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PAPER

Mechanism of epoxide hydrolysis in microsolvated nucleotide bases adenine, guanine and cytosine: A DFT study†

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Six water molecules have been used for microsolvation to outline a hydrogen bonded network around complexes of ethylene epoxide with nucleotide bases adenine (**EAw**), guanine (**EGw**) and cytosine (**ECw**). These models have been developed with the MPWB1K-PCM/6-311++G(3df,2p)//MPWB1K/6-31+G(d,p) level of DFT method and calculated S_N2 type ring opening of the epoxide due to amino group of the nucleotide bases, *viz*. the N6 position of adenine, N2 position of guanine and N4 position of cytosine. Activation energy (E_{act}) for the ring opening was found to be 28.06, 28.64, and 28.37 kcal mol^{-1} respectively for **EAw**, **EGw** and **ECw**. If water molecules were not used, the reactions occurred at considerably high value of E_{act}, *viz*. 53.51 kcal mol^{-1} for **EA**, 55.76 kcal mol^{-1} for **EG** and 56.93 kcal mol^{-1} for **EC**. The ring opening led to accumulation of negative charge on the developing alkoxide moiety and the water molecules around the charge localized regions showed strong hydrogen bond interactions to provide stability to the intermediate systems **EAw-1**, **EGw-1** and **ECw-1**. This led to an easy migration of a proton from an activated water molecule to the alkoxide moiety to generate a hydroxide. Almost simultaneously, a proton transfer chain reaction occurred through the hydrogen bonded network of water molecules and resulted in the rupture of one of the N–H bonds of the quaternized amino group. The highest value of E_{act} for the proton transfer step of the reaction was 2.17 kcal mol^{-1} for **EAw**, 2.93 kcal mol^{-1} for **EGw** and 0.02 kcal mol^{-1} for **ECw**. Further, the overall reaction was exothermic by 17.99, 22.49 and 13.18 kcal mol^{-1} for **EAw**, **EGw** and **ECw**, respectively, suggesting that the reaction is irreversible. Based on geometric features of the epoxide–nucleotide base complexes and the energetics, the highest reactivity is assigned for adenine followed by cytosine and guanine. Epoxide-mediated damage of DNA is reported in the literature and the present results suggest that hydrated DNA bases become highly S_N2 active on epoxide systems and the occurrence of such reactions can inflict permanent damage to the DNA.

Introduction

DNA is the major cellular target for chemical carcinogenesis.[1–20] Polycyclic aromatic hydrocarbons (PAHs), derived from the incomplete combustion of organic materials, are potent environmental carcinogens that can form covalent adducts with DNA bases leading to initiation of tumorigenesis.[6,12,21–32] It has been shown that wide range of PAHs are metabolically activated *in vivo* to diol epoxides and the benzylic carbocations generated from these electrophilic diol epoxides by opening of the *O*-protonated epoxide ring are capable of forming covalent adducts with the nucleophilic sites in DNA and RNA, leading to alteration of genetic material.[12,33–38] The major site of covalent adduct formation of benzylic carbon are characterised to be the amino groups of deoxyadenosine (N6) and deoxyguanosine (N2) residues in the DNA. There are some reports of minor adduct formation with the amino group of deoxycytidine and the seventh position of deoxyguanidine.[19,39–46] The reaction mechanism at the amino groups in the DNA is quite different from the usual reaction of DNA alkylating agents which primarily occur at the ring nitrogen atoms in the bases. Reactions leading to both hydrolysis and covalent reaction with DNA occur through formation of a prereaction noncovalent dihydrodiol epoxide–DNA complex. The reactions leading to both hydrolysis and covalent adduct formation with DNA are reported to occur through a noncovalent diol epoxide DNA complex prior to the reaction.[47,48]

The factors that control the binding mechanism of epoxides to nucleotide bases are not very clear. However, it is well established that the rate-limiting step for reaction of the epoxy type carcinogens with the nucleophilic sites of DNA and proteins is the epoxide ring opening.[47–52] The intermediate then picks up

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the proton from the aqueous environment, which is believed to be a fast step. It is likely that electrophilic attack of DNA by epoxides is S_N2-type and proceeds through proton-stabilized transition states in which the hydrocarbon exhibits significant carbenium ion character.

