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Original Scientific Paper

Carcinogenicity of Styrene Oxide: Calculation of Chemical Reactivity*

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Abstract. In this article the calculations of the activation free energy for a chemical reaction between styrene-7,8-oxide and DNA, in particular guanine at position N7, are reported. Calculations were performed by Hartree-Fock and DFT methods in conjunction with flexible basis sets. Effects of solvation were considered using the Langevin dipoles method. The calculated activation free energies are in good agreement with the experimental value of 26.52 kcal mol^{-1}.

Keywords: Hartree-Fock and DFT calculations, styrene oxide, chemical reactivity

INTRODUCTION

Carcinogenesis is a complex pathological process, where normal cells become neoplastic. Mainly is the process associated with chemical modification of DNA. Chemical reactions of DNA are associated with viruses, photochemical processes and reactive chemicals.[1,2,3,4] If the chemicals are hormones or their metabolites, then they can be referred either as endogenous[5,6] or exogenous carcinogens.[7] If they come from the environment they are referred to as exogenous carcinogens.

Styrene is widely used in the chemical industry in particular in the production of unsaturated polyesters and polystyrene. Having entered the human body either via respiration or absorption through skin, styrene is metabolized into styrene-7,8-oxide primarily in the liver and to a lesser extent in kidney, intestine and lung. The main metabolic pathway for styrene is oxidation mainly via CYP 2E1 a member of cytochrome P450 mixed-function oxydase system (to styrene-7,8-oxide (STO), followed by rapid enzymatic hydration to styrene glycol or conjugation with glutathione. The styrene glycol is oxidized to mandelic acid, which is excreted in the urine. Further oxidation of mandelic acid also occurs, resulting in phenylglyoxylic acid, which is also excreted in the urine. For very recent and often updated information concerning the metabolism of styrene see the web page.[8]

STO is a colorless to light yellowish liquid. It is unstable and it polymerizes with compounds like acids and alcohols. STO is a direct alkylating agent, which can react with nucleophilic sites in DNA in particular with guanine at position N7. Therefore, it can be mutagenic, cytotoxic and carcinogenic.[9,10] Alkylation is followed by other reactions, of which depurination is a typical example. At this point it is worth to emphasize that both epoxidation and depurination are fast relative to DNA alkylation that represents the rate limiting step. Simultaneously STO degradation takes place by its hydrolysis to styrene glycol and its reaction with glutathione.[8]

STO has been detected in blood of workers exposed to styrene in the reinforced plastics industry**,** whose biomaterials showed also genotoxic effect induced by STO (*e.g.* adducts in hemoglobin and DNA, DNA single strand breaks/alkali labile sites) as reviewed by Henderson and Speit.[11] Positive genotoxic results were associated with higher overall STO levels and negative results with decreasing exposures to styrene, but several epidemiologic studies could not confirm a correlation between styrene exposure and increased incidence of cancer in human.[12–15] On a contrary, STO was found to be carcinogenic in rats and mice. If rats and mice receive styrene by oral gavage[16] or inhalation,[17] they develop benign and malignant tumors of breast and forestomach.[18] In addition, a higher incidence of hepatocellular tumors was found in male mice.[18]

* Dedicated to Professor Zvonimir Maksić on the occasion of his 70th birthday.
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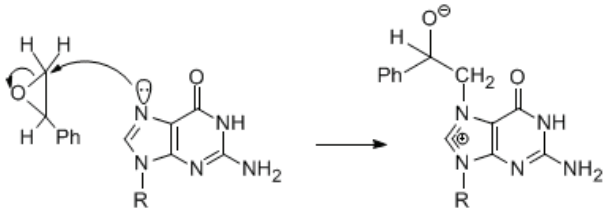

Scheme 1. Guanine alkylation by styrene-7,8-oxide. R stands for the rest of DNA that was in our calculations truncated to methyl group.

