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Notes

Ionic effects on some anticancer drugs from analysis of their metal ion and proton affinities

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The proton affinities (PA) and the metal ion affinities (MA) of nitrogen mustards have been investigated to analyze the ionic effect in alkylation reactions. The ion affinities of N4 atomic site of nitrogen mustards are significant, and the proton affinities are more significant than the metal ion affinities. However, Na^+ affinities are comparatively less than Mg^{2+} affinities. The values of ion affinities have been taken for comparing the ionic effect in alkylation reaction. The magnitudes of PA and MA (Mg^{2+}) indirectly show the possibility of inhibitory effect at the intermediate state during aziridinium ion formation of alkylation reaction. So, the presence of H^+ and Mg^{2+} may change the alkylation rate, since these ions acquire strong affinity for N4 of nitrogen mustards.

In general, the nitrogen mustards form aziridinium ion as an intermediate in alkylation reaction with the release of chloride ion (Fig. 1). Both the aliphatic group and aromatic group substituted nitrogen mustards at N4 form aziridinium ions at the intermediate state, and these nitrogen mustards acquire similar binding with sequences of DNA[1-6]. Again, the reactivity of a number of nitrogen mustards has been found to be correlated with the molecular electrostatic potential of the guanine-rich region in DNA[6b]. The increase of ionic strength in the alkylation reaction reduces the rate of reaction. It has been shown that the presence of Na^+ or Mg^{2+} ions substantially shows decrease in the rate of alkylation[7c-e]. On the basis of experimental findings on the reaction conducted at different *p*Hs, the protonation at some atomic sites appears to be an important factor for the alteration of rate of alkylation[8]. Basically, the protonation of a molecule depends on the presence of basic sites in the molecule. Thus, the proton or cations may interact with the basic sites of the drug, resulting in significant differences in the alkylation rate. It has been experimentally observed that the dissociation of chlorambucil to form aziridinium ion and chloride ion at low *p*H (acidic) is significantly less than that in neutral solution[9]. Under this condition the aziridinium ring formation step at the intermediate state may be controlled by the interaction of H^+ and cation with N4 of nitrogen mustard (Fig. 1). The cationic effect on drug is likely to play important role during alkylation by nitrogen mustards, and hence it is important to monitor such effect for examining the decrease of reaction rate in presence of ions.

Here, the first step of the alkylation reaction proceeds through intramolecular cyclization (SN1) pathway where one of the two 2-chloroethyl side chains forms aziridinium ion with the release of chloride ion [10-13]. By this reaction the tertiary amine is converted to a quaternary ammonium compound. Moreover, the aziridinium ion can also form either a carbonium ion or a transition complex by interacting with cations present in solution.

Besides the cationic effect, the proton may also influence alkylation by inhibiting the formation of this reactive aziridinium ion[2-12]. Hence, the interaction with proton may be also taken for understanding the factors responsible for the stability of cation-molecule complexes. In addition to investigating the metal ion affinities, the calculations of proton affinities of nitrogen mustards necessarily bridge the general effect of these ions in alkylation reaction[14-16]. The present study focuses on the quantum mechanical calculation of H^+ and cation affinities at N4 of nitrogen mustards.

Methodology

The *ab initio* calculations were performed at the 6-31G/MP2 and HF/6-31G** levels using Gaussian 94 programme code[17]. In the present study, we limit our computation up to 6-31G basis set for geometry optimization, and the optimized geometries were taken for calculating proton and cation affinities at 6-31G/MP2 and 6-31G** level of theories. The proton affinities (PA) and cation affinities (MA) are computed as follows :

$$\text{I. E. } (H^+) = E_{DH+} - E_D$$
$$\text{I. E. } (M^+) = E_{DM+} - E_D - E_{M+}$$
$$\text{PA} = -\text{ I. E. } (H^+)$$
$$\text{MA} = -\text{I. E. } (M^+)$$