In this work we have performed a model DFT study to elucidate the mechanism of the ring opening of the epoxy system when it interacts with the exocyclic amino group of DNA bases. Ethylene oxide is taken as a simple model for epoxy system and its reaction with adenine, guanine, and cytosine are considered. The mechanism of carcinogenicity of ethylene oxide is not well characterized. This molecule is categorized as "carcinogenic to humans" by International Agency for Research on Cancer (IARC), based largely on studies in experimental animals and limited evidence exists for carcinogenicity in humans. The human evidence for carcinogenicity of ethylene oxide is still being debated due to a lack of clear understanding of the carcinogenic mode of action and limited epidemiological data. It may be noted that the main product of the reaction of ethylene oxide with DNA is *N*7-(2-hydroxyethyl) guanine with O6-(2-hydroxyethyl) guanine and *N*3-(2-hydroxyethyl) adenine as minor adducts produced in smaller amounts.[53–56]

Methods

We propose a proton transfer mechanism for epoxide binding with DNA using a model system consisting of ethylene oxide, a nucleotide base in DNA (adenine, guanine or cytosine) and six water molecules. The six water molecules form a network of hydrogen bonds which surround the weakly bonded epoxide and nucleotide base to provide a microsolvation environment. Full geometry optimization of epoxide–adenine–water (**EAw**), epoxide–guanine–water (**EGw**), and epoxide–cytosine–water (**ECw**) systems, related transition states and intermediates were performed at the MPWB1K/6-31+G(d,p) level of theory as implemented in the Gaussian03 suite of programs.[57] MPWB1K method is a hybrid DFT method with reasonably good performance for thermochemistry, thermochemical kinetics, hydrogen bonding, and weak interactions, and they give excellent saddle point geometries.[58,59] Harmonic frequency calculations were performed on all optimized structures. Transition states were characterized by a single imaginary frequency (first order saddle points) while all frequencies were real for reactants. Further, evaluation of the performance of different DFT methods have been carried out by single point energy calculations using generalized gradient approximation (GGA) functionals PBE[60] and BP86,[61,62] meta-GGA functional TPSS,[63] hybrid-GGA functionals B3LYP[64,65] and PBE0,[66] and hybrid-meta-GGA functional M05.[67] For all these calculations the 6-311++G(3df,2p) basis set is used. Throughout this paper the energetics of the reactions of **EAw**, **EGw** and **ECw** are discussed on the basis of the MPWB1K-PCM/6-311++G(3df,2p)//MPWB1K/6-31+G(d,p) level zero-point energy (ZPE) corrected SCF energies. PCM stands for the polarizable continuum model which uses a self consistent reaction field approximation to incorporate solvation effect.[68,69] For PCM calculation, united atom topological model for Hartree Fock (UAHF) is used for assigning atomic radii.

Results and discussion

(a) EAw, EGw, and ECw

Optimized structures of the microsolvated base-ethylene epoxide systems are shown in Fig. 1. In all cases, the orientation of water molecules are in such a way that hydrogen bond interactions extend between the NH bonds of amino group and oxygen atoms of water molecules. Further, in the lone pair directions of the epoxy oxygen, water molecules form hydrogen bonds. Apart from these, the **EAw**, **EGw**, and **ECw** show hydrogen bonding of the water molecules at (N1 and N7), (N3 and N1H) and N3, respectively (Fig. 1). In all the three cases, epoxide shows weak interaction with the amino nitrogen where the distance of the corresponding N···C interaction is 3.112 in **EAw**, 3.358 in **EGw** and 3.382 in **ECw**. In **EGw** and **ECw**, the epoxide C–C bond is placed directly above the plane of the ring structure of the nucleotide base while in **EAw**, one of the carbon atom of the C–C bond is well outside the face of the ring (Fig. 1). This geometric feature is worth noting because the orientation of the epoxide as found in **EGw** and **ECw** may be difficult to realise in DNA systems due to stacked structure of base pairs whereas the orientation of epoxide in **EAw** may be easy as it will not be affected by stacking of base pairs. The microsolvated structures **EAw**, **EGw**, and **ECw** are expected to be adequate for the present study as they nearly fulfil the most dominant hydration sites. The orientation of water molecules in diol epoxide hydrolysis is well described in a simulation study by Rabinowitz *et al.*[70] They demonstrate that at any time of simulation there were not more than seven water molecules that individually bound to the epoxide derivatives. Orientation of a second water molecule close to the epoxide oxygen is also described in their study. Hence, the initial arrangement of various molecules in the micro solvated base- ethylene epoxide systems is justifiable. The N···C interaction is expected to trigger a nucleophilic attack of the nitrogen lone pair on the C–C bond of the epoxide.

