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Dependence of the biological activity and mass spectrometric pattern on the structure peculiarities of the molecule of alkylating drug thiotepa

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Abstract

Structural and electronic parameters of the chemotherapeutic alkylating drug thiotepa obtained by MNDO and MINDO/3 quantum mechanical calculations are used to explain some physical, chemical and biological properties of this compound. On the basis of the revealed difference between the preferential conformations of the thiotepa molecule in crystal and vacuum, a conclusion is made concerning the precautions in the choice of the appropriate molecular geometry in the search of structure–activity correlations. The theoretical data are also applied to the explanation of some peculiarities of soft ionization mass spectra of thiotepa, in particular its sensitivity to high field effects and the absence of protonation. The modeling of some reactions of thiotepa directly in the conditions of field ionization mass spectrometric experiment is discussed.

Keywords: Chemotherapeutic alkylating drug thiotepa; MNDO quantum mechanical calculations; Hydration; Soft ionization mass spectrometry; Structure–activity links

1. Introduction

In the search of the correlations between the structure and physical, chemical and biological properties of biologically active substances, the data obtained by means of various experimental and theoretical methods are used. In our previous studies aimed at the elucidation of the relations between the structure of the chemotherapeutic alkylating drug thiotepa $(S{=}P\text{–}(\text{–}N\triangleleft)_3)$, mechanisms of its action on molecular level (DNA and nitrogen bases) [1–3], and its behavior under different mass spectrometric ionization conditions (such as field ionization (FI), field desorption (FD), fast atom bombardment (FAB), electron impact (EI)) [4–6], it was perceived that some peculiarities of the mass spectra of thiotepa are inconsistent with its structure in crystal determined by means of X-ray analysis [7]. Earlier the thiotepa structure derived from the X-ray data was used in CNDO/2 calculations [8] of electronic parameters of thiotepa to correlate its activity with the interactions with its main target, the DNA molecule. The ques-

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tion now arises as to the structure of thiotepa in the non condensed state.

The features of the mass spectra of thiotepa, which prompted us to doubt the structure of thiotepa molecule in vacuum, are summarized in Table 1. One of the general traits of FI mass spectra of many polar substances is the presence along with the molecular ion $[M]^{+\cdot}$ of the protonated one $[MH]^+$, arising from the interactions of the analyzed molecules with one another or with any proton donor agent [9] both in the gaseous state or on the FI emitter surface [10]. As it is easy to see, there are four proton acceptor centers (electronegative atoms of sulfur and nitrogens with the exposed lone pairs) in the structure of thiotepa derived from X-ray data [7] and presented at Fig. 1a. The more unexpected feature was that protonated $[MH]^+$ ion was never observed under normal FI conditions [1,4–6]. At the same time in another ionization mode, namely FAB, where the analyte comes from the liquid state (being preliminary dissolved in the glycerol matrix), the protonated ion $[MH]^+$ is quite intensive [5]. As to the soft ionization methods, it has been established that the main features of ion formation are very similar for all of them, but in the case of thiotepa its FI/FD [6] and FAB [5] spectra differ significantly (Table 1), which points to the enhanced sensitivity of thiotepa to the high field effect, the explanation of the latter may also be deduced from the structure of the molecule.

To resolve these contradictions we embarked on quantum mechanical calculations of the structure of thiotepa molecule in isolated state in vacuum.

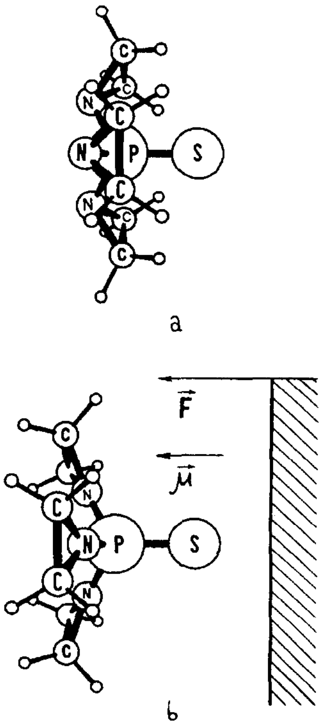


Fig. 1. Conformations of thiotepa molecule: (a) structure in crystal imaged using X-ray data [7]; (b) structure of the isolated molecule in vacuum, optimized in MNDO calculations. Preferential orientation of the molecule in the high electric field near the FI emitter surface is depicted.