So, there is inadequate evidence in human for the carcinogenicity of STO, whereas there is sufficient evidence for the carcinogenicity of STO in experimental animals. Overall evaluation: styrene is classified as a possible carcinogenic compound according to International Agency for Research on Cancer classification (IARC) – group 2B, whereas its main metabolite styrene-7,8-oxide is classified as probably carcinogenic to humans (IARC – group 2A).[19]

As previously mentioned, the N7 of guanine is the major site of STO alkylation. It is well established that the rate limiting step for reaction of the ultimate carcinogens of the epoxy type with the nucleophilic sites of DNA and proteins is the epoxide ring opening.[20] The intermediate picks up the proton from the protein rich aqueous environment and this step is believed to be fast.[21]

The kinetics of guanine alkylation was studied experimentally,[22] and from the rate constant determined free energy of activation was 26.52 kcal mol^{-1}. The free energy of activation was calculated from the rate constant using the transition state formula.

$$k = \frac{k_B T}{h} \cdot e^{-\frac{\Delta G^{\neq}}{k_B T}} \quad (1)$$

In the above equation k_B represents Boltzmann constant, h Planck constant, and T the absolute temperature. Transition state theory is based on the assumption that reactants and transition states form a thermal equilibrium. The reaction rate constant has been determined in a whole blood solution. HPLC in conjunction with UV spectroscopy was used to measure the time-dependent concentrations of adducts. Realistic simulation of chemical reactivity of nucleic acids in aqueous solution is a challenge for computational chemistry. Schrader and Linscheid demonstrated that in addition to N7 guanine adduct STO forms adduct also with O6 and N2 of guanine and nucleophilic sites of adenine.[10] It remains a major challenge to model those reactions in particular the factors controlling the selectivity.

In this study, we calculated activation free energy for alkylation of guanine by styrene-7,8-oxide and compared it to the experimental free energy of activation. We applied Hartree-Fock and DFT methodology in conjunction with flexible basis sets. The effects of solvation were included by using Langevin dipoles method of Florian and Warshel.[23]

The organization of this article is as follows. The applied computational methods are described in section 2, results are collected in section 3, and discussion is in section 4.

COMPUTATIONAL METHODS

For calculation of the Born-Oppenheimer hypersurface and consequently the rate constant for the reaction between STO and guanine, we performed *ab initio* Hartree-Fock and DFT calculations. The distance between the β-carbon atom of the STO linked to N7 of guanine was chosen to be the reaction coordinate. We optimized all degrees of freedom except the fixed value of the reaction coordinate for each calculation; the highest energy point on this path represents the approximation of the transition state. For the reactants a full geometry optimization was performed. The transition state structure was refined by the methodology built in Gaussian-03.[24] For optimization of the transition state the keywords opt=(ts,noeigentest,calcfc) were used. Geometries and atomic charges for reactants and transition states can be obtained from us on request. The difference between energy of the transition state and the reactants is activation energy. For reactants and transition state we performed vibrational analysis in the harmonic approximation. For reactants all frequencies were real, while the transition state had one imaginary frequency predicted by all levels of theory.

Calculation of the Born-Oppenheimer surface for chemical reactions is not a trivial task. It is generally believed that one needs relatively flexible basis sets and inclusion of electron correlation. Our calculations were performed on the Hartree-Fock level in conjunction with the following basis sets 6-31G(d), 6-31+G(d,p) and 6-311++G(d,p). Calculations beyond the Hartree-Fock level (*e.g.* MP2) were not possible because of large size of the system. Therefore we considered the DFT method B3LYP that has exchange functional introduced by Becke[25] and correlation functional introduced by Lee, Yang and Parr[26] in conjunction with the same basis set. In addition, we applied the semiempirical MO method PM3. The latter method we applied because of its low CPU cost, which allows for QM/MM applications and thermal averaging. We are aware that DFT methods

Table 1. Calculated energies of activation for reaction between styrene-7,8-oxide and guanine using different methods

Method	$\Delta E^{\#(a)}$ / kcal mol^{-1}	$ZPE(\mathrm{TS})^{(b)}$ / kcal mol^{-1}	$ZPE(\mathrm{R})^{(c)}$ / kcal mol^{-1}	$\Delta ZPE^{(d)}$ / kcal mol^{-1}	$\omega^{(e)}$ / i cm^{-1}
HF/6-31G(d)	48.73	192.30	192.58	−0.28	568
HF/6-31+G(d,p)	46.10	191.14	191.46	−0.32	562
HF/6-311++G(d,p)	46.11	191.14	191.45	−0.31	562
B3LYP/6-31G(d)	36.20	178.47	178.75	−0.28	468
B3LYP/6-31+G(d,p)	33.86	177.62	177.88	−0.26	447
B3LYP/6-311++G(d,p)	33.83	177.61	177.89	−0.28	447
PM3	49.07	173.08	174.08	−1.00	727

(a) Classical activation energy.
(b) Zero point vibrational energy for the transition state.
(c) Zero point vibrational energy for the reactants.
(d) Zero point energy of the transition state minus zero point energy of the reactants.
(e) Imaginary frequency value corresponding to the transition state.

