Density functional studies of endosulphan and its interaction with glycine and GABA[#]

C N RAMACHANDRAN^a, BRIJESH KUMAR MISHRA^b and ASHWANI KUMAR TIWARI^{c,*}

^aDepartment of Chemical and Biological Engineering, University at Buffalo, The State University of New York, Amherst, NY 14260, USA

^bEnvironmental Chemistry Modeling Laboratory, GR C2 544, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland

^cDepartment of Chemical Sciences, Indian Institute of Science Education and Research Kolkata, Mohanpur, 741 252, India
e-mail: ashwani@iiserkol.ac.in

Abstract. Density functional theoretic (DFT) methods are employed to study the interactions between endosulphan and two amino acids, namely glycine and γ -aminobutyric acid (GABA). Two conformers of each isomer α - and β -endosulphan are considered in the study. The DFT methods B3LYP, M05, M05-2X, M06 and M06-2X in conjunction with the basis set 6-31++G** are used. The complexes of α - and β -endosulphan with amino acids are stabilized by a strong hydrogen bond. In addition, there are several weak C-H...O interactions present between the two moieties. Among the DFT methods used, M06-2X method shows the highest stabilization energy for all the complexes. The M06-2X/6-31++G** method predicts that among the four conformers of endosulphan, the α conformer in which the S=O points up, forms the most stable complex with both glycine and GABA, with stabilization energies -15.24 kcal/mol and -14.39 kcal/mol, respectively. The β conformer in which the S=O points down, forms the least stable complex with both amino acids with stabilization energies -7.14 and -7.85 kcal/mol, respectively.

Keywords. Endosulphan; density functional; amino acid; glycine; γ -amino butyric acid; GABA.

1. Introduction

Endosulphan, a polychlorinated organic pesticide, has been used for many years in agricultural production in India and several other countries. Due to its adverse effects a ban on its use was negotiated under the recent Stockholm convention. Endosulphan is considered as a primary pollutant and is released into the atmosphere during its application and volatilization.¹ The excess use of endosulphan has posed a threat to the aquatic environment.² The presence of endosulphan and its oxidized product endosulphan sulphite contaminates the surface water.^{3,4} Endosulphan has high affinity for soils.^{5,6} It was reported that endosulphan causes significant histopathological alterations in fish.⁷

The toxic effects of the endosulphan in mammals (for example rats, rabbits and humans) have been investigated in the past.^{8–10} It was reported that the endosulphan may adversely affects the male reproductive system.¹¹ Endosulphan primarily targets the GABA-

receptors, which respond to the neurotransmitter GABA in the vertebrate central nervous system. Normally, the binding of GABA to these receptors is accompanied by a change in their shape, thereby allowing the ions, particularly the chloride ions pass through their central channel. However, the presence of endosulphan can influence the binding of GABA to GABA-receptors.

The endosulphan exists in two isomeric forms (i) α -endosulphan and (ii) β -endosulphan. Nuclear magnetic resonance (NMR) and X-ray crystallographic studies have shown that the α -endosulphan is asymmetric whereas the β -endosulphan is symmetric.^{12,13} The ratio between the two isomers depends on the environment in which they are present. For example, it has been reported that the α -isomer is predominant in the air samples, whereas the β -isomer in the rain samples.^{14–19} It has also been found that α - and β -isomers differ in their biological activity.²⁰

The aim of the present work is to investigate the interaction between endosulphan and amino acids employing density functional methods. Two conformers of both α - and β -endosulphan were considered in the present study. Two amino acids, glycine and GABA were chosen, as they are known to be chief neurotransmitters in the central nervous system. It has been shown

[#]Dedicated to Prof. N Sathyamurthy on his 60th birthday

*For correspondence

that glycine and GABA exists as zwitterions in solution phase while as a neutral molecule in gas phase.^{21,22} The scope of this study is limited to the gas phase and hence we have considered the interaction of neutral glycine and GABA with endosulphan. To the best of our knowledge, so far no one has reported an interaction of endosulphan with amino acids using quantum chemical methods.