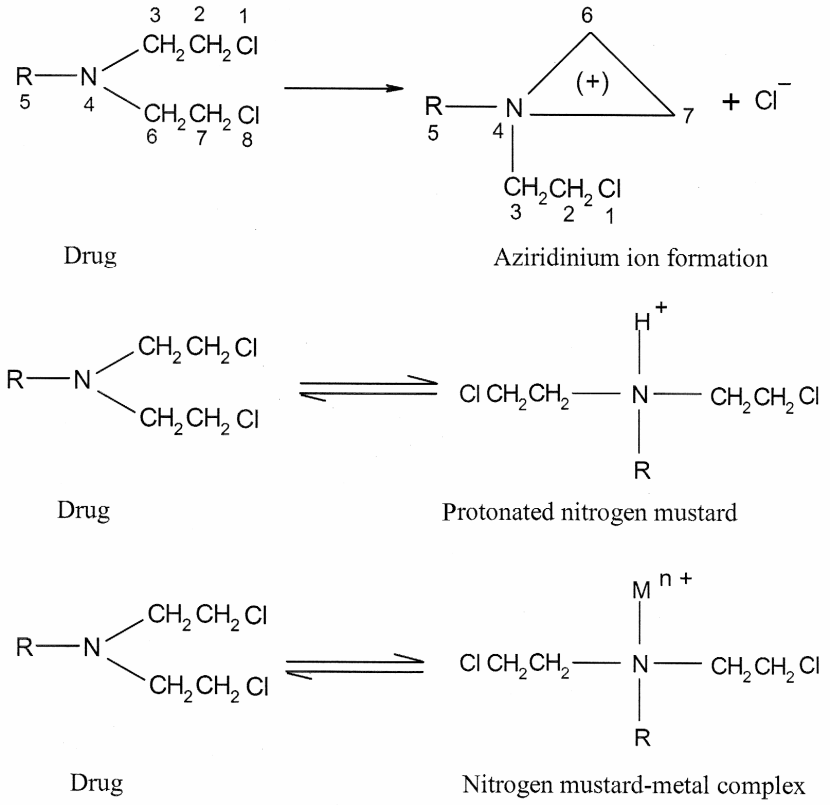

Fig. 1 – Formation of aziridinium ion formation, protonated nitrogen mustard and nitrogen mustard-metal complex.

I. E. (H^+) and I. E. (M^+) are the interaction energies of proton and cations, E_{DH+}, E_{DM+}, E_D and E_{M+} are the corresponding energies of drug-H^+, drug-cation, drug and metal ion respectively.

Figure 1 presents the structures of the nitrogen mustards involved in the equilibrium equation for the reaction with H^+ and metal ion. All the structures of drugs and drug-ion complexes were fully optimized before computing the corresponding values of PA and MA.

Results and discussion

The proton and metal ions (Na^+ and Mg^{2+}) have been considered for analyzing the ionic effect on alkylation reaction. Na^+ and Mg^{2+} are important ions present in biological systems. Also, these ions are used in many alkylation reactions. The metal ion affinities as well as proton affinities are computed with different levels of theories and geometrical features of ion-drug complexes are analyzed.

Proton-drug and metal ion-drug complexes

The geometrical features of the proton-drug and metal ion-nitrogen mustards are shown in Figs 2–5. On the basis of these structures, analysis has been carried out on the complexity of cation interaction with drugs as compared with the proton interaction. Such a study may show better understanding of the metal ion interaction with different nitrogen mustards.

In the protonated mustine, the proton is found well attached to N4 (nitrogen) of mustine, and this drug acquires large PA value (Table 1, Fig. 3a). Similarly, in the protonated melphalan and chlorambucil, the proton interacts specifically with N4 but the computed PA value of mustine is slightly more than those of melphalan and chlorambucil. Moreover, the variability of PA obtained from different level of theories is similar (Table 1). The proton interacts with N4 of mustine, melphalan and chlorambucil at the distance of 1 Å approximately, and the PA values are higher than the MA values. The Mulliken net charges on N4 of these drugs before and after interaction with proton are shown in Tables 2 and 3.