(b) Alkylation of adenine

Alkylation of adenine is analyzed by identifying all the key intermediates and transition states in the reaction (Fig. 2). The close proximity of N6 and epoxide carbon in **EAw** suggests an S_N2 type nucleophilic attack of adenine on the epoxide giving rise to opening up of the epoxide ring. **EAw-TS1** is the transition state located for such a reaction and the required activation energy (E_{act}) is 28.06 kcal mol^{-1}. In **EAw-TS1**, many hydrogen bond lengths are significantly shortened compared to those in **EAw**, suggesting a considerable stabilizing interaction from the water cluster on the zwitterionic transition state. Particularly noteworthy are the two NH···O interactions from the amino group and two OH···O interactions from the epoxide oxygen. In the intermediate product **EAw-1** of this S_N2 reaction, further strengthening of the above mentioned hydrogen bonds are observed. Interestingly, the O···H distance of 1.462 Å involving w1, depicted in Fig. 2 is much shorter than a typical hydrogen bond length and thus indicates a highly activated O–H bond of that water molecule. In transition state **EAw-TS2**, a proton from the activated water molecule w1 is migrated to the negatively charged alkoxide oxygen to produce the alcohol functionality in **EAw-2**. Thus in **EAw-2**, w1 assumes the character of OH^- which is stabilized by interaction from a nearby water molecule w2. In the next step, a proton from w2 migrates

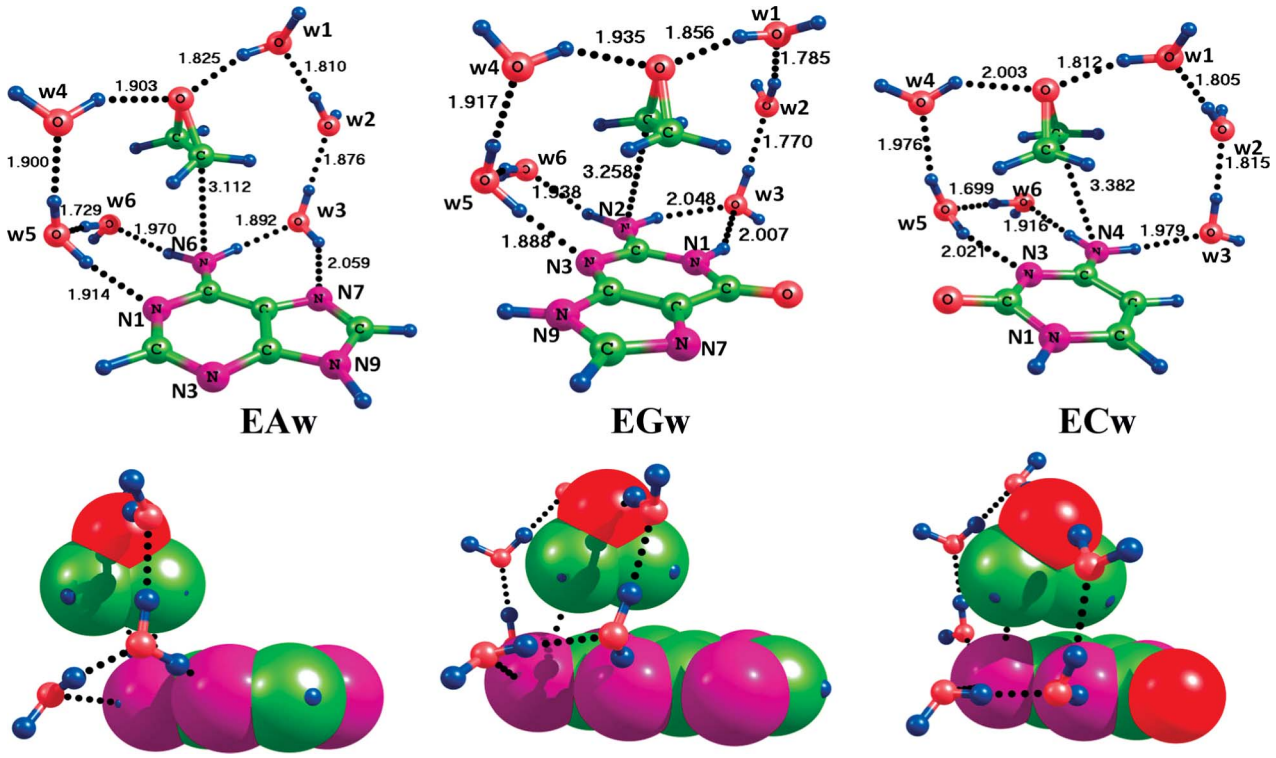

Fig. 1 Optimized geometries of microsolvated nucleotide base-ethylene epoxide systems **EAw**, **EGw**, and **ECw** at MPWB1K/6-31+G(d,p) level. All bond lengths in Å. Pictures at the bottom are side views wherein large spheres are used to represent the base and the epoxide. Nitrogen atoms are numbered according to usual conventions and water molecules are labelled from w1 to w6.

to OH^- and simultaneously w2 accepts a proton from w3 while w3 gets a proton from N6 center. The transition state **EAw-TS3** explains this proton transfer chain reaction and yields the final alkylated hydroxide derivative of adenine (**EAw-3**).