Table 1
The main types of ions, present in the EI, FAB and FI/FD mass spectra of thiotepa [4–6]

m/z	Types of ions		
	EI	FAB	FI/FD
379		$[2MH]^+$	
378			$[2M]^{+\cdot}$
190		$[MH]^+$	
189	$[M]^{+\cdot}$		$[M]^{+\cdot}$
173			$[2M{-}S]^{++}$
147	$[M{-}N\triangleleft]^+$	$[M{-}N\triangleleft]^+$	$[M{-}N\triangleleft]^+$
115	$[M{-}N\triangleleft{-}S]^+$	$[M{-}N\triangleleft{-}S]^+$	
94.5			$[M]^{++}$
78.5			$[M{-}S]^{++}$
63	$[M{-}3N\triangleleft]^+$	$[M{-}3N\triangleleft]^+$	
42	$[N\triangleleft]^+$	$[N\triangleleft]^+$	$[N\triangleleft]^+$

2. Experimental

Mass spectrometric experiments were performed with the help of a sector magnetic mass spectrometer MI-1201B ('Electron' Works, Sumy, Ukraine). FI ion source, described elsewhere [11,12], was employed. The design of this ion source was specially dedicated to the studies of intermolecular interactions in vacuum for which purpose the possibility of supply of two individual samples from two separate glass evaporators to the interaction zone near the needle-type tungsten emitter was provided. In the experiments both evaporators were kept at ambient temperature, as thiotepa is volatile enough and readily sublimates in vacuum, and monomolecular flows of H_2O and D_2O were provided by the method described elsewhere [12,13] from the crystalline hydrates of $CuSO_4 \cdot 5H_2O$ and $CuSO_4 \cdot 5D_2O$.

Thiotepa (N, N′,N″-thryethylenethiophosphoramide) was purchased from Olaynski Chemico-Pharmacological Plant (USSR) and was doubly recrystallized from benzene–hexane [4].

Quantum mechanical calculations were performed employing two semiempirical methods MNDO (sp basis set) and MINDO/3 (spd basis set) with full optimization of geometrical parameters [14,15].

3. Results and discussion

For quantum mechanical calculations the initial structure of thiotepa molecule was taken from the X-ray data [7]. One can suggest, that the structure obtained after optimization of geometrical parameters (Table 2, Fig. 1b) corresponds to the most favorable conformation of the free molecule in vacuum, which is consistent with the vacuum conditions of a mass spectrometric experiment. So further discussion of the results will be carried out considering that one of the aims of these calculations was to find out what structure peculiarities of thiotepa in vacuum determine the pattern of its mass spectra (Table 1).

It may be seen at once, that the structure of the molecule in crystal differs significantly from the one calculated in vacuum approximation (Table 2). This result points to the fact, that the packing of thiotepa molecules in crystal is strongly influenced by the crystalline field forces and is accompanied by the shift of its conformation to a more compact form. The main difference in the two structures is connected with the orientation of the ethyleneimine rings. In the conformation corresponding to the crystal state all three nitrogen atoms have pyramidal configurations, the planes of ethyleneimine rings are directed towards sulfur atom thus masking P–N bonds, and lone electron pairs of nitrogens are exposed for formation of H-bonds (Fig. 1a). In the calculated structure the sum of the valence angles near the nitrogen atoms is close to 360°, ethyleneimine rings are turned away from the sulfur atom (Fig. 1b) and their hydrophobic hydrocarbon groups shield to some extent electronegative nitrogen atoms.

