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Biocatalysis of azidolysis of epoxides: Computational evidences on the role of halohydrin dehalogenase (HheC)

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Abstract. Biocatalytic azidolysis of 9 unsymmetrical epoxides by halohydrin dehalogenase enzyme (HheC) in gas phase and uncatalysed azidolysis of the same epoxides in gas phase and in aqueous solution have been modelled at DFT level. Aliphatic epoxides (1–6) and aromatic epoxides (9) undergo β cleavage while styrene oxide (7) and *p*-nitro styrene (8) oxide prefer α cleavage in the gas phase. Inclusion of aqueous solvation effect via Polarizable Continuum Model (PCM) increases the activation barrier and makes the reaction endothermic due to extensive solvation of azide anion and oxido anionic products, but does not alter the regioselectivity. Halohydrin dehalogenase from Agrobacterium radiobactor ***AD1*** catalyses (E1–E9) ring opening of all these epoxides by azide ion with β selectivity and the reversal of selectivity in epoxide 7 and 8 is notable. These reactions follow, in both enzymatic and non-enzymatic environment, S_N2 mechanism. Calculations while agreeing totally with experimental results offer better insights on the factors determining the regioselectivity and particularly the role of enzyme. Active site model and crystal structure data reveal that the Tyr145 and Ser132 form weak hydrogen bonds with epoxide oxygen lone pair and form reactant enzyme complex (REC). The enzyme complex activates the epoxide ring towards azidolysis. The NBO deletion and second order perturbation analyses clearly bring out the role of catalytic duo Tyr145 and Ser132 and particularly shed light on the dominant contribution of Tyr145 in selectively activating C_β–O bond. The present results indicate that Arg149 or other residues in the pocket do not seem to have any significant effect on the reaction.

Keywords. Halohydrin dehalogenase; azidolysis of epoxide; density functional theory; biocatalysis.

1. Introduction

Halohydrin dehalogenase (HheC) has been shown to exist in a wide variety of organisms from insects to fungi, bacteria, plants and mammals.[1a–e,2] Halohydrin dehalogenase catalyses first the conversion of halohydrin to epoxide and then it catalyses cleavage of epoxide to a more soluble and pharmaceutically interesting compounds like 1, 2-azido alcohols by azide ion.[3,4] These azido alcohols further hydrolyse to give 1, 2 diols. These diols are usually prepared through ring opening of epoxides followed by protonation of the oxyanion by using different azides in suitable solvents. The azidolysis of symmetrical aliphatic epoxides gives stereochemically identical products while unsymmetrical epoxides leads to enantioconvergent yield with S_N2 attack at the least substituted carbon (C_β) atom.[5] In non-enzymatic conditions, the aryl epoxides like styrene oxide form an exception, they prefer α cleavage. This is due to stabilization of positive charge formed at C_α by delocalization into the phenyl ring in the transition state.[6] At the same time the biocatalytic (HheC) azidolysis of epoxide have notable unique selectivity during the course of epoxide ring cleavage process.

Biocatalytic azidolysis of epoxides received considerable attention in view of the biological interests as well as their unique regioselective ring opening.[7a–d] HheC from RADIOBACTER ***AD1***, is one of the enzymes known to catalyse the epoxide ring opening by various small nucleophiles.[8–10] The high regio- and stereoselective nature of HheC catalysed ring opening of epoxides by azide anion created lot of interest focusing on the biocatalytic mechanism.[11–13] These ubiquitous enzymes are vital to the process of biocatalytic organic synthesis. Azidolysis of epoxide by HheC occurs in a two-step via S_N2 mechanism. Cofactors or metal ions are not needed for enzymatic activity. Variety of experimental[11–13] and theoretical[7a–d] studies

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have been reported so far focusing on the biocatalytic conversion of various epoxides by HheC. Recently Fahmi Himo *et al*. have studied[7a] the role of Tyr145 and Ser132 in the regioselective azidolysis of *p*-nitro styrene oxide catalysed by HheC and they have also reported, based on the PBD structure of HheC, that Tyr177, Tyr187, and Leu178 residues make weak interactions with the nucleophile, so that the nucleophile could not approach C_α during the reaction. They have emphasized the role of Arg149 in transferring a proton to Tyr145 during the reaction. These points are re-examined. Further it is not clear from the earlier studies if Tyr145 or Ser132 makes a dominant contribution to the activation, and what causes β selectivity in these catalysed reactions. These questions are mainly addressed in this paper. A broad range of epoxides have been chosen to check the validity of the mechanism and model and azidolysis of all of them have been modelled in both enzymatic and non-enzymatic environment to clearly bring out the role of enzyme on the reaction.