Table 2. Calculated free energies of hydration using Langevin dipoles method and calculated free energies of activation

Method	$\Delta G_{\mathrm{hydr}}^{\mathrm{LD}}(\mathrm{TS})^{(a)}$ / kcal mol^{-1}	$\Delta G_{\mathrm{hydr}}^{\mathrm{LD}}(\mathrm{R})^{(b)}$ / kcal mol^{-1}	$\Delta G_{\mathrm{hydr}}^{\mathrm{LD}}(\mathrm{TS-R})^{(c)}$ / kcal mol^{-1}	$\Delta G_{\mathrm{LD}}^{\#(d)}$ / kcal mol^{-1}
HF/6-31G(d)	−40.25	−22.84	−17.41	31.04
HF/6-31+G(d,p)	−41.84	−23.63	−18.21	27.57
HF/6-311++G(d,p)	−41.71	−23.56	−18.15	27.65
B3LYP/6-31G(d)	−31.79	−20.14	−11.65	24.27
B3LYP/6-31+G(d,p)	−35.99	−21.44	−14.55	19.05
B3LYP/6-311++G(d,p)	−35.84	−21.41	−14.43	19.12

(a) Free energy of hydration for the transition state.
(b) Free energy of hydration for the reactants.
(c) Free energy of hydration of the transition state minus free energy of hydration of the reactants.
(d) Free energy of activation obtained by Langevin dipoles method. Zero point correction is considered.
$\Delta G^{\#}$(experimental) = 26.52 kcal mol^{-1}, $k_{\mathrm{r}} = 1.97 \times 10^{-7}$ mol^{-1} s^{-1}.

have also significant empirical character, nevertheless they include to some extent electron correlation.

Free energy of hydration for reactants and transition state was calculated using Langevin dipoles (LD) method parameterized by Florian and Warshel.[23] For LD calculations, Merz-Kollman atomic charges were determined for each level of theory. *Ab initio* and semiempirical MO calculations were performed by Gaussian-03 suite of programs,[24] while the LD calculations were obtained using the LD program ChemSol 2.1.[23] Calculations were performed on a cluster of Linux workstations with Intel Pentium 2 GHz processors. We estimated that about 50 days of single processor CPU time was used.

RESULTS

The calculated activation energies, zero point energies and data for imaginary frequencies are collected in Table 1. Langevin dipoles calculated free energies of hydration are collected in Table 2 that includes also calculated free energy of activation.

From Table 1 it is evident that for Hartree-Fock calculations of the barrier height we achieved convergence in terms of the basis set size. It looks like that addition of diffuse function on heavy atoms and polarization functions on both heavy atoms and hydrogens is crucial for prediction of the reaction barrier. The predicted Hartree-Fock level barriers are between 46.10 and 48.73 kcal mol^{-1}.

Application of DFT significantly reduces the barrier. Again the barrier does not change anymore with addition of basis functions when polarization functions are used on heavy atoms and hydrogens, while diffuse functions are only on heavy atoms.

Semiempirical MO method PM3 yield the barrier comparable to HF level and seems to promising for QM/MM calculations that require thermal averaging.

It was demonstrated that the PM3 method performs well for energetics associated with the reaction catalyzed by xylose isomerase.[27] Table 1 also shows that the zero point vibrational energy correction of the reaction barrier is almost negligible. In addition, one can see from the Table 1, that the DFT calculated BO surfaces are shallower than the HF calculated surfaces what is reflected in lower zero point vibrational energy values.

In Table 2 are collected the hydration free energies calculated with Langevin dipoles. Reduction of the barrier relative to the corresponding *in vacuo* values is due to the zwitterionic nature of the transition state.

The HF calculated activation free energies together with more flexible basis sets in conjunction with the LD free energies of hydration are in almost perfect agreement with the experiment. The corresponding B3LYP barriers are too low.

DISCUSSION

In this article we studied a chemical reaction between the probable ultimate carcinogen styrene-7,8 oxide and guanine. The structures of the transition state and reactants, calculated on the B3LYP/6-311++G(d,p) level are shown in Figure 1. DNA was truncated to methylated guanine, where the sugar moiety is mimicked by the methyl group.

