Comparative Molecular Field Analysis (CoMFA) and Comparative Molecular Similarity Index Analysis (CoMSIA) Study of Mutagen X

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Mutagen X (MX) exists in our drinking water as the bi-products of chlorine disinfection. Being one of the most potent mutagen, it attracted much attention from many researchers. MX and its analogs are synthesized and modeled by quantitative structure activity relationship (QSAR) methods. As a result, factors affecting this class of compounds have been found to be steric and electrostatic effects. We tried to collect all the data available from the literature. With both CoMFA and CoMSIA various combinations of physiochemical parameters were systematically studied to produce reasonable 3-dimensional models. The best model for CoMFA gave $q^2 = 0.90$ and $r^2 = 0.97$, while for CoMSIA $q^2 = 0.85$ and $r^2 = 0.94$. So the models seem to be reasonable. Unlike previous result of CoMFA, in our case steric parameter alone gave the best statistics. Although the steric contribution was found to be the most important in both CoMFA and CoMSIA, steric parameter along with electrostatic parameter produced slightly better model in CoMSIA. Overall, steric contribution is clearly the most important single factor. However, when we compare chlorine and bromine substitution, chlorine substitution can be more mutagenic. This indicates that other factors such as electrostatic effect also influence the mutagenicity. From the contour maps, steric contribution seems to be focused on rather small area near C6 substituent of the furanone ring, rather than C3 substituent. Therefore the locality of steric contribution can play a significant role in mutagenicity.

Key Words : Mutagen X, Mutagenicity, CoMFA, CoMSIA, QSAR

Introduction

Chlorine bleaching disinfects our drinking water by reducing the water-mediated diseases. However, some of the bi-products caused by this disinfection process are highly mutagenic.[1] Although how MX (3-chloro-4-(dichloro-methyl)-5-hydroxyl-2(5H)-furanone) is produced in water is not clearly understood,[2] MX is a potent mutagen ever tested in Ames test with test strain TA100.[3] The mutagenicity of MX has been reported 3430-13800 induced reversants per nanomole in the Ames assay without S9 mix. This unusual high mutagenicity attracted considerable attention from many researchers.[4] Until recently, MX was assumed to pose little carcinogenic risk due to its low exposure, high reactivity and short residence time.[5] But recent identification of DNA adducts[6] and evidence of carcinogenicity along the gastro-intestinal lining in rodents following MX exposure has heightened concern for this class of chemicals. MX can alter the metabolic pathway when it is administered in rats in high dosage.[7] It is also found to induce apoptosis of HL-60 cells.[8] A relatively large number of MX analogs have been synthesized,[9] tested for mutagenicity,[10] subject to many experimental studies. As a result, the resultant MX analogs show wide range of mutagenicity.[11] They are modeled by structure-activity relationship methods.[12] In spite of this multitude of studies, basic questions concerning the nature of the 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. At pH 5.5 the ratio of ring form and open form is 1 : 1. The relative concentration of open form becomes high as the solution gets more basic. This is a fast equilibrium process.[13] To study factors affecting the mutagenicity, there have been a few quantitative structure activity relationship (QSAR) studies. The structural and electronic properties were calculated using the semi-empirical AM1 (Austin Model 1) method. The lowest unoccupied frontier orbital (LUMO) was found to be important by using

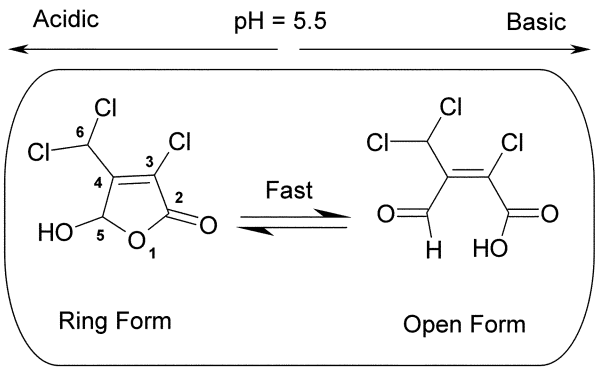

Figure 1. Two forms of MX in equilibrium.