In Fig. 3a, the reaction profile for the alkylation reaction of adenine is depicted. Rate limiting step is the initial S_N2 reaction leading to the epoxide ring opening. The second (**EAw-TS2**) and third (**EAw-TS3**) steps of the reaction are nearly barrier-less (in fact, with ZPE-correction, **EAw-TS2** will disappear because the product **EAw-2** showed higher energy than **EAw-TS2**), suggesting spontaneous formation of the final hydroxide once the reactants gain sufficient energy to reach the transition state of **EAw-TS1** and thus, the reaction can be considered as occurring in one step. The reaction is exothermic by 17.99 kcal mol^{-1} which suggests a substantial stabilization of the product. In other words, the N–C bond formation will permanently damage the nucleotide base as the reverse process is impossible in physiological conditions. If the mediation of water molecules is not sought in the reaction, the ring opening of the epoxide can occur only by absorbing a high amount of energy (53.51 kcal mol^{-1}) and the reaction is highly endothermic.

It may be noted that in the DNA system, the N6–H and N1 positions of adenine are involved in base pair interactions with thymine which means that these positions of the nucleotide base may not be available for a water mediated proton transfer reaction. In the model presented in Fig. 2, the N–H bond interacting with w6 will be the one involved in interactions with thymine. Therefore, the N–H bond interacting with w3 is the right choice for the proton transfer. The calculated reaction model falls in line with this observation as the water molecules w4, w5, and w6 do not undergo major bonding changes while a chain of proton transfer occurs with mediation of w1, w2 and w3.

(c) Alkylation of guanine

In **EGw**, the N2···C interaction distance (3.258 Å) is 0.246 Å longer than that of **EAw**. Further, the nucleophilic attack of guanine on the epoxide *via* **EGw-TS1** is only 0.58 kcal mol^{-1} more energy demanding (E_{act} = 28.64 kcal mol^{-1}; Fig. 5a) than the corresponding reaction of adenine. The zwitterion **EGw-1** forms strong hydrogen bond interactions with water molecules at the alkoxide site and also at the N–H bonds of the quaternized nitrogen atom. In **EGw-1**, the N–H bond connected to w6 shows stronger interaction than the N–H bond connected to w3 whereas the hydrogen bonds of w4 are weaker than those of w1. In the second transition state **EGw-TS2**, the O–H bond of w4 as well as N–H bond connected to w5 are activated which lead to the formation of the alkylated hydroxide product **EGw-2**. Overall, the reaction is exothermic by 22.49 kcal mol^{-1} which is nearly same as the alkylation of **EAw**.

In DNA, the N2–H, N1–H and C=O positions of guanine interact respectively with C=O, N3 and N4–H positions of cytosine and therefore those positions of guanine may be shielded from a proton transfer reaction. It is gratifying that N1–H and N2–H positions interacting with w3 and the connected w2 and w1 do not undergo major changes in bonding (Fig. 4) and thus the model follows a realistic pattern of the proton movement through chain of water molecules w4, w5 and w6. The reaction profile given

