Ethanediol, ▼-water,).

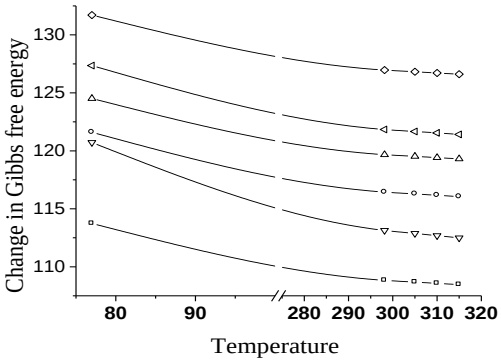


Figure IV(a): Gas phase

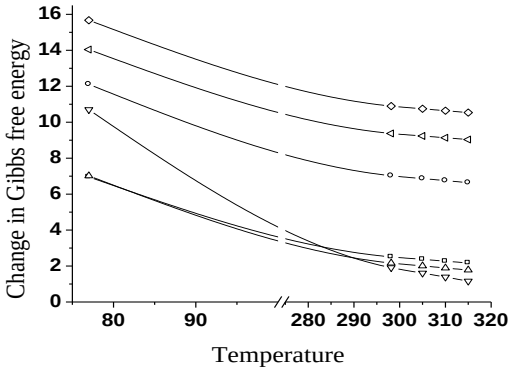


Figure IV(b): Aqueous phase

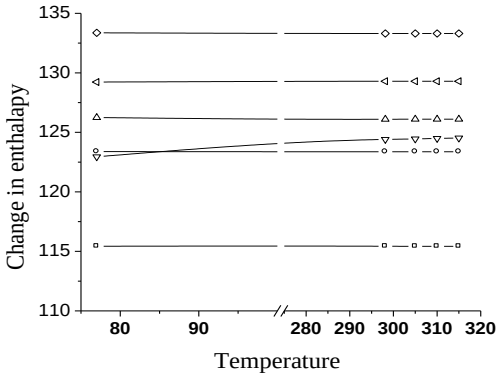


Figure IV(c): Gas phase

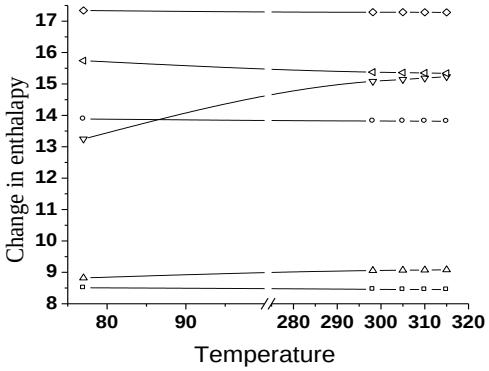
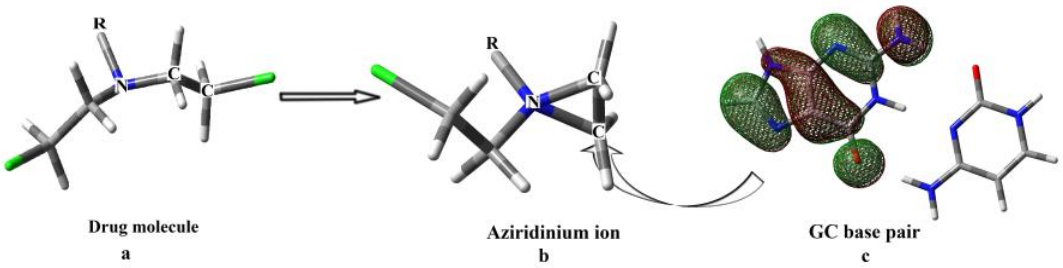


Figure IV(d): Aqueous phase

Figure IV: Variation of enthalpy (ΔH) and Gibbs free energy (ΔG) (in kcal/mol) with temperature (in Kelvin) in gas and aqueous phases (∇-spiromustine, ♢- uracil mustard, ◁-melphalan, □ - bendamustine, ○- chlorambucil, Δ- mustine).



Scheme I: Transfer of electron density from guanine to aziridinium ion during alkylation.