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SPECTROSCOPIC STUDIES ON A POTENTIAL COMPLEX BETWEEN TNT AND FOLIC ACID: A DFT STUDY

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Abstract. The interaction between TNT and an indispensably substantial biological molecule, folic acid (FA), has been investigated at the level of density functional theory. Two models are assembled: (i) an intimate pair of TNT and FA and (ii) π-complex of them. The calculations (in vacuum conditions) have showed that these molecules in the intimate pair model orient themselves in an angular geometry. A π-complex formation between these molecules is quite likely in the case of parallel arrangement of aromatic rings. The calculated UV and NMR spectra support the idea of a strong interaction between TNT and FA.

Keywords: folic acid, TNT, NMR, UV-VIS, complex, DFT

Introduction

Folic acid, FA, (Fig. 1), also acknowledged as folate (the natural form in the body), vitamin B_9 (Grandall et al., 1995), vitamin B_c (Dawson et al., 1989), pteroyl-L-glutamic acid, pteroyl-L-glutamate, and pteroylmonoglutamic acid (Hathcock, 1997), is a water soluble vitamin (Herbert & Zalusky, 1962) with molecular formula $C_{19}H_{19}N_7O_6$ (Scptt, 1999). The solubility of FA in water is 1.6 mg/L at 25°C. Folic acid itself is not biologically active, but its biological significance is due to tetrahydrofolate and other derivatives after its transformation to dihydrofolic acid in the liver (Bailey & Ayling, 2009).

Folic acid is vital for many biological activities. The human body requires FA to synthesize DNA, repair DNA, and methylate DNA as well as to act as a cofactor in certain biological reactions (Weinstein et al., 2003). It is particularly significant in assisting rapid cell division and growth, such as in infancy and pregnancy. Children and adults both need folic acid to produce healthy red blood cells and prevent anemia (Bruice, 2003).

A lack of dietary folic acid initiates folate deficiency in the body. A thorough lack of dietary folate takes months before deficiency develops, because normal individuals have about 500–20,000 μg (Duthie, 1999) of folate in body stocks (Hoffbrand & Weir, 2001), This insufficiency can give rise to many health problems; the

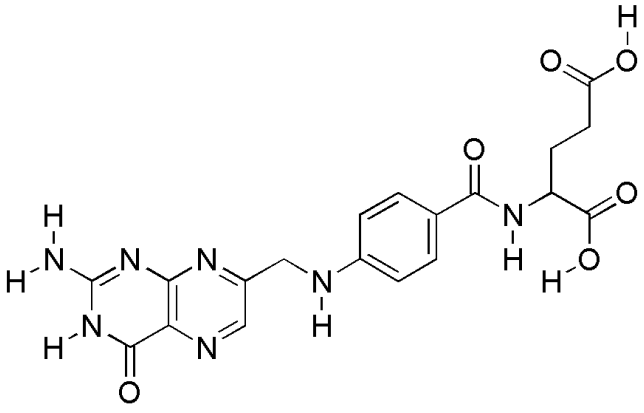

Fig. 1. Structure of folic acid

most outstanding one is the neural tube defects in developing embryos. Common symptoms of folate deficiency include diarrhea, macrocytic anemia with weakness or shortness of breath, nerve damage with weakness and limb numbness (peripheral neuropathy), pregnancy complications (Goh & Koren, 2008), mental confusion, poor memory (Krebs et al., 2009) or other cognitive declines, mental depression (Coppen & Bolander-Gouaille, 2005), sore or swollen tongue, peptic or mouth ulcers, headaches, heart palpitations, irritability, and behavioral disorders. Low levels of folate can also lead to homocysteine accumulation (Keshava et al., 1998). DNA synthesis and repair are weakened, which may result in cancer development (Taylor et al., 2004).

Trinitrotoluene (TNT), or more specifically, 2, 4, 6-trinitrotoluene, is a chemical compound with the formula $C_7H_5N_3O_6$. This yellow-colored material is infrequently employed as a reagent in chemical synthesis, however it is best known as a useful explosive material with appropriate handling properties. The explosive yield of TNT is considered to be the standard measure of strength of bombs and other explosives. In chemistry, TNT is employed to generate charge transfer salts (Urbański, 1964).