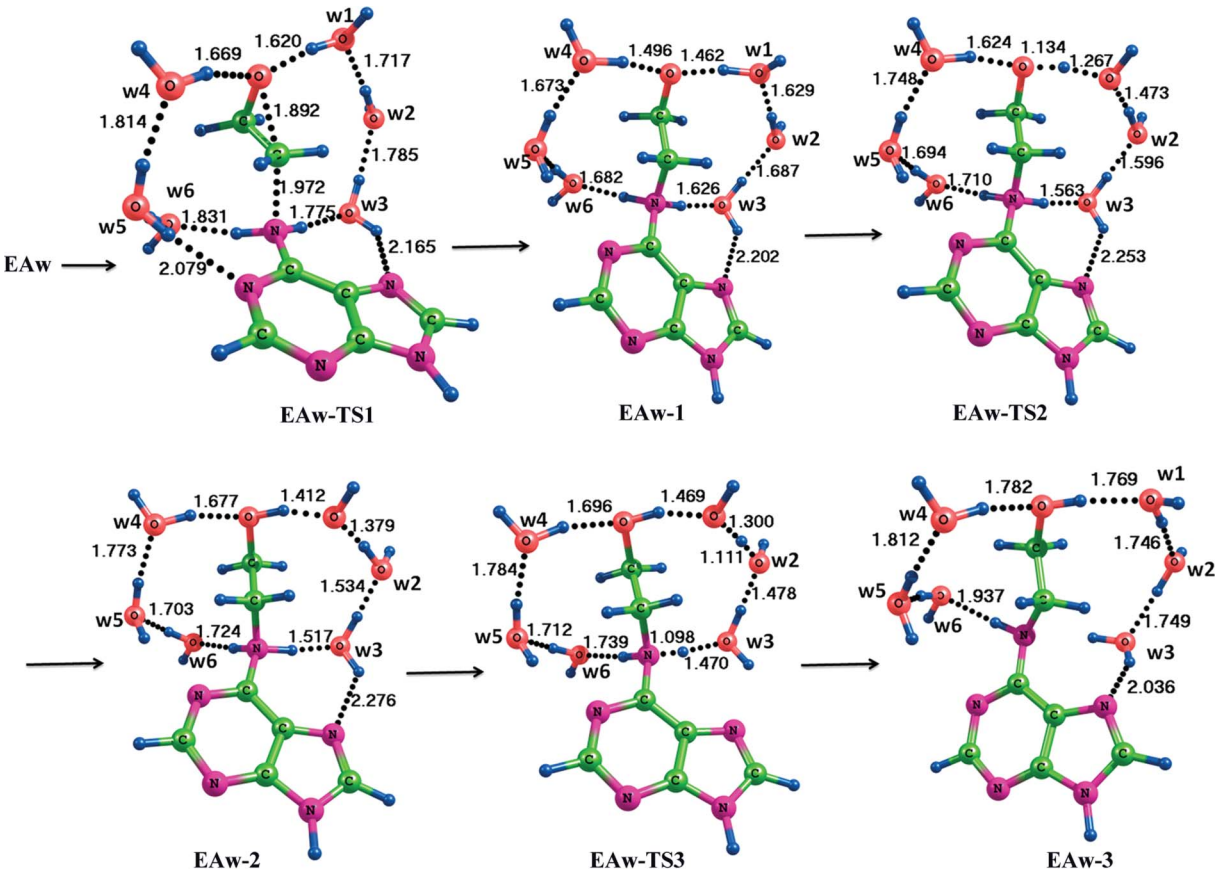

Fig. 2 Reaction of adenine with ethylene oxide. All values in Å (MPWB1K/6-31+G(d,p) level).

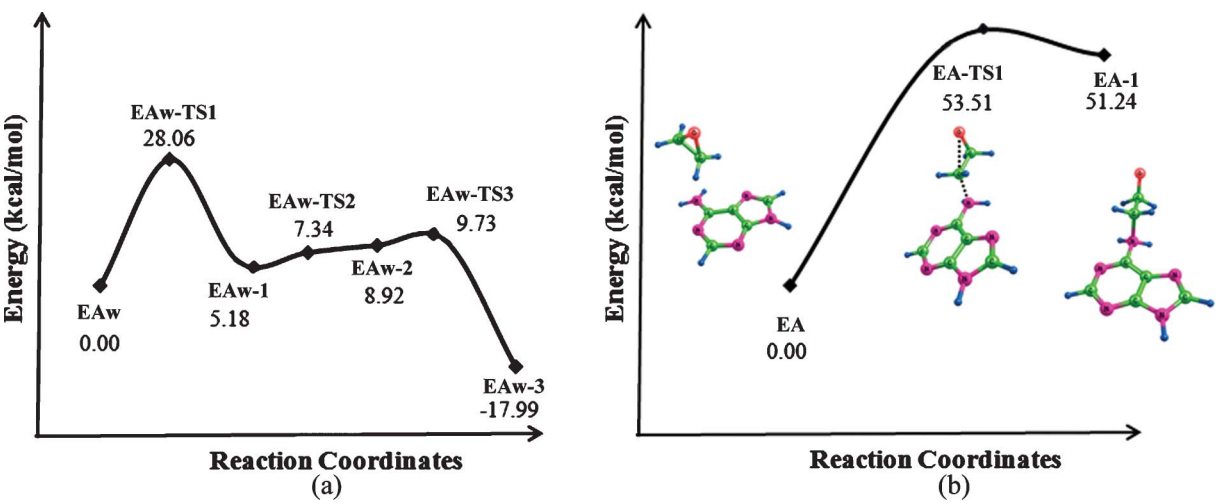

Fig. 3 Energy profile diagram for (a) S_N2 type ring opening of epoxide by nucleotide base in **EAw**, calculated at MPWB1K-PCM/6-311++G(3df,2p)//MPWB1K/6-31+G(d,p) level (b) direct S_N2 type ring opening of epoxide by adenine, calculated at MPWB1K/6-311++G(3df,2p)//MPWB1K/6-31+G(d,p) level. Activation energy (E_{act}) is calculated with respect to the initial pre-reactant complex.

in Fig. 5a clearly suggests that unlike adenine, the water-assisted alkylation of guanine is a two step process. If water mediation is not present, the reaction can occur only at very high temperature as the observed E_{act} for the S_N2 type ring opening (Fig. 5b) is very high (55.76 kcal mol^{-1}) and the alkoxide formed is highly unstable.