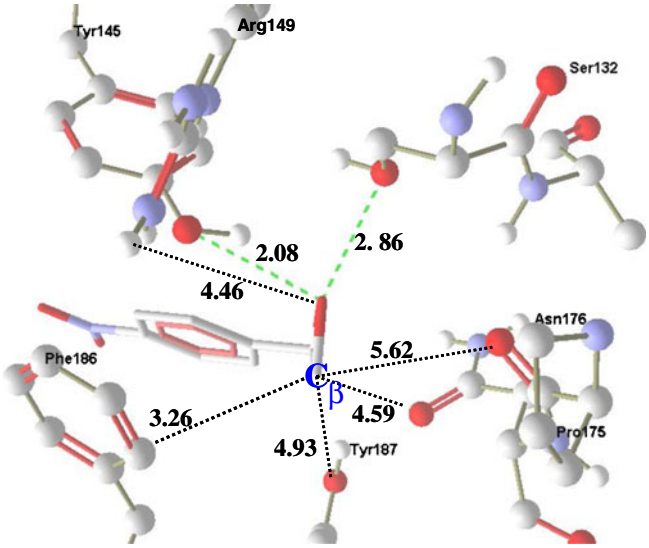


Figure 1. The crystal structure information on the binding of *p*-nitro styrene oxide in HheC pocket (PDB ID: 1ZMT).

2. Computational details

Nine uncatalysed (1–9) and HheC catalysed (E1–E9) azidolysis of epoxides have been considered here. For all of them both α-cleavage and β-cleavage paths have been traced. All species on the potential energy surfaces of the uncatalysed reactions have been optimized at B3LYP/6-31+G (2d, p). Species on the PES have been characterized by harmonic analysis and IRC calculations have been performed to confirm transition states. The free energies have been calculated using thermochemistry in Gaussian 03.[14] The relative free energy calculation of enzyme catalysed azidolysis, the enzyme reactant complex (REC) has taken as reactant. Reactants and products have all real frequencies and transition states have one imaginary frequency. Solvent effect on the barrier and reaction free energies have been computed using PCM model with water as solvent ($\varepsilon = 78.39$).

2.1 *Active site model*

The active site for the HheC catalysed azidolysis of epoxides have been designed on the crystal structure information on the binding of *p*-nitro styrene in the enzyme pocket (figure 1). There are seven closer residues in the pocket and the distances shown in figure 1 indicate that the epoxides oxygen form strong hydrogen bonding only with Tyr145 and Ser132 and other remaining five residues are substantially at a longer distance to make a significant contribution to control the reactivity. The role of Arg149 in making hydrogen bond with epoxide oxygen is ruled out since the distance is larger. Further the residues Phe186, Tyr187, Asn176 and Pro175 surround the β-carbon from below and the distances of them with β-carbon

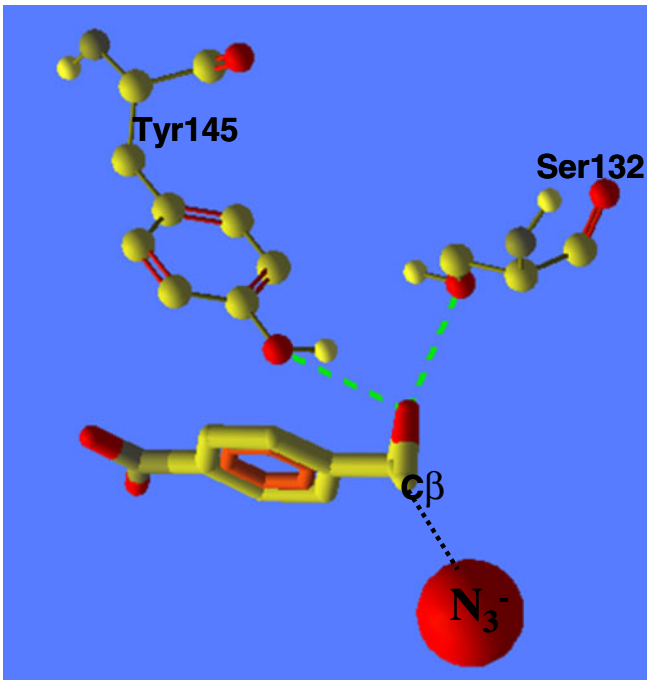


Figure 2. The active site model view of *p*-nitro styrene oxide – HheC complex and antiperiplanar orientation of C_β and N_3^- to Tyr145.