Styrene-7,8-oxide is biologically relevant *per se* and is also a model compound for numerous larger ultimate carcinogens, like benzpyrene. DNA was truncated to guanine, the chemically relevant part that enters the reaction. The reaction proceeds via mechanism common to all epoxy ultimate carcinogens.

Effects of solvation were calculated using the Langevin dipoles.

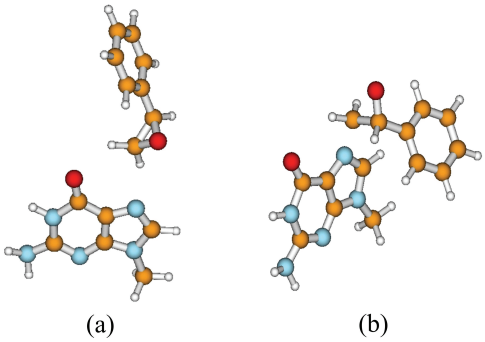

Figure 1. The structures of the reactants (a) and the transition state (b), calculated on the B3LYP/6-311++G(d,p) level for alkylation of guanine with styrene-7,8-oxide. Methyl group of guanine represents sugar moiety.

We demonstrated that Hartree-Fock calculated barrier when combined with Langevin dipoles method for calculation of hydration free energies gives very reasonable agreement with the experimental free energy of activation. On the other hand B3LYP calculations predict too low activation free energy in conjunction with LD solvation model. Disagreement between the experimental and calculated activation free energies can be also explained by considering only part of DNA (guanine) and not treating water and counterions in atomic details.

Guengerich and coworkers demonstrated that alkylation of oligonucleotide guanine proceeds slower than alkylation of DNA guanine (the difference in free energy is 1−2 kcal mol^{-1}),[28] what can be attributed to praeorganized electrostatics ineherent to DNA. The catalytic effect is considerably smaller than in the case of enzymes.[29] To properly understand these effects one has to proceed with advanced quantum chemical calculations and QM/MM in conjunction with free energy calculations. Methodology is developed and ready to be used.[29–36]

Carcinogenesis is a complex biomolecular process involving many chemical reactions.[1] It is therefore a major challenge to understand and model those reactions. We have impression that the B3LYP functional systematically underestimates the reaction barrier. A possible explanation is that there were no epoxy species in the parametrization set. Due to the size of the system we did not manage to perform post Hartree-Fock calculations what remains a challenge for the future. Semiempirical method PM3 performed surprisingly well for this system.

All in all, we found very good agreement between the experimental and calculated free energy of activation for alkylation of guanine by styrene-7,8-oxide by combination of Hartree-Fock calculation using flexible enough basis sets and Langevin dipoles calculation of hydration free energies. Carcinogenicity is a very complicated process. Carcinogenic compounds can be either genotoxic or non-genotoxic. The first step for inducing genotoxic cancerogenicity is the alteration of the cellular DNA by the reactive form of carcinogen. This initial reaction leads to translocation and amplification of specific genes (protooncogenes), which translate into transformation from normal to altered cell. The altered cell may remain dormant or under specific circumstances may proliferate into praeneoplastic and ultimately progress to neoplastic cell.

Beside rate constant for a chemical reaction between the ultimate carcinogen and DNA are important also the reactions of the former with water, proteins and the ultimate carcinogen scavengers such as polyphenols.

Moreover, pharmacodynamics of the ultimate carcinogens including above threshold carcinogen level as well as contact time of the ultimate carcinogen with target organs are also a very important factors for carcinogenicity. Ultimately, we expect that only a complex computer simulation of pharmacodynamics of carcinogens where reactive steps will be included will yield a measure for carcinogenicity and will contribute toward understanding, prevention and treatment of cancer.[1,37,38]

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SAŽETAK

Kancerogenost stiren oxida: proračun kemijske reaktivnosti

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U ovom radu su opisani računi slobodne energije aktivacije za kemijsku reakciju između stiren-7,8-oksida i DNK, posebice gvanina na položaju N7. Računi su provedeni sa Hartree-Fock i DFT metodama uz korištenje fleksibilnih osnovnih skupova. Efekti solvatacije su razmatrani korištenjem Langevin dipoles metode. Izračunate slobodne energije aktivacije su u dobrom slaganju sa eksperimentalnom vrijednoću od 26.52 kcal mol^{-1}.