TNT is acknowledged to be a poisonous substance harmful for almost all cells, particularly those of liver, bone marrow and kidney. Pathologic results are acute yellow atrophy of the liver, aplasia of the bone marrow, petechial hemorrhages and toxic nephritis (Dreisbach, 1971). TNT possibly disturbs progressively many biochemical activities critically which are yet unknown symptomatically.

Although the general poisonous effects of TNT are clinically known, their mechanisms at the molecular level have not been revealed yet. In the current article, interaction of TNT with folic acid (FA) has been considered at the molecular level. The interaction of folic acid and TNT has been considered in the form of two models as

(i) an intimate pair form, (ii) a π-complex form. The π-complex formation has been considered due the tendency of three nitro bearing compounds to make π-complexes with electron-rich aromatics, and phenyl moiety in the middle of folic acid fulfills this condition.

Method

The initial geometry optimizations of all the structures leading to energy minima were achieved by using MM2 (molecular mechanics) method followed by semi-empirical PM3 self-consistent field molecular orbital (SCF-MO) method (Stewart, 1989a; 1989b) at the restricted level (Leach, 1997). Then, the geometry optimizations were achieved by using various restricted Hartree-Fock (RHF) methods successively and finally optimizing within the framework of density functional theory (DFT,B3LYP) (Kohn & Stam, 1965; Parr & Yong, 1994) at the level of 6-31G(d,p). The exchange term of B3LYP consists of hybrid Hartree-Fock and local spin density (LSD) exchange functions with Becke's gradient correlation to LSD exchange (Becke, 1988; Vosko et al., 1980). The correlation term of B3LYP consists of Vosko, Wilk, Nusair (VWN3) local correlation functional (Lee et al., 1988) and Lee, Yang, Parr (LYP) correlation correction functional (Lee et al., 1988).

Vibrational analyses and the calculation of total electronic energies were performed using B3LYP/6-31G(d,p) type calculations for closed-shell systems. The normal mode analysis yielded no imaginary frequencies, which indicates that each compound had at least a local minimum on the potential energy surface. The total electronic energies were obtained by single point B3LYP/6-31G(d,p) calculations over DFT optimized geometries and corrected for zero point vibrational energies (ZPE). All the computations were performed using Spartan 06 software.[1)]

Results and discussion

In the present article, intimate pair of folic acid (FA) and TNT is labeled and referenced as "FA + TNT" and π-complex of them as "FA-TNT" or simply the "complex".

Geometries

FA is a polar molecule with an aromatic phenyl ring in the middle, an aminopteridinone group and an amino group containing carbon based chain. The presence of these groups creates a dipole moment of 14.69 Debye from the aminopteridinone moiety to the other side of the molecule.

TNT has an aromatic toluene ring with three nitro groups. It has a dipole moment of 1.55 Debye from the methyl group to the nitro group across it.

The geometry optimized FA + TNT intimate pair and FA-TNT complex have been presented in Fig. 2. When intimate pair is considered, the aromatic rings of both FA and TNT are in an angular orientation in space. This angularity should be

the result of some steric repulsive interaction between the methyl group of TNT and hydrogen atoms of phenyl ring of FA and some attractive interaction between hydrogens of amine groups in both sides of FA with the nitro groups of TNT.