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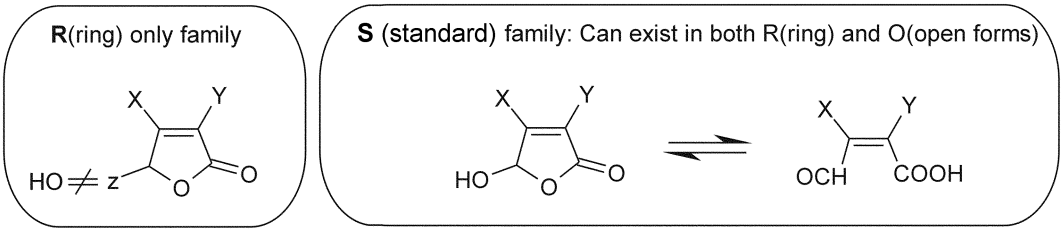

Figure 2. Two families of MX analogs.

this quantum mechanical method.[12b,c] This may imply that MX acts as an electron acceptor. In particular, LUMO electron density and partial charge of the C3 correlated with mutagenicity. Electron density near C3 also showed negative linear dependency by NMR study. Comparative molecular field analysis (CoMFA) indicated that the steric properties of MX analogs with their electron-accepting ability explain their mutagenic activity almost completely.[14] However, these studies are based on a few reports and some of the structurally relevant compounds were never considered for QSAR studies. In this study, we tried to include all the data available from the literature and summarized in Table 1. At a glance, as the degree of halogen substitution increases, the mutagenicity also increases.

Table 1. The Mutagenicity of MX analogs

	X	Y	Z	ln(TA100)	N
Standard Family					
S1 (MX)	$CHCl_2$	Cl		8.62	9
S2 (BMX2)	$CHBr_2$	Cl		8.61	1
S3 (BMX3)	$CHBr_2$	Br		6.41	2[a]
S4 (CMCF)	CH_2Cl	Cl		6.37	5
S5 (BMBF)	CH_2Br	Br		6.04	1
S6 (MCA)	Cl	Cl		1.87	6[a]
S7 (MBA)	Br	Br		1.71	1
S8	CH_2Cl	H		1.35	3
S9 (MBF)	CH_3	Br		0.41	1
S10 (MCF)	CH_3	Cl		0.21	4
S11	H	Cl		-1.61	1
S12 (MF)	CH_3	H		-3.51	2
Ring Family					
R1	$CHBr_2$	Cl	OCH_3	8.65	1
R2	$CHCl_2$	Cl	OCH_3	8.65	1
R3	$CHBr_2$	Cl	H	5.20	1
R4	$CHBr_2$	Br	H	4.86	1
R5 (RMX)	$CHCl_2$	Cl	H	4.54	6
R6	CH_2Br	Br	H	2.11	1
R7	CH_2Cl	Cl	H	1.70	4
R8	CH_2Cl	Br	H	1.37	1
R9	CH_2Br	Cl	H	1.37	1
R10	Cl	Cl	OCH_3	0.99	1
R11	CH_3	Cl	OC_2H_5	0.74	1
R12	Br	Br	H	0.17	1
R13	H	Cl	OC_2H_5	-0.22	1
R14	CH_3	Cl	H	-0.78	2[b]
R15	Cl	Cl	H	-0.62	2
R16	CH_2Cl	H	H	-1.59	3[a]
R17	$CHCl_2$	H	H	-2.41	2[b]

Data in this table comprise of 15 reports. N is the number of reports that have mutagenicity data. X, Y and Z are substituents for MX analogs as shown in Figure 2. ln(TA100) is the natural log for experimental values (rev/nm in Ames test). When there are more than two reports, after the logarithms have been taken, the values are averaged, and the resultant values are listed in this table. [a]The maximum value is more than one order larger than the minimum value in magnitude. [b]One of the reports indicates that the compound is not mutagenic and logarithms are taken for remaining value.