(d) Alkylation of cytosine

In the case of **ECw**, S_N2 type nucleophilic attack of the amino group on the epoxide molecule (**ECw-TS1**) requires E_{act} of 28.37 kcal mol^{-1} which is 0.31 kcal mol^{-1} higher and 0.27 kcal mol^{-1} lower compared to adenine and guanine systems, respectively.

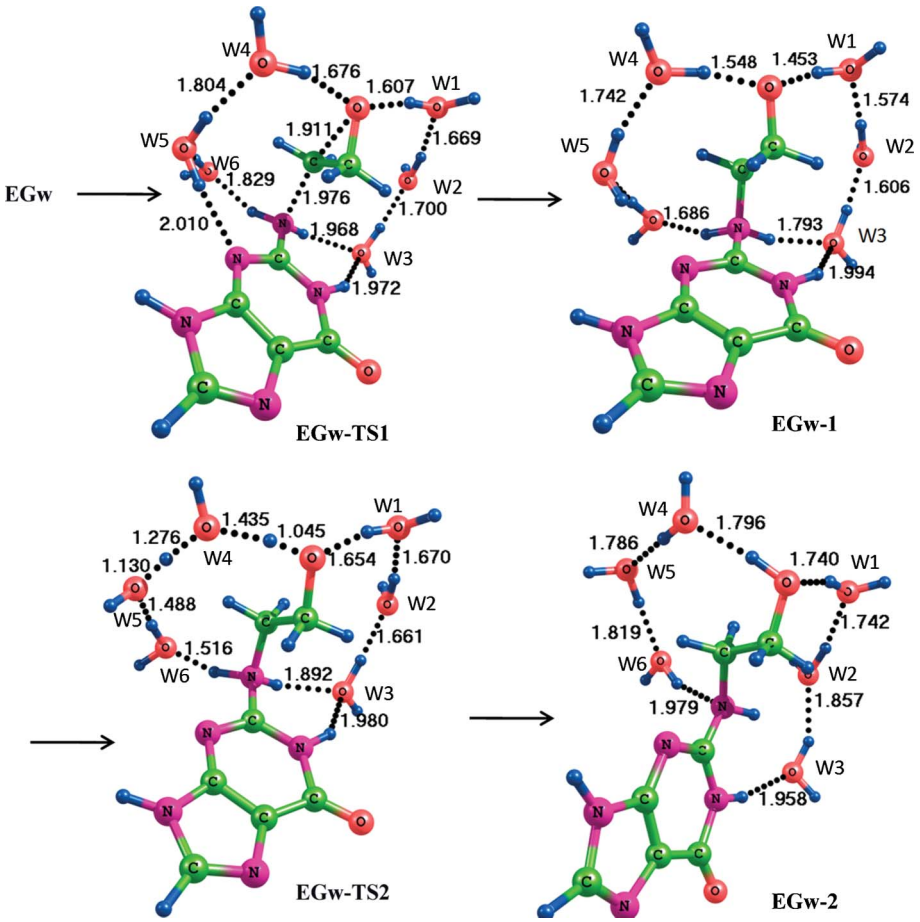

Fig. 4 Reaction of guanine with ethylene oxide. All values in Å (MPWB1K/6-31+G(d,p) level).

