Computational Study of Mutagen X

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Mutagen X (MX), 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone is one of the most potent directing acting mutagen ever tested in SAL TA100 assay. Although MX analogues have been synthesized, tested for mutagenicity and modeled by structure-activity relationship (SAR) methods, the mechanism of interaction of these compounds with DNA to produce their remarkable mutagenic potency remains unresolved. MX exists as an equilibrium mixture of both ring and open form in water. This equilibrium is very fast for Ames test. Because the mixture is not separable by experimental methods, it is not clear which one is really responsible for the observed mutagenicity. There have been many debates that which one is really responsible for the observed mutagenicity. We used *ab initio* methods for the MX analogues. It seems both ring and open form could react with DNA bases as electrophiles. However, every open form has consistently lower LUMO energy than corresponding ring form. It is reasonable to assume that the major reaction will go through *via* open form for MX analogues. This suggest that the open form is more likely really mutagenic.

Key Words : Mutagen X, Mutagenicity, SAR, *Ab initio*, Molecular modeling

Mutagen X (MX), 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone was identified in bleached pulp mill effluents.[1-3] It is one of the most potent directing acting mutagen ever tested in SAL TA100 assay. It is implied from many reports that this high mutagenicity comes from the reaction with DNA.[4] Although MX analogues have been synthesized, tested for mutagenicity and modeled by structure-activity relationship (SAR) methods, the mechanism of interaction of these compounds with DNA to produce their remarkable mutagenic potency remains unresolved.

MX exists as an equilibrium mixture of both ring (**B**) and open (**C**) form in water as shown in Figure 1.[5] This equilibrium is very fast for Ames test. Because the mixture is not separable by experimental methods, it is not clear which one is really responsible for the observed mutagenicity. There have been many debates that which one is really responsible for the observed mutagenicity. There are analogues those resemble MX. Because there is no tautomeric proton in 3-chloro-4-(dichloromethyl)-5-methoxy-2(5H)-furanone (**A**), it can not be readily converted into corresponding open form. This still shows comparable mutagenicity (5700 rev/nm) as MX (5000 rev/nm). EMX (**D**) is geometric isomer against central double bond of MX (**C**) and because the hydroxyl group and aldehyde groups are apart, it can not be readily converted into ring form. This molecule shows much less mutagenicity (320 rev/nm).[6] Thus by the analogue studies, it seems that the ring form is responsible for the observed mutagenicity. These are indirect data supporting that ring form is being more mutagenic. There is also some spectral data that open form reacts directly with DNA bases. MX by its open form reacts with DNA base at physiological conditions. However this takes too long time (several days) and the yield was too low. Therefore it still remains unsolved that which one of the two forms is responsible. To solve this puzzling problems there have also been many studies of using quantitative structure activity relationship (QSAR) study. Tuperainen *et al.* showed that there is high correlation between observed mutagenicity and energy level of LUMO (lowest unoccupied molecular orbital). Using only ring form, They showed that LUMO energy level inversely correlates with mutagenicity ($r = 0.985$). This implies that the MX analogues act as electrophiles to react with DNA bases.[7-10] It is interesting that the mutagenicity did not show significant correlation with logP values. LaLonde *et al.* also studied both open form and ring form. They also have high correlation with LUMO and mutagenicity. In their study both the open form and ring form showed high correlation with the LUMO energy

Figure 2. General Structure of MX (See Table 1 for the substitutents.)

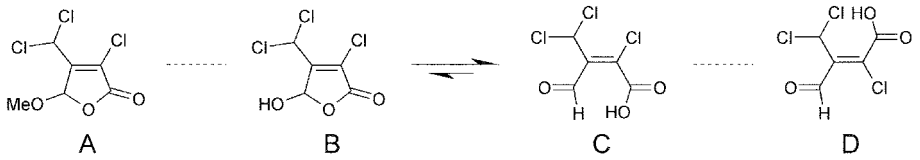

Figure 1. Various Forms of MX (**B**, **C**, **D**) and its analogue (**A**)

Table 1. The Mutagenicity and Relative LUMO Energy Level of Ring and Open Form

Common name	X	Y	ln (TA100)	ΔE_{LUMO}
MX	$CHCl_2$	Cl	8.62	11.8
BMX2	$CHBr_2$	Cl	8.61	17.2
BMX3	$CHBr_2$	Br	6.41	7.2
CMCF	CH_2Cl	Cl	6.37	11.7
BMBF	CH_2Br	Br	6.04	18.4
MCA	Cl	Cl	1.87	14.5
MBA	Br	Br	1.71	22.0
	CH_2Cl	H	1.35	21.2
MBF	CH_3	Br	0.41	25.6
MCF	CH_3	Cl	0.21	10.6
	CH_3	Cl	-1.61	12.5
MF	CH_3	H	-3.51	16.7

X and Y are for Figure 2. ln(TA100) is the natural log for experimental values (rev/nm in Ames test). Whenever there are more than two reported values, natural logarithms are taken for them and then averaged. ΔE_{LUMO} is the difference of open and ring form. Open form is always lower in energy level (kcal/mol).

level.[11-17] QSAR studies mostly showed that MX analogues are electrophiles and they attack electron rich DNA bases. In this work we considered all the possible conformations for both open and ring forms. In Figure 2 the general structures of ring and open form of are shown. The substitutents of 3,4-positions are listed in Table 1.

All the calculations were done with JAGUAR 3.0 suite of programs. Molecular geometries were fully optimized within SPARTAN using the semiemprical AM1 methods for full conformational analysis, followed by geometry optimization of the lowest energy AM1 conformer at the *ab initio* HF/3-21G* level. LogP values were calculated using the Grose-Crippen method within Spartan. All statistical calculations were carried out using multiple linear regression techniques. We took biological activity as natural log of the number of reversant in Ames study (with test strain 100). Whenever multiple experimental data available for the same molecule, average values are chosen as representative values.[5,6,18-22] For membrane permeability, we considered Van Der Waal's molecular surface area, volume, and logP values. For electronic parameters, we used diploe moment, Mulliken charge, energy level of HOMO (highest occupied molecular orbital) and LUMO and their energy difference. The final result for ring form is shown in equations.

$$\ln(TA100) = -12.03 - 103.3 \times E(LUMO) + 0.130 \times SA \quad \text{for ring form,}$$

$$\ln(TA100) = -16.94\ 124.6 \times E(LUMO) + 0.146 \times SA \quad \text{for open form,}$$

where E(LUMO) is energy level of LUMO and SA is surface area.

The square of correlation constant (r^2) is 0.91 for both ring and open form. This is comparable with other reports from Tuperainen *et al*. and LaLonde *at al*. E(LUMO) alone also gives r^2, 0.66 for ring form and 0.77 for open form. This may mean that this single parameter may not be sufficient. Unlike previous report, our results show some correlation with surface area. This could mean the importance of lipophilicity or volume. However, The LUMO energy level was the most important parameter. When we compared with the LUMO data of open and ring forms, Every MX analogue in Table 1 shows that open form has always lower LUMO energy level than that of corresponding ring form. The LUMO energy level implies the importance of electrophilicity. The MX analogues would attack electron-rich DNA bases, resulting in observed mutagenicity. In conclusion, it seems both ring and open form could react with DNA bases as electrophiles. However, our data supports that open form is the real mutagen at physiological conditions. The majority of species in solution are open forms.[23] Furthermore, the open forms have very reactive aldehyde group. As a result, every open form has consistently lower LUMO energy than corresponding ring form. It is reasonable to assume that the major reaction will go through *via* open form for MX analogues. This may indicate that the open form is real mutagenic species for MX analogues.

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