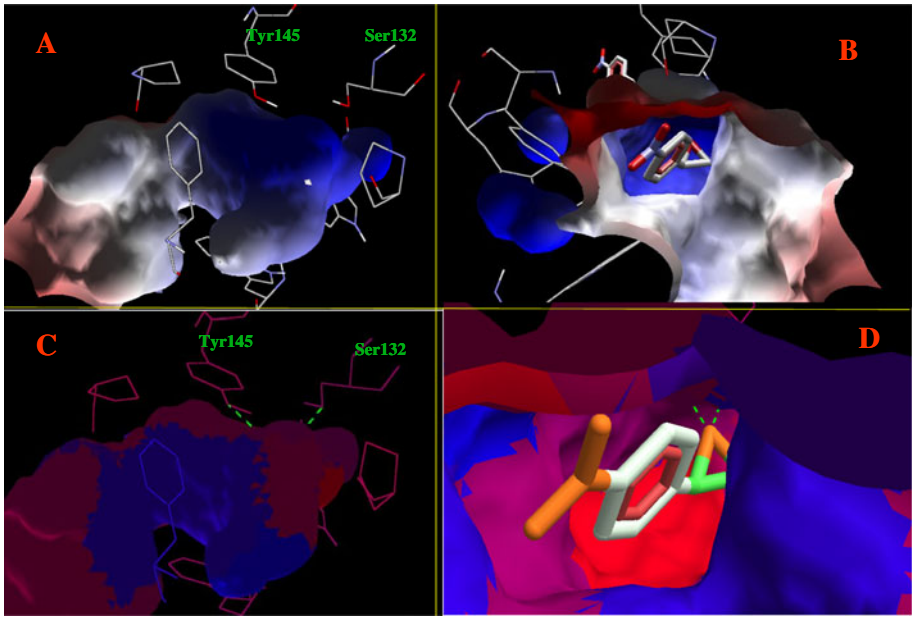


Figure 3. The graphical view of REC8 (*p*-nitro styrene oxide complexed with HheC enzyme (PBD ID = 1ZMT)) surfaces and its hydrogen bonding interactions. (**A**. ESP surface, **B**. cutting view of ESP surface, **C**. hydrophobicity surface, **D**. cutting view of hydrophobic surface.) constructed by using Molegro Molecular viewer.

(figure 1) indicate that they do not tightly crowd around C_β controlling the movement of azide ion towards C_β. Therefore in the active site model only two residues namely, Tyr145 and Ser132 have been considered and in the reaction the azide attacks the C_β in an antiperiplannar approach (figure 2) to the Tyr145 hydrogen bonding interaction with lone pair of epoxide. The graphical view of electrostatic potential (ESP) surface and hydrophobicity surfaces of enzyme reactant complex REC8 presented in figure 3 also support the above decision. The blue colour of ESP surface (figures 3 A and B) indicates the non-bonding interaction of active site residues with *p*-nitro styrene oxide and the red colour of hydrophobicity surface (figures 3 C and D) indicates the hydrophobic interaction of active site residues with *p*-nitro styrene oxide.

To determine the effect of HheC on azidolysis of epoxide, the above active site model of the enzyme has been designed; terminal part of Ser132 and Tyr145 residue have been included in the model. The coordinates of Tyr145 and Ser132 have been truncated from PDB[15] file and used without any modification in the active site model. During enzyme catalysis, the epoxide is steadily drawn into the enzyme fold and in close encounter, the epoxide form hydrogen bonds with Ser132 and Tyr145 residues. During this approach, epoxide and their residues make smaller movements and to mimic such dynamical action the Ser132 and Tyr145 residues and the epoxides are allowed to move without freezing the coordinates of them. Such a flexible approach has been followed by Houk *et al.*[16a] and Claeyssens *et al.*[16b] Because of the bigger size of the system, the geometry optimization for the HheC catalysed reactions (E1–E9) were performed on the active site model at B3LYP/6−31+G (2d,p) level of theory. Further to explain the role of hydrogen bond between