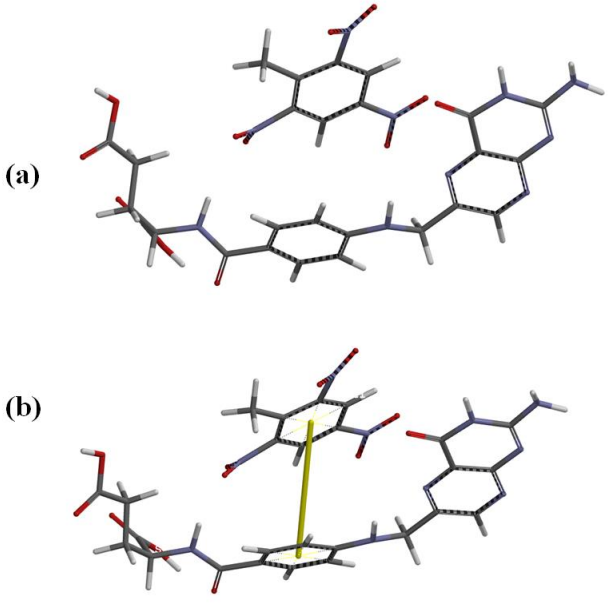

Fig. 2. Geometry optimized structures of a) FA+TNT pair b) FA-TNT complex

The interaction of FA with TNT might originate from charge-charge, charge-dipole and dipole-dipole interactions or all of them. Another probable interaction could be π-complex formation between electron accepting TNT molecule and a relatively electron rich phenyl moiety of FA molecule. In the present study, there are some supports in the following sections for the π-complex formation by NMR and UV-VIS characterization methods.

Energies

Table 1 shows the Zero Point Energy (ZPE) and total electronic energy (ZPE corrected) values of the structures in the present study. The energy values have been obtained by single point calculations over B3LYP/6-31G(d,p) optimized geometries. For the energy calculations of individual FA and TNT, the geometries of the partners in the complex or in the intimate pair have been conserved and a single point energy calculation in the absence of its partner has been carried out (for instance, the complex has been decomposed and one partner is deleted leaving the

other partner having the same geometry of it in the complex).

Examination of Table 1 shows that the sum of separate energies of TNT and FA (-6407730.96 kJ/mol) is less negative (less stable) than those of both the intimate pair and the complex. This is a strong indication of the interaction between TNT and FA. The total energy values of the intimate pair and the complex are found to be very close to each other.

Table 1. ZPE and total energy values of the molecules

	ZPE (kJ/mol)	Total Energy (kJ/mol)
TNT	355.85	-2310372.96
FA+TNT	1387.05	-6407794.84
FA-TNT	1387.36	-6407793.26
FA	1026.83	-4097358.00
The sum of separate energies of TNT and FA is -6407730.96 kJ/mol		

Frontier molecular orbitals (FMO)

Fig. 3 represents the HOMO and LUMO pattern of the π-complex (within the constraints of DFT). The HOMO is almost completely spread over the phenyl moiety of FA molecule.

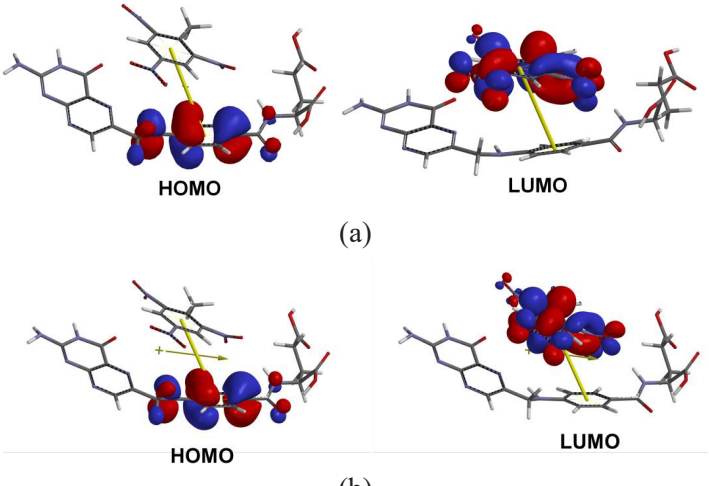

Fig. 3. (a) The HOMO and LUMO of the complex (DFT-6-31G(d,p));
(b) The HOMO and LUMO of the complex (HF-6-31G(d,p))

Conversely, the LUMO is spread over TNT molecule. Both of the frontier mo-

lecular orbitals (FMO) in great extent exhibit π-symmetry. Table 2 shows the FMO energy values in eV. The energies of the complex and the intimate pair slightly differ. The band gaps (Δε) of intimate pair and the complex are smaller than those of both TNT and FA.

Table 2a. The HOMO, LUMO energies and band gap ($\Delta\varepsilon = \varepsilon_{LUMO} - \varepsilon_{HOMO}$) of the molecules calculated DFT-6-31G(d,p).