The compounds are collected from the available reports and categorized into two groups as shown in Figure 2. Compounds which belongs to standard family (**S**) contain the structure of 5-hydroxy-2(5H)-furanone. These compounds are capable of conversion between hydroxyl ring form and aldehyde open form like MX. If an analog has a ring form and does not have 5-hydroxyl group, then it cannot be converted into the corresponding open form. Therefore it belongs to ring family (**R**). The mutagenicity of MX is the average value of 9 different studies.[11] All the activity values are within the order of magnitude (3430-13800). Thus the average value can be considered highly reliable. The whole set comprises of 29 compounds. The range of activity is fairly well spread for any particular family as well as for the whole set. All the compounds have α,β-unsaturated acidic moiety as a common structure. This structural resemblance might imply that these compounds induce mutagenicity with the same mechanism.

Methods

Quantitative structure-activity relationships (QSARs) are important tools to understand why the active compounds exhibit certain biochemical activities.[15] The challenge is to improve the accuracy and predictability of QSAR model by taking into account the structural and physicochemical features of the concerned compounds. One of the most widely used tools in 3D QSAR study is comparative molecular field analysis (CoMFA).[15] CoMFA is based on the assumption that changes in the biological activity correlate with changes in the steric and electrostatic fields of molecules. CoMFA calculates steric fields using a Lennard-Jones potential, and electrostatic fields using a Coulombic potential. While this approach has been widely accepted and scientifically feasible, it is not without problems. Both potential functions are very steep near the van der Waals surface of the molecule, causing rapid changes, and

requiring the use of cut-off values. So changes in orientation of the superimposed molecules, relative to the calculation grid, can cause significant changes in CoMFA results. In addition, a scaling factor is applied to the steric field, so both fields can be used in the same PLS analysis. In CoMSIA (Comparative Molecular Similarity Index Analysis), a recently developed technique,[16] five different fields are calculated; steric, electrostatic, hydrophobic, hydrogen bond donor and hydrogen bond acceptor. These fields were selected to cover the major contributions to ligand binding. Similarity indices are calculated at regularly spaced grid points for the pre-aligned molecules. Using Gaussian type of function, CoMSIA is less sensitive on the grid spacing and its relative orientation of the aligned molecules and grid. These two methods have often been used together as complementary methods. The CoMFA and CoMSIA analyses were performed on a Silicon Graphics workstation (IRIX 6.5 operating system) with SYBYL 6.9.2. The steric and electrostatic CoMFA descriptors were calculated with the standard Tripos forcefield at every point of the three dimensional lattice, using the sp^3 carbon probe with +1 charge with standard CoMFA cutoff values. For standard family, we do not know either ring form or open form is responsible for the mutagenicity. In this work, since ring family can exist only in ring form, ring form was used for standard family for these 3D QSAR techniques. This ensures the consistency and maximum overlap between two families. The three-dimensional molecular structures of the compounds in the data sets were fully optimized and atomic charges were calculated with AM1 (Austin Model 1) Hamiltonian. The resultant charges were used for electrostatic parameter calculations. All the possible conformations were generated and selected based on the minimum energy. The energy levels of LUMO were derived from these conformations.[17] Then the chosen conformers were superimposed as shown in Figure 3 by matching corresponding atoms in the 5-membered ring. CoMFA standard scaling was applied whenever scaling was necessary between different parameters. CoMFA standard scaling often gives results better than those obtained using uniform weighting.[18]

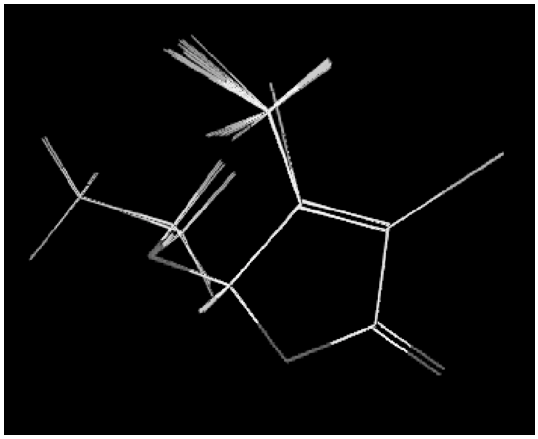

Figure 3. Superposition of MX analogs used in CoMFA and CoMSIA studies.