Both the Na^+ and Mg^{2+} ions interact with the N4 atomic sites of mustine, but Mg^{2+} is at a closer distance than Na^+ ion in the complexes of this drug. Also, the MA (Mg^{2+}) is much more than MA (Na^+) (Table 3, Fig. 3(b & c)). Thus, Mg^{2+} may produce more inhibition than the Na^+ ion in the formation of aziridinium ion.

From the computed proton and the metal ion affinities of melphalan, the proton affinities are found to be more than the metal affinities (Table1). Similar to protonated mustine, the proton interacts specifically with N4 of melphalan whereas Mg^{2+} and Na^{+} interact with multiple atomic sites (Fig. 4(a & c)). The structure of Na^{+}- melphalan complex indicates three interaction sites with Na^{+} ion, the N4 and the carbon atoms of aromatic ring (C1 and C2) (Table 3). The net charges on N4, C1, C2 and ions obtained from Mulliken population analysis are shown in Table 2. This shows that Na^{+} acquires affinity for both the lone pair electrons of N4 and the nearby π electrons of the aromatic ring (Fig. 5b). Hence the MA values of Na^{+} and Mg^{2+} ions depend on these multiple interactions and the presence of these ions may retard the formation of aziridinium ring due to strong interaction with N4. In addition to having multiple interactions with the π electrons of aromatic ring other than N4 atomic site, MA (Na^{+}) is still found to be less than PA (Table 1). The MA (Mg^{2+}) values of these drugs are significantly larger than MA (Na^{+}) values. Also, there is certain similarity in the mode of interaction of Mg^{2+} and Na^{+} with melphalan, where the ions interact with N4 as well as with the π electrons of aromatic ring. This may be due to the bivalent nature of Mg^{2+}. If we compare the interaction distances of Mg^{2+} and Na^{+} in melphalan-Mg^{2+} and melphalan-Na^{+} complexes, Mg^{2+} lies closer to N4 than Na^{+}. Again, MA (Na^{+}) is much less than MA (Mg^{2+}), and hence the result indirectly indicates that the effect of Na^{+} in the process of aziridinium ion formation may be negligible as compare to the effects of Mg^{2+} and H^{+}. We have carried out test calculations for Mg^{+} interaction with nitrogen mustards so that the large difference between the MA values of Na^{+} and Mg^{2+} may be analysed

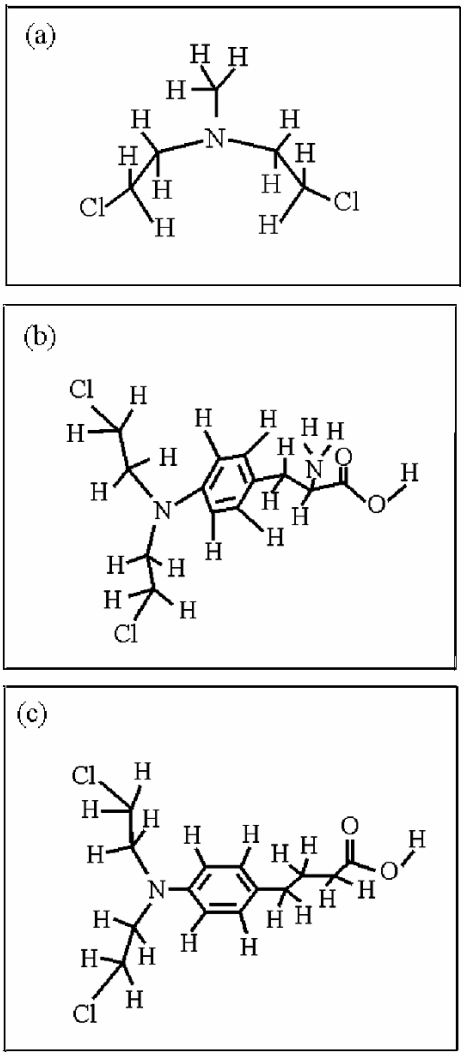

Fig. 2—Structures of nitrogen mustards. [(a) Mustine; (b) Melphalan; and (c) Chlorambucil].