	HOMO (eV)	**LUMO (eV)**	**Δε**
TNT	-11.56	-0.05	11.51
FA+TNT	-8.53	-0.46	8.07
FA-TNT	-8.53	-0.44	8.09
FA	-8.24	1.2	9.44

Table 2b. The HOMO, LUMO energies and band gap ($\Delta\varepsilon = \varepsilon_{LUMO} - \varepsilon_{HOMO}$) of the molecules calculated HF-6-31G(d,p)

	HOMO (eV)	**LUMO (eV)**	**Δε**
TNT	-11.54	-0.02	11.52
FA+TNT	-8.36	0	8.36
FA-TNT	-8.36	0.02	8.38
FA	-8.25	1.32	9.57

UV-VIS spectra

UV-VIS spectrum is a useful technique in complex structure characterization. Fig. 4 shows the calculated (B3LYP/6-31G(d,p)) UV-VIS spectra and Table 3 shows the relative absorptivity and λ_{max} values of TNT, FA, FA-TNT pair and the complex, respectively. The % relative absorptivity values have been determined assigning the first absorption peak of FA as 100%. As seen in the Figure 4, the UV-VIS spectrum of TNT shows a single absorption peak at 340 nm. FA shows double absorption peaks at around 300 nm and 450 nm. Both the spectra of pair and complex shows triple absorption peaks (Table 3).

Table 3. The relative absorptivity (RA %) and λ_{max} values of TNT, FA, intimate pair and the complex

	RA1 (%)	λmax,1 (nm)	RA2 (%)	λmax,2 (nm)	RA3 (%)	λmax,3 (nm)
TNT	88	340	-	-	-	-
FA	100	300	85	450	-	-
FA+TNT	55	440	61	530	76	670
FA-TNT	82	450	67	510	82	660

The UV-VIS spectra of both intimate pair and complex are very different from those of TNT and FA. There is a considerable bathochromic shift (as compared to the spectra of partners) detected as the interaction occurs between the FA and TNT. The absorbance peak of complex and the intimate pair begins at around 380 nm and shows similar patterns. The differences in the intensity and the absorbance peak points in the spectrum of the intimate pair and the complex supports the idea of π-complex formation.