Results

As shown in Table 2, we have tried 5 grid spacings for CoMFA. It started with the default 2.0 Å grid spacing, then we increased the model resolution up to 0.1 Å. If the grid spacing is large *e.g.*, the default of 2 Å, the results can be sensitive to the alignments with respect to the grid. Reduction of the grid spacing would reduce this sensitivity. Also with more grid points, a better model could be expected. As expected, there is a tendency that high resolution of grid spacing would give higher q^2 and r^2. When we compare the grid spacings of 0.1 Å and 0.2 Å, both results are almost the same, indicating saturation of grid points. If we consider single parameter, steric factor (**S**) gave highest predictive power as well as explanatory one over any other parameters (**E**, $\mathbf{E_{LUMO}}$). Combination of these parameters did not really improve the statistical parameters except for S, E_{LUMO}, at 1.0 Å. The best model was chosen based on the predictive power (q^2). If q^2 values are equal, then explanatory power (r^2) were considered. Thus the best model was obtained when steric factor alone was used at grid spacing 0.2 Å. With CoMSIA, we considered 5 parameters, so there are 31 possible combinations of parameters. These are listed in Table 3. As in the cases of CoMFA, steric parameter seems to be most important when we consider single parameter. Combining two or more parameters did not really much improve statistical values. There are 4 cases those gave q^2 values more than 0.7. Steric parameter is involved in all four cases as shown in Table 3. The effect of grid spacing is also considered and listed in Table 4. Varying the model resolution did not change the statistical values much. The best model was obtained with steric parameter along with electrostatic parameter was $q^2 = 0.80$ and $r^2 = 0.92$. In Table 5, the predicted values for the compounds along with residuals are listed using the best CoMFA and CoMSIA models. For CoMFA the absolute values of residual for R17 and R14 were greater than the double of standard error (0.703). So, these two molecules can be outliers for this model with more than 95% of confidence. There are contradictory reports for these two values. While in one report the mutagenicity of R17 is 0.09 rev/nmol, in the other it is not mutagenic. Likewise, R14 also have two different values; one is 0.46 rev/nmol, the other not mutagenic. The mutagenicity of R17 and R14 in Table 5 is listed based on one report that they are mutagenic. There are chances that these values are incorrect. Therefore we performed CoMFA excluding these two potential outliers. The resultant CoMFA with the steric parameter gave $q^2 = 0.897$ and $r^2 = 0.968$. For CoMSIA only R17 has the highest absolute residual value and was greater than double of standard error (0.982). Therefore R17 was excluded in the CoMSIA model. The resultant CoMSIA with the steric and electrostatic parameters gave $q^2 = 0.854$ and $r^2 = 0.939$. Using these final models for CoMFA and CoMSIA, contour plots are drawn in Figure 4. **A** is the steric field map of

Table 2. Grid Spacing Variation and Statistical Parameters (CoMFA)

	S	E	E_{LUMO}	S, E	S, E_{LUMO}	S, E, E_{LUMO}
2.0 Å						
q^2	0.822	0.769	0.702	0.782	0.811	0.782
number of comp.	8	5	1	5	5	5
r^2	0.951	0.900	0.743	0.919	0.914	0.930
F	48.331	41.605	78.106	51.917	48.994	61.530
contribution ratio						
Steric	1.000			0.450	0.559	0.298
Electrostatic		1.000		0.550		0.295
E_{LUMO}			1.000		0.441	0.407
1.0 Å						
q^2	0.836	0.803	0.702	0.820	0.843	0.825
number of comp.	6	7	1	5	7	6
r^2	0.954	0.954	0.743	0.934	0.956	0.948
F	75.610	61.646	78.106	64.831	64.980	66.258
contribution ratio						
Steric	1.000			0.563	0.747	0.421
Electrostatic		1.000		0.437		0.307
E_{LUMO}			1.000		0.253	0.281
0.5 Å						
q^2	0.848	0.762	0.702	0.841	0.848	0.841
number of comp.	7	6	1	7	7	7
r^2	0.946	0.894	0.743	0.945	0.933	0.939
F	52.880	31.017	78.106	51.102	41.858	46.105
contribution ratio						
Steric	1.000			0.503	0.633	0.360
Electrostatic		1.000		0.497		0.291
E_{LUMO}			1.000		0.367	0.350
0.2 Å						
q^2	0.848	0.792	0.702	0.828	0.843	0.828
number of comp.	5	4	1	4	5	4
r^2	0.951	0.912	0.743	0.930	0.949	0.931
F	90.040	62.255	78.106	79.993	85.522	80.689
contribution ratio						
Steric	1.000			0.437	0.730	0.279
Electrostatic		1.000		0.563		0.403
E_{LUMO}			1.000		0.270	0.318
0.1 Å						
q^2	0.847	0.792	0.702	0.832	0.847	0.832
number of comp.	5	4	1	5	5	5
r^2	0.951	0.912	0.743	0.941	0.949	0.945
F	90.040	62.255	78.106	73.166	85.522	78.996
contribution ratio						
Steric	1.000			0.432	0.730	0.273
Electrostatic		1.000		0.568		0.447
E_{LUMO}			1.000		0.270	0.280