Table 2
Geometrical parameters of the thiotepa molecule

Parameter		Theory		Experiment X-ray [7]
		MNDO	MINDO/3	
Bond	S–P	1.870	1.825	1.91
length,	P–N	1.632	1.645	1.64
Å	N–C	1.443	1.452	1.50
	C–C	1.547	1.547	1.48
	C–H	1.101	1.101	–
Bond	S–P–N	113.8	114.1	118
angles,	P–N–C	147.8	146.9	119
degrees	N–C–H	118.5	119.2	–

Table 3
Charges on the atoms of the thiotepa molecule

Charge atom	Isolated molecule		Structure in crystal, CNDO/2 [8]
	MNDO	MINDO/3	
S1	−0.45	−0.46	−0.21
P2	1.34	1.31	0.191
N3	−0.69	−0.71	−0.15
N4	−0.69	−0.71	−0.15
N5	−0.69	−0.71	−0.17
C6–C11	0.14	0.14	–
H12–H23	0.02–0.03	0.02–0.03	

As to energy and electronic characteristics, the values obtained by both MNDO and MINDO/3 methods are very close, e.g. the heat of formation ΔH_f for optimum structure is 106.5 and 112.3 kcal/mole in two methods respectively. It should be noted that the heat of formation for fixed thiotepa structure characteristic of crystal obtained by the same MNDO procedure is 41.2 kcal/mole higher, which favors the calculated structure for free molecules in vacuum from the energy point of view. Ionization potentials V_i are -8.61 eV (MNDO) and -8.79 eV (MINDO/3). An analysis of the abundances of the atomic orbitals to the energy of the highest occupied molecular orbital, which determines an ionization potential V_i, reveals the highest yield of p_x and p_z orbitals of the sulfur atom. When applied to FI this means, that the tunnel loss of electron may occur with the highest probability namely from the sulfur atom.

The charges on atoms presented in Table 3 also nearly coincide in MNDO and MINDO/3 and differ significantly from those calculated by CNDO method [8] for the fixed geometry in crystal. A negative charge, as it was expected, is localized on the sulfur and nitrogen atoms; a positive charge on the phosphorus atom is much greater for vacuum conformation maybe due to delocalization of the negative charge towards nitrogen atoms, thus the acidity of phosphorus being almost an order higher for isolated molecules. Nevertheless total negative charge on three nitrogen atoms, lying in one plane, is higher than that on sulfur atom, the vector of dipole moment $\vec{\mu}$ is directed from sulfur to nitrogens (almost parallel with P=S bonds) due to substantial compensation of the negative charge by positive ones on hydrocarbon groups. A numerical value of dipole moment is almost twice as high in vacuum (5.71 D, MNDO, and 5.38 D, MINDO/3) as for crystal (2.08 D, CNDO/2 [8]) conformation. In vacuum conformation hydrocarbon groups are more remoted from sulfur atom, thus enhancing the polarity of the molecule. On the other hand, in crystal, thiotepa has a more compact form, the polar groups being shielded by nonpolar ($-CH_2-CH_2-$) groups to a greater extent (the positions of six carbons resembling these in cyclohexane); such structure and small dipole moment may be responsible for easy sublimation of thiotepa.

It is well known, that one of the necessary steric prerequisites to the high alkylating activity of ethyleneimine derivatives is the high conformational mobility of aziridine rings. The latter was calculated earlier by the Monte Carlo method [16] in terms of distribution of the density of probability of the states of the system ρ in relation to the rotation angle of each aziridine ring around P–N bond at 300 K. It was shown, that when we consider a single molecule with geometry corresponding to crystalline state, the rotation of the rings is absent due to the existence of completely forbidden states in the system, and only vibrations of each ring in an interval no greater than $\pm 60^\circ$ are allowed. For the optimized geometry in the gaseous state there are no conformational restrictions at all, thus free rotation of the rings being possible. As regards high alkylating activity of thiotepa one can suppose, that conformational mobility of the molecules in solution is also high, facilitating the adjustment of the alkylating groups to their molecular receptors in DNA, the latter established in [1–3].