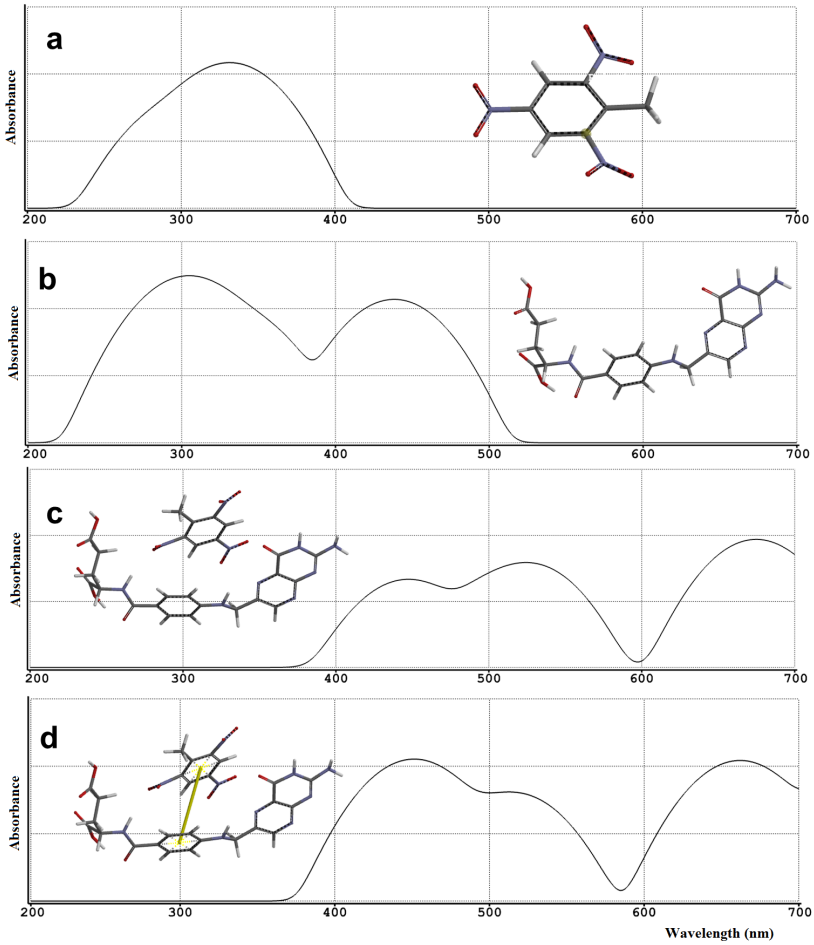

Fig. 4. The calculated UV-VIS spectra of a)TNT, b)FA, c)FA-TNT pair and d) the complex

In the intimate pair, the electrostatic field of each partner affects the electron distribution of the other through space while itself being affected likewise. Consequently, the molecular orbital energies and electron population of the molecules change. The calculations specify that in the case of intimate pair, the previously stated changes occur in such a way that a strong bathochromic effect results compared to separate spectrum of FA and TNT.

In the case of complex formation, relatively electron rich aromatic phenyl moiety of FA donates some of its π-electrons to electron poor TNT π-system. This donor-acceptor interaction apparently changes the molecular orbital energies and electron population as compared to separate partners. Therefore, the intensity and the absorbance peak points in the UV-VIS spectrum of the complex are different from that of intimate pair.

NMR spectra

Fig. 5 shows the ^{1}H-NMR spectra of TNT, FA, the intimate pair and the complex. The spectra for TNT and FA stand for the structures obtained from the complex by deleting one of the other components each time. Thus the geometries of the components in the complex are kept constant.

TNT has two signals on the NMR spectrum. The one at around 3.9 ppm stands for the methyl protons, the other at around 10 ppm stands for the aromatic hydrogen of the phenyl ring. FA structure possesses eighteen different protons, accordingly eighteen signals are observed on the NMR spectrum. The peaks between 2-4 ppm are the signals of two methylene (-CH_2) groups between two carboxyl group and the primary amino group on aminopyrimidinone moiety. The peaks between 4-6 ppm belong to methine (-CH-) group of FA, to secondary amino group (-R-NH_2) attached to the phenyl ring and to methylene group attached to aminopteridinone moiety. The peaks between 6-7 ppm are the signals of protons of phenyl ring in the middle of the molecule. The peaks between 7-9 ppm indicate secondary amino (R_2-N-H) group near carbonyl group, hydrogen of pyrazine and secondary amino group on aminopyrimidinone moiety. The peaks between 9-12 ppm are the signals of carboxylic acid groups.

It is clear from the spectrum of intimate pair that some peaks of FA have been shifted to downfield due to the presence of TNT. For example, the methyl peaks between 2-4 ppm has shifted to downfield slightly. One of the peaks between 6-8 ppm has shifted to downfield (between 8-9 ppm). The spectrum of FA has 18 peaks; TNT has 2 peaks; however spectra of intimate pair and the complex have 21 peaks instead of 20 peaks as expected. A new peak has been observed at around 5.8 ppm. These shifts in ppm values and the observation of a new peak have been interpreted as an interaction between FA and TNT.

In the complex, these protons do not resonate at the same δ values. It is obvious from the comparison of the individual spectra of FA and the complex that the pro-

ton of TNT resonating at around 10 ppm shifts towards high field slightly. Since the protons of TNT are in the shielding zone of the phenyl moiety of FA, this shielding is expected. However, this effect could be also a result of transfer of some electron population to TNT from the π-system of FA.