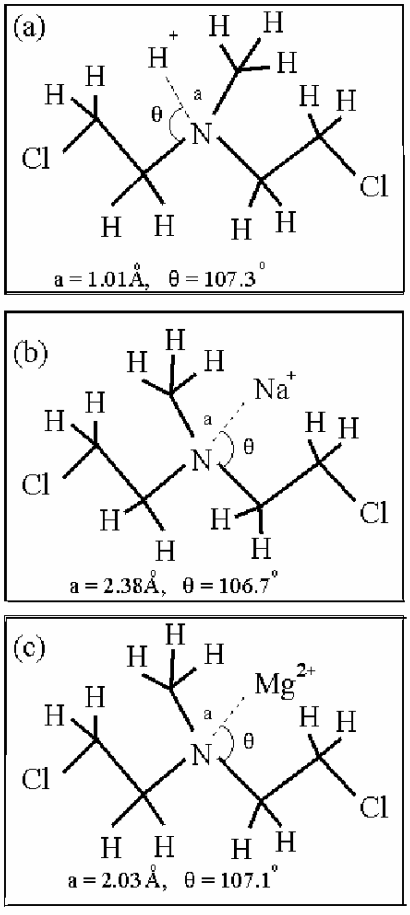

Fig. 3 – (a) Protonated; (b) Na^{+}; and (c) Mg^{2+} interacted structures of mustine at N4.

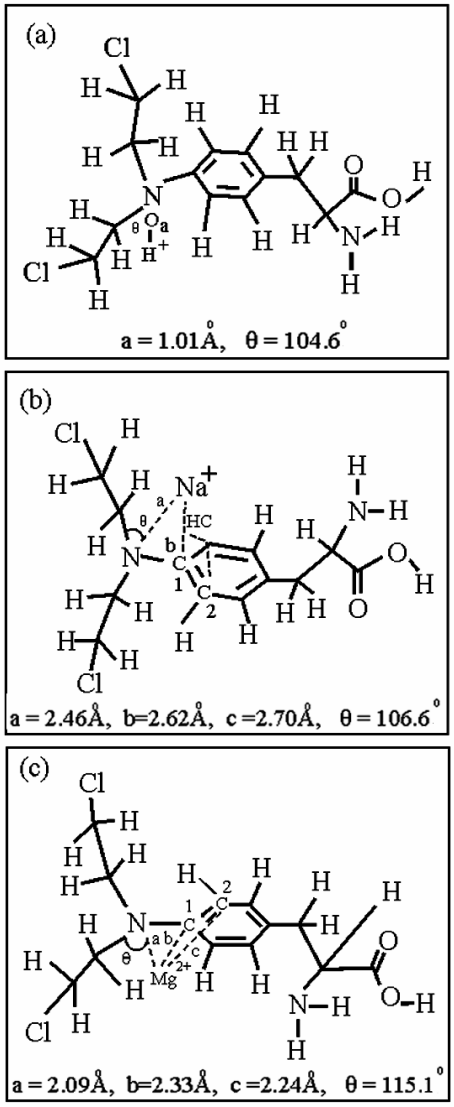

Fig. 4—(a) Protonated; (b) Na^+; and (c) Mg^{2+} interacted structures of melphalan at N4.

(Table 1). It has been found that MA(Mg^+) for mustine is still larger than that of MA(Mg^{2+}), but the MA values of chlorambucil and melphalan could not be calculated due to convergence problem in energy. The computed MA (Mg^{2+}) for melphalan with 6-31G/MP2 routes is approximately 137 kcal/mol whereas the PA value is 226 kcal/mol (6-31G/MP2), and the Mg^{2+} ion and proton are expected to exhibit some influence during SN1 reaction step.

