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## *Ab Initio* Study of Mutagen X: Importance of Ionization and Solvation


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The halogenated oxyfuranones are a class of drinking water disinfection by-products that include 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (Mutagen X), one of the most potent direct-acting mutagens ever tested in Salmonella TA100.[1] Because of the extremely high mutagenic potencies, it is estimated that Mutagen X(MX), along with its chlorinated and brominated structural analogues, accounts for up to 70% of the total mutagenicity of drinking water samples, in spite of relatively low concentration exposure levels.[2] Until recently, MX was assumed to pose little carcinogenic risk due to its low exposure, high reactivity and short residence time. But recent identification of DNA adducts and evidence of carcinogenicity along the gastro-intestinal lining in rodents following MX exposure has heightened concern for this class of chemicals. A relatively large number of MX analogues have been synthesized, tested for mutagenicity, subject to mutational spectral analysis and DNA adducts studies, and modeled by structure-activity relationship methods.[3] In spite of this information, a number of basic questions pertaining to the nature of the ultimate reactive species and the mechanism of interaction of these compounds with DNA to produce their remarkable mutagenic potency in SAL TA100 remain unresolved.

MX exists as an equilibrium mixture of both ring and open form in water as shown in Figure 1. The relative concentration of ring and open form depends heavily on the pH of the solution. If the aqueous solution is highly acidic, the ring form is dominant species.[4] The relative concentration of open form becomes high as the solution gets more basic. This is a very fast process. On the other hand, the existence of regioisomer of open form is also observed. Fifty percent of open form was converted spontaneously into extended form in aqueous solution in several days.[5] This implies that the extended form is more stable than the open form at the physiological conditions.

There have been many debates which one of three species shown in Figure 1 is responsible for the high mutagenicity in

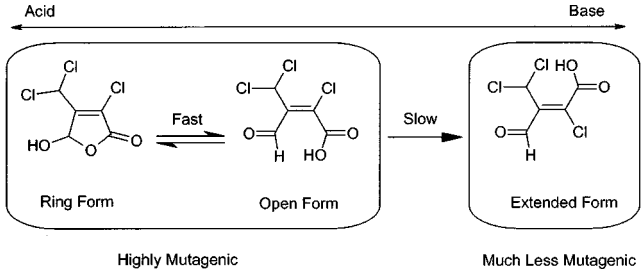


**Figure 1**. Various forms of mutagen X.

SAL TA100. Experiments showed that the extended form is not much mutagenic.[5] The observed mutagenicity comes from the experimentally not separable mixture of open and ring form. However, the exact mechanism has never been clarified and which one of two species is responsible for the observed mutagenicity. Understanding the origin of chemical stability of these species is interesting. This may also help to solve this puzzling problem of mutagenic mechanism. It is reported that this equilibrium does not depend on the substituent of 2,3-position of oxyfuranone.[4] All the chemicals shown in Figure 2 favor open form over ring form.[4] Therefore it is reasonable to expect that the 5-hydroxy-2(5H)-furanone exists mainly in its open form. In summary, it is reasonable to assume that extended form is the most stable, open form is

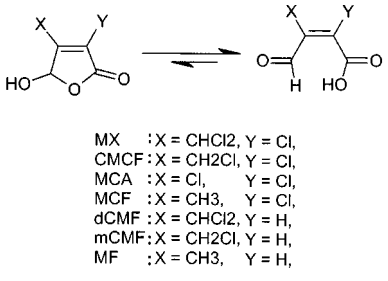


**Figure 2**. All the MX analogues exist open forms at physiological conditions.

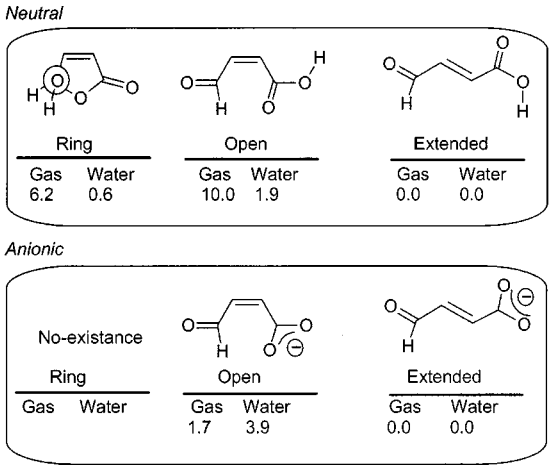


**Figure 3**. Various forms of 5-hydroxy-2(5H)-furanone. For each form, the most stable conformer is drawn. The numbers are relative energies against the most stable one. Units are in kcal/mol.

less stable and ring form is least stable at the physiological conditions, regardless of substituents (X, Y, in Figure 2).

All The calculations were done using program JAGUAR 3.0 and Spartan at the LMP2/6-31++G** level of theory.[6] To model solvation, Self Consistent Reaction Field method was used. Solvation was calculated by Pöisson-Boltzman solver which is based on continuum model. First gas phase conformations were fully optimized without any constraint. Then using these geometries, the solvation energies were calculated at the same level of theory. The anionic forms are also studied because the open form and extended form may be in anionic state in aqueous solution.

For simplicity, 5-hydroxy-2(5H)-furanone (X=H, Y=H. See figure 2) were calculated. For this molecule, its ring form, open form, and extended form are calculated. For each form, all the possible conformers for both neutral and anionic species are calculated as described above. For ring form, there exist only two conformers. Normally, there exist three conformers for single bond. The extremely strong stereoelectronic effect may be responsible for disappearance of one conformer.[7] For open form, 6 conformers were found. Because it is fully conjugated system and there are three single bonds, it is expected that 8 conformers are possible. The two conformers do not exist because of steric repulsion. For extended form, we could find 8 conformers. Anionic species were simple to calculate. The anionic ring form does not exist. It spontaneously opens up to corresponding open form.[8] Anionic open form has only one conformer. There are two anionic extended conformations. The most stable conformers are chosen for both neutral and anionic forms (Figure 3).

For neural form, the most stable one is extended form. When we compare ring and open form, the ring form is more stable. This is not consistent with experiments. However, when we consider the anionic species, the open form is much more stable. The ring form does not even exist. It is well known that carboxylic acids are ionized in physiological conditions. When we consider the anionic forms, the relative energy nicely corresponds with experimental data. *i.e.* the stability order is extended, open, and ring form. We have also calculated the relative energy of solvation of both neutral and anionic species. Ionization in the gas phase gives a lot less stable species (175 kcal/mol higher in energy). However solvation energy is about 287 kcal/mol. Thus when they are solvated, ionized species becomes very stable in aqueous solution by about 112 kcal/mol.[9] The results shows that in the gas phase, ring form is more stable than open one. However solvation and ionization reverses the order of stability. In conclusion, it is interesting that the experimental finding of open form stability comes not from the inherent stability of this compound. Rather, ionization and solvation plays a decisive role in the stability of open form over ring form in physiological conditions.

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8. We have tried to obtain this anionic ring form structure. When we depronated from neutral form, there was no local minimum. The energy of ring form is about 30 kcal/mol higher than corresponding open form structure.
9. All the energies are electronic energies.