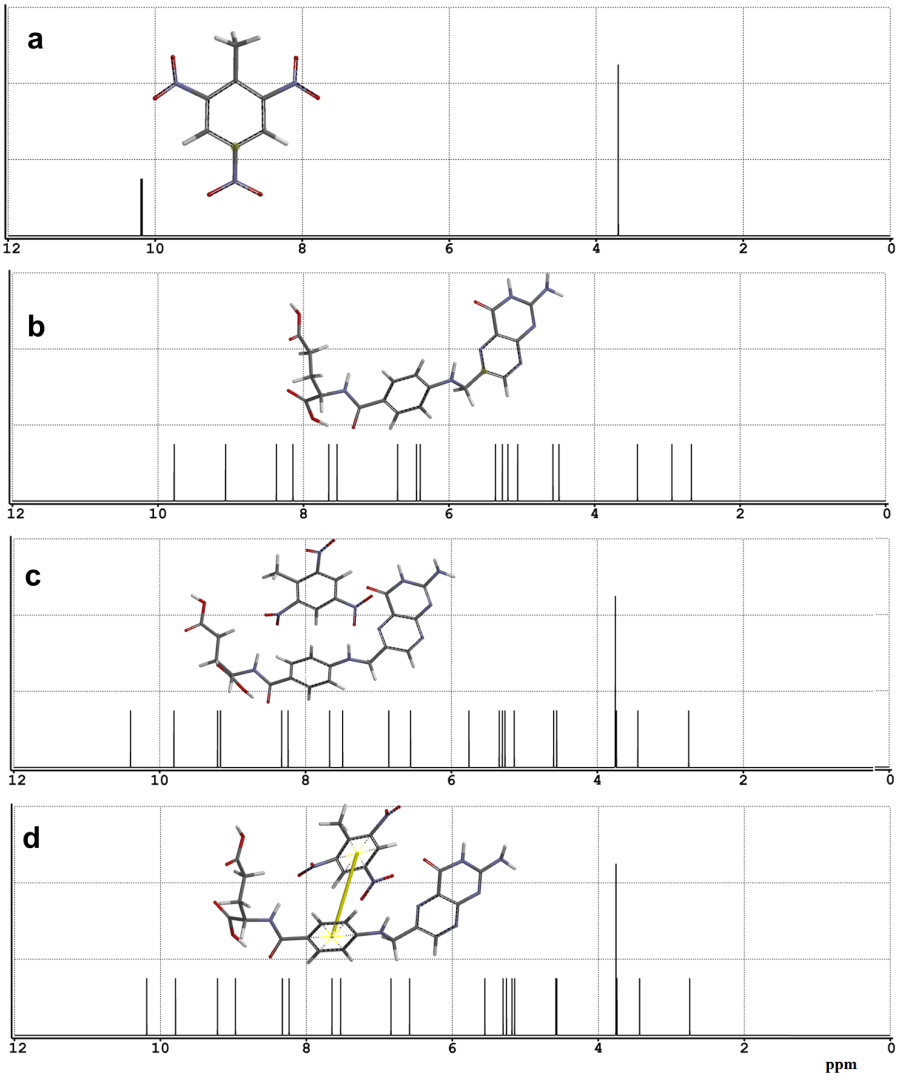

Fig. 5. ^{1}H-NMR spectra of a) TNT, b) FA, c) FA-TNT pair and d) the complex

The data presented above clearly shows that TNT and FA strongly interact in both models described presently. Although our calculations reveal the situation in the vacuum conditions, it is quite feasible that the interaction continues in the solvent case. The question of which model of interaction actually occurs needs some experimental work. The sensitivity to solvent polarity of a complex is an important experimental characteristic. The energy required for absorption decreases as the solvent polarity increases (Turro, 1991; Reichardt, 2004). One can get some idea by assessing the shift of maximum absorption bands (λ_{max}) by changing the solvent.

Conclusion

Results of present research have indicated that there is an interaction between renowned explosive TNT and a biological compound FA. The type of interaction between FA and TNT could be in the form of an intimate pair (having angular geometry) or a π- complex (parallel geometry). In both cases, the interaction is strong. FA is known to take place in the synthesis of DNA, in renovation of DNA, and in the methylation of DNA as well as a cofactor in certain biological reactions. TNT may interfere with these activities upon interaction with FA to a certain extent as shown in the present article. This interference should be investigated medically. Particularly, the chronic poisoning of TNT may give symptoms which are not attributable to TNT poisoning. Authors hope that the present work will be a helpful first step for medical researchers interested in effects of TNT on FA biochemistry.

NOTES

1. http://downloads.wavefun.com/06WinMacLinuxManual.pdf

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