q^2: leave-one-out crossvalidation, F: F ratio, E_{LUMO}: energy level of LUMO

CoMFA with steric parameter alone while **D** is that of CoMSIA. The green polyhedra indicate sterically favorable contribution while yellow areas are disfavored. The favorable regions are located near C3 and C6 substituents. When both electrostatic and steric contribution were considered for model derivation, the steric contribution near C3 substituent disappears, only favoring C6 position (**B**, **E**). This indicates that the area near C6 is sterically more favored than that near C3 position. In electrostatic interaction field maps (**C**, **F**), the blue polyhedra indicate the regions where positive charge enhances the mutagenicity while red ones indicate that negative charge does. The electrostatic contour maps indi-

Table 3. CoMSIA and various combination of parameters

	q^2	r^2	number of comp.
S	0.762	0.869	5
E	0.629	0.949	7
H	0.648	0.798	4
D	0.163	0.325	3
A	-0.081	0.192	3
S & E	0.794	0.921	5
D & A	0.260	0.588	4
S, E	0.371	0.752	4
S, H	0.728	0.880	6
S, D	0.227	0.673	6
S, A	0.764	0.879	6
E, H	0.612	0.920	7
E, D	-0.093	0.148	1
E, A	0.410	0.838	6
H, D	0.153	0.418	2
H, A	0.604	0.805	3
D, A	0.278	0.631	5
S, E, H	0.621	0.917	6
S, E, D	0.034	0.332	3
S, E, A	0.331	0.662	3
S, H, D	0.540	0.838	4
S, H, A	0.576	0.773	3
S, D, A	0.169	0.798	7
E, H, D	-0.167	0.163	1
E, H, A	0.571	0.921	5
E, D, A	0.017	0.485	7
H, D, A	0.126	0.435	2
S, E, H, D	-0.182	0.274	2
S, E, H, A	0.581	0.919	6
S, E, D, A	0.043	0.471	4
S, H, D, A	0.554	0.852	5
E, H, D, A	-0.193	0.154	1
S, E, H, D, A	-0.144	0.115	1

S: steric, **E**: electrostatic, **H**: hydrophobic, **D**: hydrogen bond donor, **A**: hydrogen bond acceptor

cated that we need some negative charge near the C3 position probably indicating electronegative substituents.

Conclusion

Although electrostatic contribution may slightly improve the statistics, CoMFA and CoMSIA gave consistent result that steric contribution is the most important. When we carefully look into the data in Table 1, there are cases that chlorine substitution enhances mutagenicity than sterically more bulky bromine substitution. *i.e.*, S4 vs. S5, etc. Therefore, there could be some other minor effects such as electrostatic effect. The sterically important regions are somewhat localized on the area near C6 rather than C3 position, indicating C6 substitution might change mutagenicity more dramatically than C3. This is consistent with the conclusion of LaLonde *et al.*[11e] that the halogen-by-hydrogen replacement at C6 induces the greatest mutagenicity reduction.