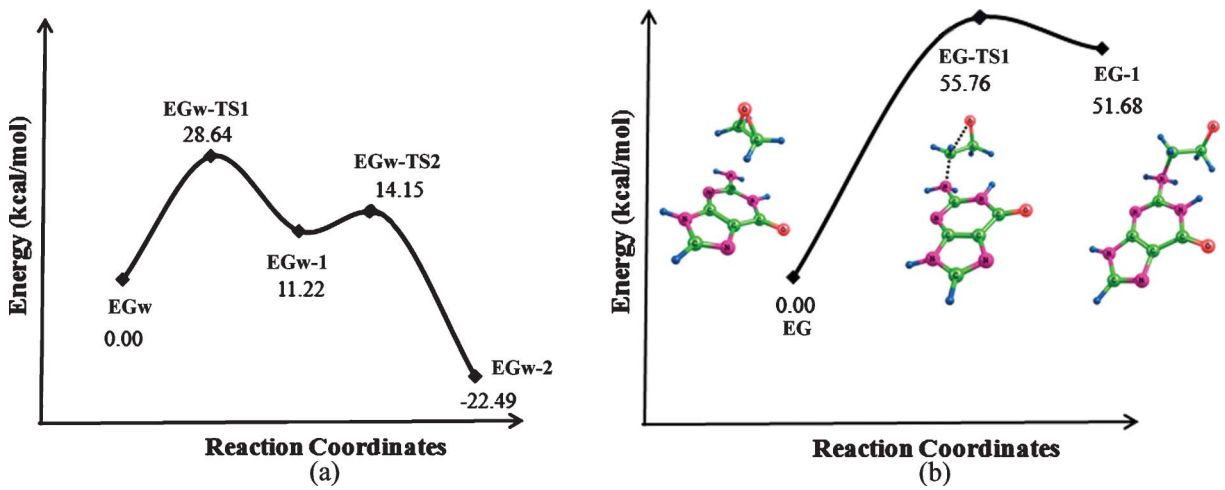

Fig. 5 Energy profile diagram for (a) S_N2 type ring opening of epoxide by nucleotide base in **EGw**, calculated at MPWB1K-PCM/6-311++G(3df,2p)//MPWB1K/6-31+G(d,p) level (b) direct S_N2 type ring opening of epoxide by guanine, calculated at MPWB1K/6-311++G(3df,2p)//MPWB1K/6-31+G(d,p) level. Activation energy (E_{act}) is calculated with respect to the initial pre-reactant complex.

In the intermediate zwitterion **ECw-1**, w1 and w4 form strong hydrogen bonds around the alkoxide moiety while the N–H bonds of the amino group form bonding interactions with w6 and w3. The transition state **ECw-TS2** shows the migration of the proton from w1 to the alkoxide moiety. This proton migration also strengthens the hydrogen bond interactions of w2 and w3. The product of **ECw-TS2** is the alkylated hydroxide **ECw2** which suggests that migration of a proton from w1 to the alkoxide moiety triggers simultaneous transfer of a proton from w2 to w3 and another one from w3 to w2 and a third one from the quaternized nitrogen to w3 (Fig. 6). The energy profile for this reaction is given in Fig. 7a. The reaction can be considered as a one step process

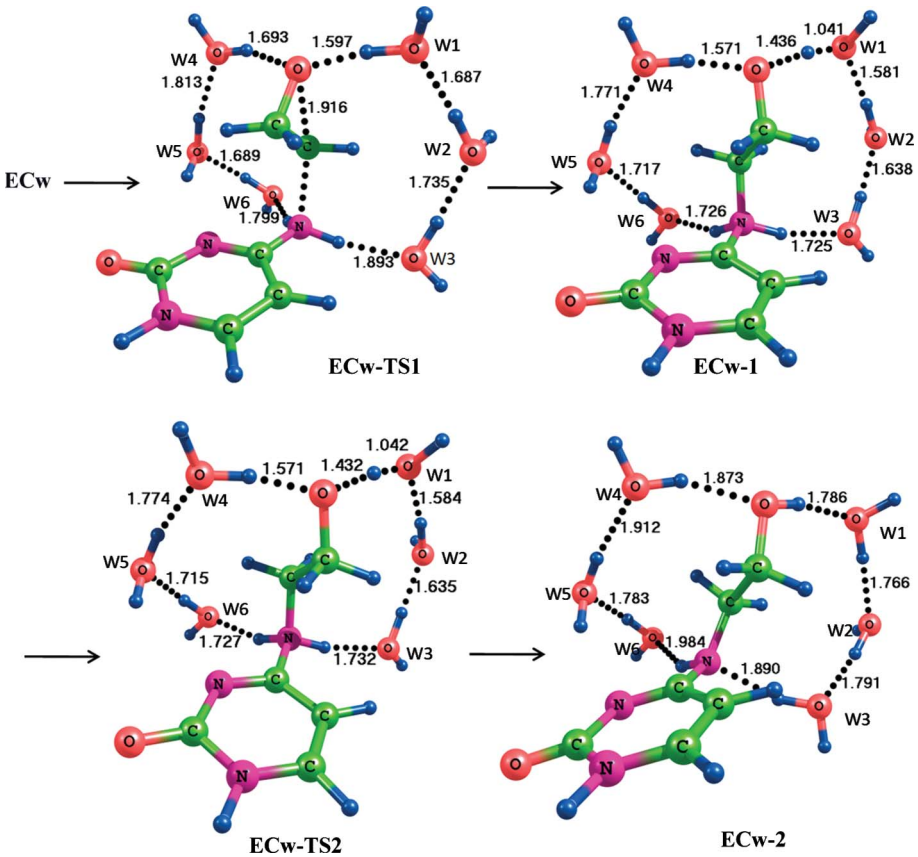

Fig. 6 Reaction of cytosine with ethylene oxide. All values in Å (MPWB1K/6-31+G(d,p) level).