The results presented above indicate, that such essential molecular parameters as conformation, charge distribution and dipole moment differ greatly for the free molecule and the one in the crystal. At the same time biological activity of drugs is usually tested neither in vacuum nor in crystal, but in water solution, and the structure of the molecule in liquid may also differ from two other aggregate states; however the conformation in liquid may be assumed to be closer to the gaseous case in the sense that both are not rigidly restricted by the crystalline field forces.

On the basis of all said above a conclusion can be made that one should not use X-ray data for thiotepa molecule in the solid state for estimations of its structure–activity correlations, as it is inconsistent with the most favorable structure in vacuum and, maybe, in liquid.

Now let us discuss how structure peculiarities of thiotepa molecule may be reflected in its FI mass spectra. At first we have to state, in what sense FI is structure and conformation sensitive. In early studies on FI it was established, that in the conditions of high electric field polar molecules, while approaching an emitter surface, are directed in such a way that the vector of their dipole moment $\vec{\mu}$ becomes parallel with the field strength lines $\vec{F}$ [9]. Such orientation predetermines, from which atom the electron will be tunneled to emitter material or which atom will be bound to the surface in the course of chemisorption, the latter being important for direction and steriochemistry of field-induced reactions [9,10]. To change this orientation high thermal excitation energy must be conveyed to the molecule, causing its rotation and due to this virtual appearance of new pathways of reaction and new peaks in the spectrum [10]. The most favorable orientation of thiotepa molecule (with optimized calculated geometry) near the FI emitter surface is shown at Fig. 1b, the vector of its dipole moment $\vec{\mu}$ being parallel with the $\vec{F}$ vector. Such orientation and the highest yield of molecular orbitals of sulfur to ionization potential V_i favors greatly the tunnel loss of electron namely from S atom. This event may be accompanied by chemisorption of the molecule on the emitter surface by sulfur atom via mechanisms described in references [9] and [17]. Namely the latter process is responsible for the main distinctions of FI spectra from those obtained in other ionization modes, as a chemisorbed (and thus immobilized) molecule may be readily involved in field induced bond-breaking and bond-forming reactions, absent in other methods. E.g. the loss of the second electron from the sulfur of such chemisorbed particle leads to formation of a double charged molecular ion M^{2+}, m/z 98.5; the weakening of P–S bond during this process causes the fragmentation with formation of the fragment $[M-S]^{2+}$, m/z 78.5 (sulfur remains bound to the surface) [6]. One more ion specific for FI only is $[2M-S]^{2+}$, m/z 173, which is formed via field polymerization mechanism [9] as follows: under the high field conditions the polarization of the molecule is enhanced and rearrangement of charges takes place in the chemisorbed particle; positive charge tends to be shifted further from the positive emitter, in the present case to phosphorus atom which becomes a site for the substitution of the sulfur atom of the second molecule, resulting in the formation of a covalent dimer. The loss of the second electron and fragmentation analogous to that of the monomer produces the mentioned $[2M-S]^{2+}$ ion. The aim of such detailed discussion of the field induced reactions was to show and to prove, that for sulfur atom there are two pathways of interactions: its involvement both in chemisorption and dimerization favorable from steric and probably energy points of view. One may suggest that these processes are competitive to binding with proton donor centers, if any, on the emitter surface, and are much more favorable in the employed FI conditions. As nitrogen atoms are shielded by hydrophobic groups in 'vacuum' geometry, there are no more accessible proton acceptor centers left in the thiotepa molecule, which is the cause of the absence of its protonation in FI experiments.

In contrast to this, if the conformation characteristic of crystal would be realized, the reactions of proton transfer with involvement of lone pairs of any of nitrogen atoms could occur, but we did not observe this in experiment. In addition, a chemisorption via nitrogens might happen, which may lead to decay of P–N bonds and substantial increase of the corresponding $[M-N\triangleleft]^{+}$ fragment ion, which also was not observed. Thus we have the right to conclude, that namely the calculated optimized geometry of thiotepa molecule is the main conformation (regarding also the free rotation of ethyleneimine rings) in vacuum conditions and agrees well with FI data.