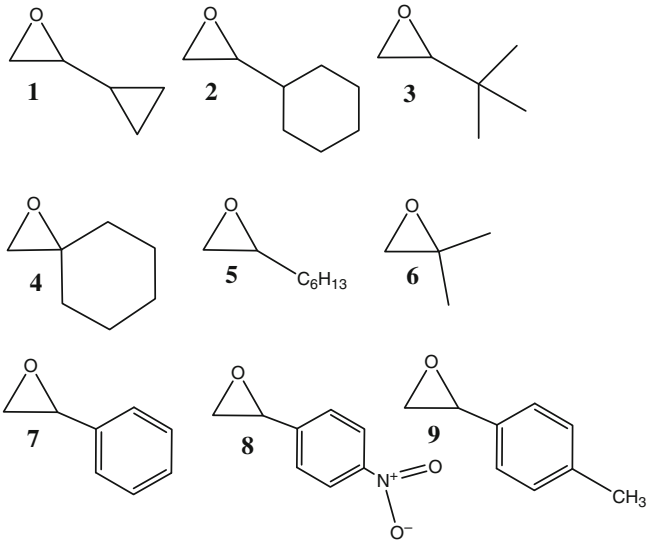
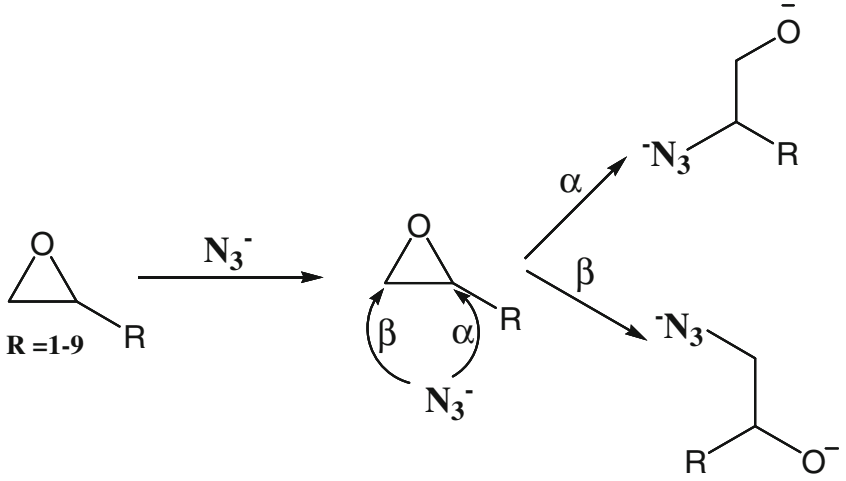
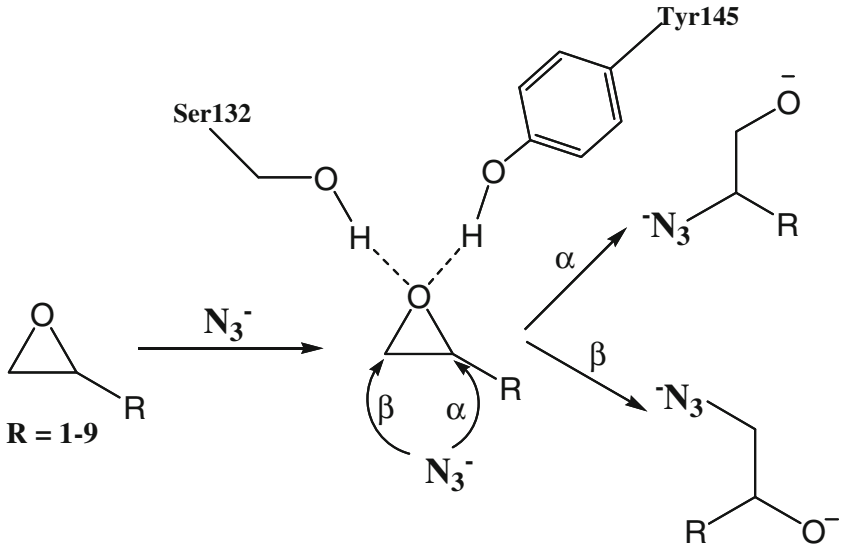


Figure 4. Epoxides used as substrates in both uncatalysed and HheC catalysed azide mediated ring opening reaction.



Scheme 1. Mechanism for uncatalysed azidolysis of epoxides.



Scheme 2. Mechanism for HheC catalysed azidolysis of epoxides.

Table 1. B3LYP/6−31+G(2d,p) relative free energies of activation and reaction for the reactions 1−9 in gas phase and in solution.