2. Computational methods

All the computations were performed using the Gaussian 09 suite of programs.²³ The geometry optimizations of the monomers and the complexes were carried out using various DFT methods. The DFT methods employed in this study are B3LYP, M05, M05-2X, M06 and M06-2X in conjunction with the 6-31++G** basis set. These functionals (except B3LYP) were shown to perform adequately in accounting for non-covalent interactions for a wide variety of systems.²⁴ Vibrational frequency calculations were performed to ascertain that the optimized geometries correspond to minima in the potential energy surface. The geometry optimization at the second order Møller-Plesset perturbation (MP2) method has also been carried out wherever it is possible. The stabilization energy was computed using the supermolecular approach using the equation:

$$\Delta E_{\text{stab}} = E_{\text{complex}} - (E_{\text{Endosulphan}} + E_{\text{glycine/GABA}}). \quad (1)$$

3. Results and discussion

3.1 Isomers of endosulphan

The optimized geometries of glycine, GABA, α -endosulphan ($\alpha 1$ and $\alpha 2$) and β -endosulphan ($\beta 1$ and $\beta 2$) are depicted in figure 1. Among the four conformers of endosulphan, the β -isomer, in which the S=O bond points upward ($\beta 1$), was found to be the most stable. The energies of the other three conformers $\alpha 1$, $\alpha 2$ and $\beta 2$, with respect to the energy of $\beta 1$, were calculated to be 4.26, 4.74 and 7.83 kcal/mol, respectively.

It was experimentally demonstrated that the two isomers of β -endosulphan ($\beta 1$ and $\beta 2$) remain in equilibrium in solution and the β -endosulphan converts to the α -endosulphan, while the reverse does not occur.¹² Using the results from the above experiments combined with the semi-empirical calculations it was also suggested that the conversion of $\beta 1$ -conformer to the α -endosulphan could take place only via its higher energy conformer $\beta 2$. We have calculated the free energy

Figure 1. Optimized geometries of glycine, GABA and endosulphan obtained at the M06-2X/6-31++G** level.

change (ΔG) associated with the formation of two conformers of α -endosulphan from the β -endosulphan at room temperature. The results obtained at the M06-2X/6-31++G** method are as follows:

$$\begin{aligned} \beta 1 &\Rightarrow \alpha 1 & \Delta G &= +3.90 \text{ kcal/mol;} \\ \beta 1 &\Rightarrow \alpha 2 & \Delta G &= +4.29 \text{ kcal/mol;} \\ \beta 2 &\Rightarrow \alpha 1 & \Delta G &= -3.45 \text{ kcal/mol;} \\ \beta 2 &\Rightarrow \alpha 2 & \Delta G &= -3.06 \text{ kcal/mol.} \end{aligned}$$

The positive values of ΔG for the formation of $\alpha 1$ and $\alpha 2$ from $\beta 1$ suggest that these transformations are not feasible. On the other hand the formation of $\alpha 1$ and $\alpha 2$ from $\beta 2$ is spontaneous which confirms the findings by Schmidt *et al.*¹²

3.2 The complexes of endosulphan with glycine and GABA

The optimized geometries of the complexes of endosulphan with glycine and GABA are depicted in figures 2 and 3. The stabilization energies of the complexes obtained using B3-LYP, M05, M05-2X, M06 and M06-2X methods are listed in table 1. The stabilization energies of the complexes clearly indicate that all the conformers of endosulphan form stable complexes with both the amino acids, by forming a strong hydrogen bond. The stabilization energies obtained

Figure 2. Optimized geometries of the complexes of endosulphan–glycine obtained at the M06–2X/6–31++G** level.

by B3LYP method are significantly less compared to those obtained in other methods. This is understandable because of the inadequacy of the B3LYP functional to describe the weak interactions present in the systems herein. In general, the stabilization energies predicted by the M05–2X and M06 methods are comparable to each other. The M06–2X method predicts the highest stabilization energies for the complexes.

Considering the unavailability of any experimental results, further discussion is based on the results obtained from the M06–2X/6–31++G** level of calculations.

With glycine, the conformer $\alpha 1$ forms the most stable complex, having a stabilization energy of -15.24 kcal/mol, followed by the complex $\beta 1$ -glycine (-13.14 kcal/mol). For the complexes $\alpha 2$ -glycine and

Figure 3. Optimized geometries of the complexes of endosulphan–GABA obtained at the M06–2X/6–31++G** level.

Table 1. The calculated values of stabilization energy for the complexes of endosulphan with glycine and GABA at various methods and using 6-31++G** basis set.