To obtain some additional confirmation to this conclusion, a special experiment was performed to stimulate intermolecular interactions in the excess of proton or deuterium donor agents, such as H_2O and D_2O. A technique developed earlier for the model studies of hydration of nucleic acid constituents [11–13] was employed. Many of organic hetheroatom containing compounds produce protonated species $[MH]^+$ due to pair interactions even with background water [9], and with its excess a series of hydrated ions $M \cdot (H_2O)_n \cdot H^+$ (usually $n = 0, 1, 2$ at room temperature) may be observed [11,12,18]. In our case for the mixtures of thiotepa with either H_2O or D_2O neither protonated (deuterated) nor hydrated ions were observed; the intensities of the peaks with m/z 190 and 191 corresponded exactly to the isotope distribution (mainly due to C and S isotopes) of pure compound [4]. These results confirm, that there are no interactions of thiotepa with water in normal FI conditions and provide support for the calculated geometry of thiotepa molecule. They also demonstrate, that interactions of this kind have a competitive character, and, evidently, a chemisorption of thiotepa molecules on the clean areas of the emitter surface and homoassociation are more favored than the interactions with water or proton donor groups virtually present on a surface.

However, in our further experiments we managed to create very special conditions, in which protonated or deuterated thiotepa species are produced. Those corresponded to the formation of a film on the emitter surface, consisting of several layers of substances; in the case of water in accord with [9,19] it represents a quasi-liquid state. The formation of the films is a generally unwanted process, as it indicates on the violation of equilibrium conditions, when balance between the delivered, ionized and removed particles is shifted to adsorption of incoming molecules due to insufficient field strength and excessive flows of substances. The beginning of such processes is immediately observed by the changes in spectra pattern and peak shapes: the absolute intensities fall due to field shielding, the peaks become unstable and broadened. The film may be easily removed by emitter heating, which cleans the surface and restores the 'gas-phase' character of the spectra. The described conditions in our case were achieved by lowering of field strength for 15–20 min interval

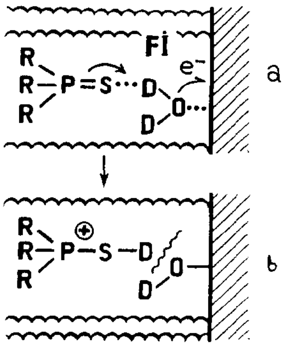
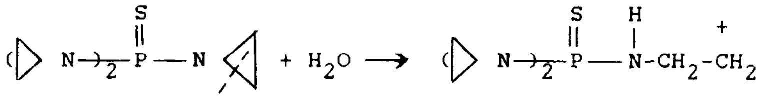


Fig. 2. Schematic representation of the insertion of the thiotepa molecule into the thin film of heavy water molecules on the FI emitter surface (a) and possible mechanism of its deuteration under such conditions (b).

and increasing of water molecules flux, after which the distorted spectrum was emerging containing along with $[M]^{+\cdot}$ also unstable $[MH]^+$, m/z 190 or $[MD]^+$, m/z 191 ions in the cases of common and heavy water respectively with relative intensities being approximately 20–60% of that of the molecular ion and the character of peaks being unstable due to the above discussed reasons. This change was reversible and, as it was mentioned, the heating of the emitter allowed to regain the original character of the spectra. A model of such processes in a film is presented in Fig. 2. As the emitter surface (or at least its certain areas) is covered with a layer of water molecules, thiotepa cannot reach the surface directly. In this case its indirect ionization may occur via an electron transfer through the chain of hydrogen bonds (Fig. 2a). The shift of ionization zone away from the emitter surface and different distances of ionization (measured by dimensions of several water molecules) are responsible for the broadening of the peaks. If we have assumed, that in normal conditions the interactions of thiotepa with emitter surface are preferential, in the described circumstances its molecules are forced to have contact with water, and so hydrogen or deuterium transfer to sulfur may occur by standard hydration mechanism (Fig. 2b). Regarding the film as a model of the bulk water it may be concluded that the behavior of thiotepa molecule in water solution will differ from that in the gas phase conditions of FI experiment, mainly due to contact with many water molecules simultaneously.