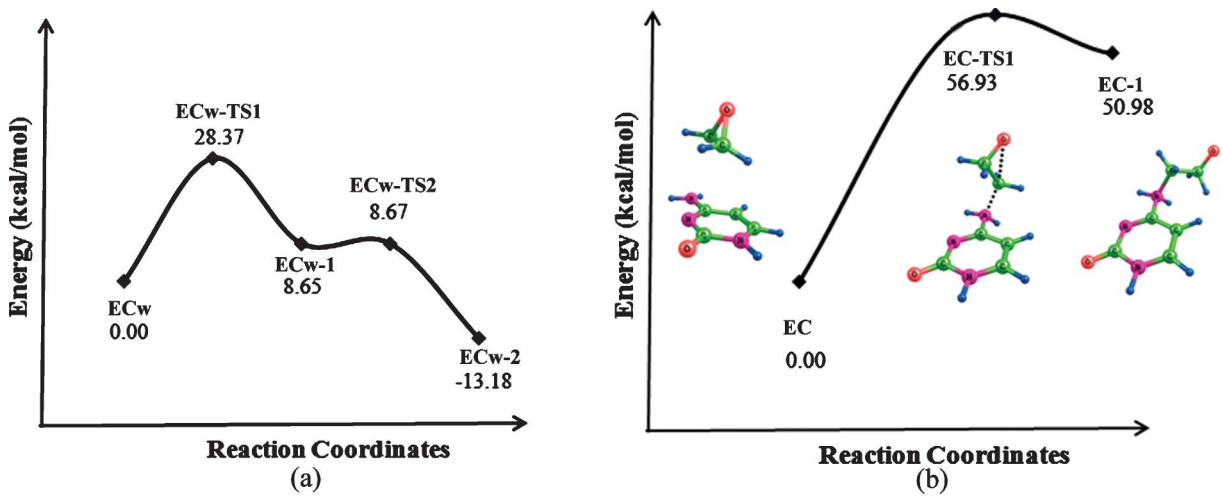

Fig. 7 Energy profile diagram for (a) S_N2 type ring opening of epoxide by nucleotide base in **ECw**, calculated at MPWB1K-PCM/6-311++G(3df,2p)//MPWB1K/6-31+G(d,p) level (b) direct S_N2 type ring opening of epoxide by cytosine, calculated at MPWB1K/6-311++G(3df,2p)//MPWB1K/6-31+G(d,p) level. Activation energy (E_{act}) is calculated with respect to the initial pre-reactant complex.

because the energy required for the chain of proton transfer due to **ECw-1** is barrier less. Overall, the reaction is exothermic by 13.18 kcal mol^{-1} which is significantly lower in value compared to the exothermicity of the adenine (17.99 kcal mol^{-1}) and guanine (22.49 kcal mol^{-1}) reactions. The ring opening of the epoxide is highly unlikely without participation of the water molecules as the bare reaction needs an E_{act} of 56.93 kcal mol^{-1} (Fig. 7b). It may be noted that the N–H bond connected to w6 may be shielded in DNA from hydration as it interacts with guanine. Hence the migration of the proton from the quaternized nitrogen to w3 can be justified as likely process to obtain the final hydroxide product.

Conclusions

The mechanism of water mediated epoxide hydrolysis by DNA bases adenine, guanine and cytosine has been studied using microsolvated structures modelled at MPWB1K-PCM/6-311++G(3df,2p)//MPWB1K/6-31+G(d,p) level of DFT method. The activation barrier for the S_N2 type ring opening of epoxide in the adenine complex **EAw** required the lowest activation energy of 28.06 kcal mol^{-1} which was substantially lower than that of the direct epoxide ring opening by adenine (53.51 kcal mol^{-1}). Similarly, **EGw** and **ECw** required 28.64 and 28.37 kcal mol^{-1} of energies for water mediated ring opening of the epoxide which were also less energy demanding than the direct pathways. Calculations using pure GGA and meta-GGA functionals have showed slightly lower activation barriers compared to hybrid-GGA and hybrid-meta-GGA functionals (s.i). In general, all the DFT methods agreed. Water clusters highly stabilized the transition states in all the three case of the bases and also provided easy pathways for the proton transfer, thus making the overall process feasible under physiological conditions. The epoxide hydrolysis in every case is irreversible as it led to a strong covalent binding of the epoxide to the nucleotide bases, causing permanent damage to the DNA.

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