The results in Table 1 shows the PA, MA (Mg^{2+}) and MA (Na^+) of chlorambucil, and the geometries of these drug-ion complexes are given in Fig. 5(a-c). Similar to that found in other drugs, the effect of Na^+ on aziridinium ion formation step from chlorambucil

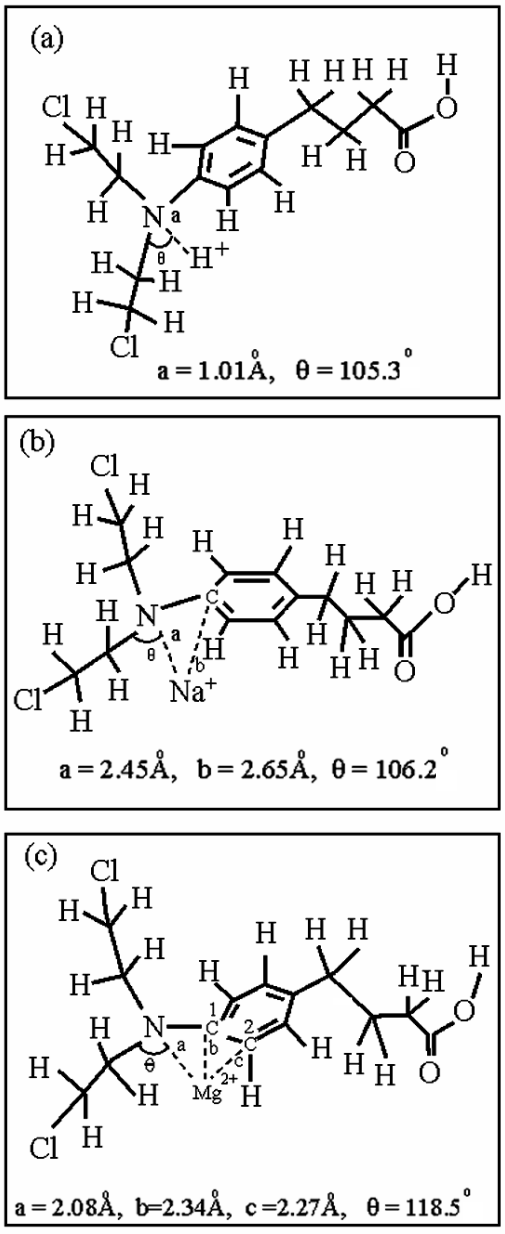

Fig. 5—(a) Protonated; (b) Na^+; and (c) Mg^{2+} interacted structures of chlorambucil at N4.

might be less. Mg^{2+}-chlorambucil complex distinctly indicates multiple interactions at N4 and π electrons. The MA (Mg^{2+}) is quite large (~131 kcal/mol), and also the distance of Mg^{2+} from N4 is found to be closer than the other atomic sites. In this case the strong influence of Mg^{2+} ion at N4 may inhibit aziridinium ion formation. The proton may also produce some inhibitory effect during alkylation reaction since the PA value obtained from 6-31G/MP2 is quite large (~223 kcal/mol).

Interpretation of ionic effects

The computed MA (Na^+) values of drugs are observed to be of the order of 20 kcal/mol – 22 kcal/mol (6-31G/MP2), and the differences between the MA(Na^+) for various nitrogen mustards are not much. However, the MA (Na^+) of drugs is taken for analyzing the role of Na^+ in the formation of aziridinium ring. The trend of MA (Na^+) is as the order of drugs, melphalan > chlorambucil >mustine**.** The MA (Mg^{2+}) of drugs are appreciably large but maintains similar order as MA (Na^+) of drugs**.** The computed values with 6-31G/MP2 route range from 108 to 139 kcal/mol.

Table 1—The computed ion affinities at N4 of mustine, melphalan and chlorambucil.

	Ion affinities of drugs(kcal/mol)		
	Mustine	Melphalan	Chlorambucil
PA	229.04[a]	228.04[a]	226.49[a]
	227.55[b]	226.52[b]	223.88[b]
	227.44[c]	226.17[c]	223.92[c]
MA (Na^+)	17.10[a]	22.40[a]	20.89[a]
	21.43[b]	25.61[b]	23.24[b]
	20.02[c]	24.22[c]	22.44[c]
MA (Mg^{2+})	100.60[a]	131.84[a]	127.01[a]
	121.16[b]	152.68[b]	—[d]
	107.69[c]	137.75[c]	131.82[c]
MA (Mg^+)	28.44[a]	—[d]	—[d]

[a] values for HF/6-31G** route,
[b] 6-31G**/B3LYP route, and,
[c] 6-31G/MP2 route.
[d] value not reported due to convergence problem in energy.