Complex	Stabilization Energy/(kcal/mol)					
	B3LYP	M05	M05-2X	M06	M06-2X	MP2
Endosulphan–Glycine						
α 1-Glycine	–9.23	–11.42	–13.51	–14.15	–15.24	–17.42 –16.10 ^a
α 2-Glycine	–6.09	–8.84	–10.65	–10.68	–11.85	–13.70 –12.50 ^a
β 1-Glycine	–8.62	–10.70	–12.12	–12.06	–13.14	–14.39 –13.41 ^a
β 2-Glycine	–4.21	–6.10	–6.72	–6.72	–7.14	–10.12 –8.73 ^a
Endosulphan–GABA						
α 1-GABA	–9.51	–11.80	–13.33	–13.58	–14.39	–14.42 ^a
α 2-GABA	–7.33	–9.14	–10.30	–9.71	–10.78	–11.71 ^a
β 1-GABA	–9.63	–11.79	–13.30	–12.97	–14.10	–13.41 ^a
β 2-GABA	–5.07	–6.97	–7.67	–7.53	–7.85	–9.44 ^a

^aThe values are calculated using the geometries optimized at M06-2X/6-31++G** level.

β 2-glycine, the computed stabilization energies are –11.85 and –7.14 kcal/mol, respectively. The stabilization energies suggest that the conformers α 1 and β 1, where S=O bond is pointing upward, form more stable complexes compared to the conformers α 2 and β 2, where S=O bond is pointing downward (figure 2). Primarily, the complexes are stabilized by the hydrogen bond interaction between the S=O group of endosulphan and the O–H group of the glycine. In addition, there are several weak interactions (electrostatic in nature) between the two units, which could be responsible for the difference in the stabilization energies of various complexes. For example, in the most stable complex α 1-glycine, there are three C–H $\cdots$ O interactions and one C–H $\cdots$ N interaction. Similarly, there are four C–H $\cdots$ O interactions in the β 1-glycine. On the other hand, the α 2-glycine complex has two C–H $\cdots$ O interactions and no such weak interactions are present in the complex β 2-glycine. Studies on the C–H $\cdots$ O interactions in close packing of molecules are available in literature.^{25,26} The favourable C–H $\cdots$ O and C–H $\cdots$ N distances corresponding to weak interactions for various complexes are illustrated in figures 2 and 3.

Similarly, GABA also forms a strong hydrogen bond with endosulphan. The stabilization energies for the complexes α 1-GABA and β 1-GABA are found to be –14.39 and –14.10 kcal/mol, respectively. In α 1-GABA and β 1-GABA complexes, GABA is attached to the S=O group of endosulphan from above. The stabilization energies for the complexes α 2-GABA

and β 2-GABA, in which GABA is bonded to the endosulphan from below, are –10.78 and –7.85 kcal/mol, respectively. As explained in the case endosulphan-glycine complexes, the binding of GABA to the endosulphan where S=O is pointing upward results in additional favourable C–H $\cdots$ O interactions. It can be seen from figure 3 that, in addition to the hydrogen bond, the complexes α 1-GABA and β 1-GABA have three and four C–H $\cdots$ O interactions, respectively. No such interactions were seen for β 2-GABA, which indeed is the least stable complex.

To understand how the stabilization energy of the complexes obtained at the density functional methods differ from those obtained at the MP2 method, we have carried out the optimization of the complexes formed between endosulphan and glycine. Table 1 also lists the stabilization energies of these complexes obtained at MP2/6-31++G** level. From the table, one can see that the stabilization energies of the complexes obtained at the MP2 level are slightly higher than those obtained at the M06-2X level. This could be due to the overestimation of the binding energy at the MP2 level as discussed widely in literature.

Considering the above as well as the large size of the molecules, we have not carried out the optimization for the complexes formed between GABA and endosulphan. However, the stabilization energies of these complexes have been calculated at the MP2 level using the optimized geometries obtained at the M06-2X method and are given in table 1. The values indicate that the stabilization energies of these complexes are increased

slightly at the MP2 level similar to the case of the glycine–endosulphan complexes.

4. Conclusions

The values of stabilization energies for various conformers of endosulphan with glycine and GABA suggest that endosulphan has a tendency to bind with amino acids and proteins. Endosulphan forms stable complexes with glycine and GABA by forming a strong hydrogen bond and their stabilization energies fall in the range of -7 to -15 kcal/mol. In the conformers where the S=O bond points upward, the C–H $\cdots$ O interaction plays an important role in the stabilization of the complexes. The formation of the stable complex between various isomers of endosulphan and GABA indicates the possibility that such a complex formation could be a reason for antagonistic effect of the endosulphan in the GABA receptors. We hope that this study would inspire more researches in this direction.