Notice that one more mechanism of hydrogen transfer in liquids under normal chemical conditions, which is responsible for the alkylating activity of thiotepa, consists in the catalytic opening of an aziridine ring with formation of carbocation [1]:



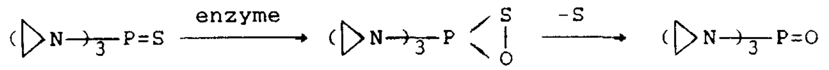
$$(\triangleright N)_2\overset{\overset{\displaystyle S}{\|}}{P}-N\triangleleft + H_2O \longrightarrow (\triangleright N)_2\overset{\overset{\displaystyle S}{\|}}{P}-\overset{\overset{\displaystyle H}{|}}{N}-CH_2-\overset{+}{C}H_2 + OH^- \quad (1)$$

As the masses of the products of reaction (1) and the process proposed in Fig. 2b coincide, we can say nothing about the relative abundances of each pathway in mass spectrometric conditions.

The realization of the reaction (1) requires the access of water molecule to nitrogen; the above discussed free rotation of the rings may greatly facilitate the accessibility of the nitrogens to proton attack. This example reveals the role of bulk water environment for the plausible protonation and indicates, that any theoretical calculations of the thiotepa reactions in the approximation of pair interactions would not adequately reflect its reactivity in solution.

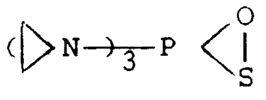
It is of interest, that in matrix-assisted method FAB, the molecular ion appears in the protonated form $[MH]^+$. The mechanisms of formation of FAB mass spectrum of thiotepa, as is discussed in [5], are not so simple, but the role of liquid surrounding of thiotepa in glycerol matrix (close in some properties to water) in formation of protonated species is evident. One more peculiarity of FAB mass spectrum of thiotepa is the so called effect of suppression of any, including matrix, signals, except of those connected with thiotepa [5]. The suppression effect in FAB in general may have several causes, the main of which is the surface activity of an analyte. In the case of thiotepa the explanation may be also found in the structure of its molecule: there are two distinctly pronounced and sufficiently separated hydrophilic (P=S) and hydrophobic (three $(-CH_2-CH_2-)$) groups which may induce to thiotepa the properties of surfactant for which suppression effect is characteristic [20] due to the filling and shielding of the matrix surface.

It was established earlier that in various processes of thiotepa transformation there exist multiple pathways of possible reactions, e.g. it was established, that in conditions of acidic hydrolysis in vitro or metabolism in vivo it gives at least half a dozen of different products. So, continuing the variation of experimental conditions, one more type of thiotepa reaction in the layer on the emitter surface may be obtained under FD. Here it should be pointed out, that the direct observation of chemical reactions induced by different ionizing agents in the conditions of mass spectrometric experiment attracts much attention in recent time [21,22], firstly as a model of the real processes and secondly as a source of new substances, which are not formed under normal conditions (the triumph of the last approach being on the news is the discovery of fullerenes [23]). In our case, under the conditions of FD, thiotepa sample (dried droplet of water solution) on the FD emitter was heated to 30–40°C at moderate field strength for 30 minutes. It was observed, that during this period certain reactions had occurred, and on the background of pure thiotepa spectrum a new peak with m/z 173 appeared. It is necessary to explain, that at m/z 173 value a small peak corresponding to doubly charged ion $[M-S]^{2+}$ ($z = 2$) is present in the thiotepa original spectrum [4–6], and its double charged character is elucidated from the peaks width and isotope at a fractional mass number. Newly appeared m/z 173^+ is much more intensive, absorbing 173^{2+}, and have parameters characteristic of singly charged ion ($z = 1$). This m/z value corresponds to one of the thioTEPA derivatives, named TEPA, in which sulfur is substituted by oxygen, and which was observed earlier as a product of thiotepa hydrolysis in vitro [4] and a product of its biotransformation in the living organism [24,25]. It is known, that some of sulfur-containing drugs undergo biotransformation on the microsomal ferments in liver by the oxidative desulfuration [26]. The scheme of such metabolism for thiotepa through the intermediate was proposed in [27]:



(2)

We consider that such a way of reaction is realized in the sample layer on FD emitter in several steps of bond-forming and bond-breaking reactions in the aggregate with remains of water, analogous to the ones presented in Fig. 2, or chemisorbed oxygen. Electric field plays a governing role in this process, as direct heating of thiotepa crystals in an evaporator for FI never causes such effect. Tentatively drawing correlations of FI mass spectrometric data with possible biological effects we usually precede from the estimation, that the field strength near the emitter surface is of the same order as that in the vicinity of ions or comparable with membrane potential [28]. On the basis of the described thiotepa behavior in the FD conditions we can make an assumption concerning its metabolism in the organism, namely, that the certain ions or ionic centers on the enzyme active site must play a substantial role in the biotransformation of thiotepa. Further efforts are necessary to isolate and confirm the existence of the intermediate of the substitution reaction. It is of interest, that in the model studies of thiotepa hydrolysis in vitro [4], in one of the fractions of chromatography of the mixture of hydrolysis products an uninterpreted ion with m/z 205 was found, which mass corresponded to the predicted intermediate of TEPA formation, but on the basis of our low resolution data we could not insist on the structure.



The latter example shows the efficiency of FI in the modeling of field-governed reactions which may be characteristic of the living cell under certain conditions, and this may form the basis for formation of a new approach in nanobiological model studies.

4. Conclusions

The main conclusion which may be derived from the revealed divergence between the calculated geometry and electronic parameters of thiotepa molecule in condensed (crystal) and isolated (vacuum) forms is, that one should not use parameters of crystalline conformation in the search of the structure–activity links for this drug. Of course, structure–activity correlation as any theoretical approach has a certain degree of approximations and definite limits of applicability, but gives good results for many classes of substances. However, thiotepa definitely drops out from this assemblage due to inherent variety of reactions pathways, strongly dependent on the external conditions.

The results of the performed mass spectrometric experiments showed that FI mass spectrometry may serve as a good probe for investigation of thiotepa properties. The observed peculiarities of thiotepa behavior under the varied mass spectrometric modes confirm the high sensitivity of this molecule to the changes of external conditions, which may be a basis of its high biological activity and specific recognition processes. The existence of several pathways of the reactions of thiotepa dependent on environment is distinctly illustrated by the modeling of its interactions with water. The disclosed absence of thiotepa hydration or even protonation by water molecules in the gas phase urges one from applying the approach of pair interactions for estimation of reactivity of the drug. Virtually, Monte Carlo simulation of small water cluster would be more appropriate, as when thiotepa is forced to have contacts with many of the water molecules in a film created on the FI emitter surface, the protonation characteristic of solution do take place. On the other hand, the deficit of water molecules may correspond to certain conditions in the living cell, e.g. when the drug approaches the surface of specific enzyme, in which case metabolic reaction of substitution of sulfur for oxygen takes place; the existence of charged sites are assumed to facilitate the process.

The data of quantum mechanical calculations allowed also to explain some peculiarities of mass spectra of thiotepa. In particular, the direction of its dipole moment parallel with P=S bond and the greatest yield of atomic orbitals of sulfur to the energy of the highest occupied molecular orbital allowed to determine the orientation of the molecule in the high electric field and assume the tunnel loss of electron from sulfur atom in FI process.

The further advancement of theoretical studies will be connected with modeling of high field effects on the structure, charge distribution and reactivity of thiotepa molecule.

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