From the geometrical features of Na^+ and Mg^{2+} complexes of various nitrogen mustards as shown in Figs. 4(b,c) and 5(b,c)**,** Na^+ and Mg^{2+} interact with N4 and with the neighboring atoms. The formation of aziridinium ion in presence of Mg^{2+} may be retarded since the MA (Mg^{2+}) is large, and the presence of free drug (uninteracted) necessary to form aziridinium ion is unlikely. Consequently, several intramolecular interactions between drugs and ions (Mg^{2+} and Na^+) and with other atomic sites in cation-drug complexes may be due to the more electrostatic behavior of Mg^{2+} and Na^+ ions. Hence, the Mg^{2+} and Na^+ affinities of chlorambucil and melphalan appear to be due to multiple interactions with atomic sites but not the absolute affinity for N4 atomic site only.

Besides MA, the protonation at N4 is also of importance for addressing the behavior of these drugs in acidic medium. Also, in many cases a comparison between metal affinities and proton affinities are made. This may indirectly indicate the stabilization of a positive charge by N4, and its further effect in the aziridinium ion formation. It is observed that the larger values of proton affinities as compared to the MA (Mg^{2+}) and MA (Na^+) of drugs, which indirectly indicate significant influence by H^+ on N4 during aziridinium ion formation. Comparison of protonation and metal interaction at N4 shows that protonation is more favorable than metal binding, and maintain the order: PA>MA (Mg^{2+})>MA (Na^+).

In the above study, the ionic effects in alkylation reaction have been analyzed with respect to H^+ and

Table 2—The computed Mulliken net charges (HF/6-31G**) on N4 and ions(X =H^+, $Na^+$and Mg^{2+}) in ion-drug complexes

Ions	Net charges on different atoms of					
	Mustine		Melphalan		Chlorambucil	
	N4	X	N4	X	N4	X
	(-0.663)		(-0.776)		(-0.775)	
H^+	-0.640	0.374	-0.692	0.379	-0.693	0.380
Na^+	-0.755	0.874	-0.783 (0.221)[a]	0.824 (-0.262)[b]	-0.787 (0.220)[a]	0.836 (--)[b]
Mg^{2+}	-0.866	1.475	-0.859 (0.269)[a]	1.277 (-0.398)[b]	-0.861 (0.261)[a]	1.297 (-0.374)[b]

The values within brackets are the Mulliken net charges in free drugs
( )[a] and ( )[b] =Mulliken net charges for C1 and C2

Table 3—Interaction distances of metal ions (M^{n+}) from N4 in drug-metal ion complexes

Ions-atomic site	Interaction distances (Å)					
	Mustine		Melphalan		Chlorambucil	
	Na^+	Mg^{2+}	Na^+	Mg^{2+}	Na^+	Mg^{2+}
M^{n+}-N4	2.38	2.03	2.46	2.09	2.45	2.08
M^{n+}-C1	---	---	2.62	2.33	2.65	2.34
M^{n+}-C2	---	---	2.70	2.24	----	2.27

cation interaction abilities of these drugs. The H^+ shows higher affinity for N4 of nitrogen mustards than the metal ions, and the order of ion affinities is PA>MA (Mg^{2+})>MA (Na^+). The computed Mg^{2+} affinities are not the absolute affinities at N4 of nitrogen mustards (except for mustine), and but the proximity effect from other atoms are also observed. The N4 site of drugs has strong affinities for proton. There is less variation of proton affinities among drugs, although the MA (Mg^{2+}) values are larger than MA (Na^+) values. Hence, the effect of H^+ and Mg^{2+} during alkylation may be more prominent than that of the Na^+.

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