Acknowledgements

The authors thank professor J Samuel Arey and the LMCE laboratory for providing computational resources. One of the authors CNR acknowledges Dr. Vivek Bajpai, Dr. Shameema and Mr. Pradeep Kumar for their valuable suggestions.

References

1. Keith L H and Telliard W A 1979 *Environ. Sci. Technol.* **13** 416
2. Peterson S M and Batley G E 1993 *Environ. Pollut.* **82** 143
3. Zhou M, Li Y C, Nkedi-Kizza P and O'Hair S K 2003 *Vadose Zone J.* **2** 231
4. Dalvie M A, Africa A, Solomons A, London L, Brouwer D and Kromhout H 2009 *J. Environ. Sci. Health B* **44** 271
5. Walse S S, Shimizu K D and Ferry J L 2002 *Environ. Sci. Technol.* **36** 4846
6. Kaur I, Mathur R P, Tandon S N and Dureja P 1998 *Environ. Technol.* **19** 115
7. Saravanan T S, Rajesh P and Sundaramoorthy M 2010 *J. Environ. Biol.* **31** 755
8. Cabaleiro T, Caride A, Romero A and Lafuente A 2008 *Toxicol. Lett.* **176** 58
9. Mora F and Ozmen O 2010 *Exp. Toxicol. Pathol.* **62** 75
10. Brandt V A, Moon S, Ehlers J, Methner M M and Struttman T 2001 *Am. J. Ind. Med.* **39** 643
11. Saiyed H, Dewan A, Bhatnagar V, Shenoy U, Shenoy R, Rajmohan H, Patel K, Kashyap R, Kulkarni P, Rajan B and Lakkad B 2003 *Environ. Health Perspectives* **111** 1958
12. Schmidt W F, Hapeman C J, Fetting J C, Rice C P and Bilboulia S 1997 *J. Agric. Food Chem.* **45** 1023
13. Schmidt W F, Bilboulia S, Rice C P, Fetting J C, McConnell L L and Hapeman C J 2001 *J. Agric. Food Chem.* **49** 5372
14. Hoff R M, Muir D C G and Grift N P 1992 *Environ. Sci. Technol.* **26** 266
15. Burgoyne T W and Hites R A 1993 *Environ. Sci. Technol.* **27** 910
16. Bidleman T F, Falconer R L and Walla M D 1995 *Sci. Total Environ.* **160** 55
17. Strachan W M J and Huneault H 1979 *J. Great Lakes Res.* **5** 61
18. Chan C H and Perkins L H 1989 *J. Great Lakes Res.* **15** 465
19. Chan C H, Bruce G and Harrison B 1994 *J. Great Lakes Res.* **20** 546
20. Videira R A, Antunes-Madeira M C and Madeira V M 2002 *Biochim Biophys. Acta* **140** 1564
21. Ramek M and Nagy P I 2000 *J. Phys. Chem. A* **104** 2199
22. Ham N S 1974 *Mol. Quantum Pharmacol. Proc. 7th Jerusalem Symposium* **7** 261
23. Gaussian 09, Revision A.02, Frisch M J, Trucks G W, Schlegel H B, Scuseria G E, Robb M A, Cheeseman J R, Scalmani G, Barone V, Mennucci B, Petersson G A, Nakatsuji H, Caricato M, Li X, Hratchian H P, Izmaylov A F, Bloino J, Zheng G, Sonnenberg J L, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Naka-jima T, Honda Y, Kitao O, Nakai H, Vreven T, Montgomery Jr J A, Peralta J E, Ogliaro F, Bearpark M, Heyd J J, Brothers E, Kudin K N, Staroverov V N, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant J C, Iyengar S S, Tomasi J, Cossi M, Rega N, Millam N J, Klene M, Knox J E, Cross J B, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann R E, Yazyev O, Austin A J, Cammi R, Pomelli C, Ochterski J W, Martin R L, Morokuma K, Zakrzewski V G, Voth G A, Salvador P, Dannenberg J J, Dapprich S, Daniels A D, Farkas Ö, Foresman J B, Ortiz J V, Cioslowski J, Fox D J, Gaussian, Inc., Wallingford CT, 2009
24. Zhao Y and Truhlar D G 2008 *Acc. Chem. Res.* **41** 157
25. Desiraju G R 1996 *Acc. Chem. Res.* **29** 441
26. Saleesh Kumar N S, Varghese S, Rath N P and Das S 2008 *J. Phys. Chem. C* **112** 8429