Table 4. Grid Spacing Variation (CoMSIA)

	S	S, E	S, H	S, A
2.0 Å				
q^2	0.762	0.794	0.728	0.764
r^2	0.869	0.921	0.880	0.879
number of comp.	5	5	6	6
1.0 Å				
q^2	0.757	0.800	0.649	0.751
r^2	0.871	0.922	0.852	0.896
number of comp.	5	6	5	7
0.5 Å				
q^2	0.761	0.798	0.705	0.777
r^2	0.869	0.919	0.870	0.891
number of comp.	5	5	6	7
0.2 Å				
q^2	0.761	0.790	0.705	0.777
r^2	0.869	0.919	0.870	0.891
number of comp.	5	5	6	7
0.1 Å				
q^2	0.761	0.798	0.705	0.777
r^2	0.869	0.919	0.870	0.891
number of comp.	5	5	6	7

Table 5. Resisuals for CoMFA and CoMSIA

	Activity	CoMFA	CoMFA residual	CoMSIA	CoMSIA residual
S1	8.62	7.77	0.85	7.77	0.85
S2	8.61	7.62	0.99	7.82	0.79
S3	6.41	7.71	-1.30	7.82	-1.41
S4	6.37	5.18	1.19	4.64	1.73
S5	6.04	5.88	0.16	5.00	1.04
S6	1.87	1.37	0.50	2.06	-0.19
S7	1.71	1.44	0.28	2.08	-0.37
S8	1.35	0.91	0.44	0.57	0.79
S9	0.41	0.93	-0.52	1.32	-0.91
S10	0.21	0.77	-0.56	1.32	-1.11
S11	-1.61	-1.12	-0.49	-1.44	-0.17
S12	-3.51	-3.84	0.33	-3.76	0.25
R1	8.65	8.39	0.26	8.19	0.46
R2	8.65	8.70	-0.05	8.25	0.40
R3	5.20	4.30	0.90	4.81	0.39
R4	4.86	4.49	0.37	4.81	0.05
R5	4.54	4.54	0.00	4.85	-0.31
R6	2.11	2.76	-0.65	2.35	-0.24
R7	1.70	1.33	0.37	1.06	0.64
R8	1.37	2.86	-1.49	2.78	-1.41
R9	1.37	2.60	-1.23	2.37	-1.00
R10	0.99	2.37	-1.38	2.78	-1.79
R11	0.74	1.36	-0.62	1.55	-0.81
R12	0.17	-0.59	0.76	-1.26	1.43
R13	-0.22	-0.43	0.21	-1.20	0.98
R14	-0.78	-2.52	1.74	-1.68	0.90
R15	-0.62	-0.64	0.02	-1.26	0.64
R16	-1.59	-2.31	0.72	-2.19	0.60
R17	-2.41	-0.63	-1.78	-0.18	-2.23

The Models of CoMFA and CoMSIA used here are the best models in Table 2 and 4.

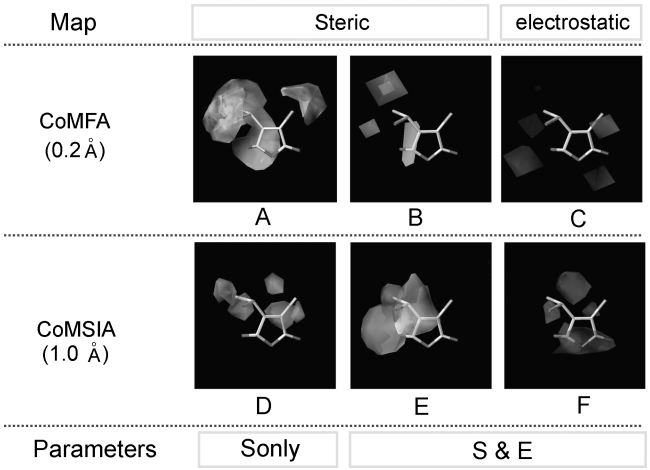

Figure 4. CoMFA steric STDDEV*COEFF contour plots. Sterically favored areas (contribution level of 80%) are represented by green polyhedra. Sterically disfavored areas (contribution level of 30%) are represented by yellow polyhedra (**A**, **B**, **D**, **E**). Positive charged favored areas (contribution level of 80%) are represented by blue ploy hedra. Negatively charged favored areas (contribution level of 30%) are represented by red polyhedra (**C**, **F**). The molecule shown in the maps is MX.

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