Reaction entry	$\Delta G^{\ddagger}$ (kcal/mol)		$\Delta G_s^{\ddagger}$ (kcal/mol)		ΔG_r (kcal/mol)		$\Delta G_{sr}^{\ddagger}$ (kcal/mol)		$\Delta\Delta G_{sr}^{\ddagger}\left(\Delta G_{\alpha}^{\ddagger}-\Delta G_{\beta}^{\ddagger}\right)$
	$\Delta G_{\alpha}^{\ddagger}$	$\Delta G_{\beta}^{\ddagger}$	$\Delta G_{s\alpha}^{\ddagger}$	$\Delta G_{s\beta}^{\ddagger}$	ΔG_{α}^{r}	ΔG_{β}^{r}	$\Delta G_{s\alpha}^{r}$	$\Delta G_{s\beta}^{r}$	(kcal/mol)
1	10.46	7.23	20.06	19.79	−0.67	−11.28	14.46	15.34	3.23
2	11.82	6.52	25.49	22.21	−1.06	−12.13	7.09	4.55	5.30
3	13.43	5.93	25.60	22.45	0.14	−2.51	9.49	13.01	7.50
4	16.89	10.35	25.91	22.68	−7.60	−27.94	13.18	6.82	6.54
5	16.44	10.64	19.69	18.77	−6.06	−17.49	−0.11	0.68	5.80
6	13.06	7.18	24.41	20.78	0.14	−2.29	6.14	4.96	5.89
7	7.05	10.12	22.76	23.33	−5.40	−22.81	6.61	1.85	−3.07
8	3.22	8.14	22.48	30.54	−7.96	−15.98	19.50	1.69	−4.92
9	8.06	4.91	26.29	22.92	−4.79	−5.04	7.68	2.21	3.15

Table 2. B3LYP/3−21+G(2d,p) level relative free energies for the HheC catalysed azidolysis of epoxide reactions E1−E9.

Reaction entry	$\Delta G^{\ddagger}$		ΔG_r		$\Delta\Delta G^{\ddagger}$
	$\Delta G^{\ddagger}_{\alpha}$	$\Delta G^{\ddagger}_{\beta}$	ΔG^{r}_{α}	ΔG^{r}_{β}	$\Delta G^{\ddagger}_{\alpha} - \Delta G^{\ddagger}_{\beta}$
E1	15.97	13.08	−47.44	49.34	02.89
E2	13.00	10.23	−45.95	−45.09	02.77
E3	13.66	08.13	−44.87	−44.87	05.53
E4	10.60	08.81	−43.60	−48.24	01.79
E5	19.21	14.97	−46.05	−46.52	04.24
E6	11.99	08.39	−46.82	−46.82	03.60
E7	08.71	04.35	−55.66	−58.64	04.36
E8	08.83	05.88	−49.97	−56.87	02.95
E9	09.32	08.39	−47.14	−49.81	00.93

the enzyme and substrate, NBO second order perturbation analysis have been performed on the enzyme catalysed transition states at B3LYP/6−31+G(2d,p) level. In order to explain the transition states' stabilization through hydrogen bonds by Ser132 and Tyr145 residues, NBO orbital deletion calculations have also been performed on the reactant enzyme complex and transition state species at the same level. All quantum mechanical calculations have been performed with the program Gaussian 03.[14]

3. Results and discussion

In this paper, HheC catalysed and uncatalysed ring opening of various epoxides by azide anion have been investigated. Nine epoxides that include six aliphatic (1–6) and three aromatic (7–9) (figure 4) have been used for the study and experimental reports where available[17] are compared. This work computationally probes the mechanism of azidolysis of epoxides and particularly the role of HheC enzyme in catalysing the reaction has been investigated. The question of regioselectivity in uncatalysed and enzyme catalysed reaction is the main focus. The active site of the enzyme is modelled to understand the nature of the enzyme substrate interactions that leads to catalysis and definite regioselectivity.

Nine epoxides chosen here are given in the figure 4 and the general scheme of the uncatalysed and HheC catalysed reactions are presented in schemes 1 and 2

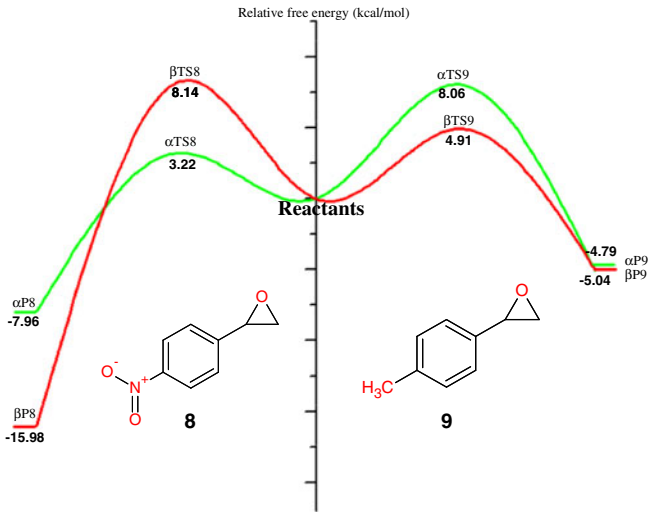


Figure 5. Relative free energies of the uncatalysed azidolysis of epoxides **8** and **9** at the B3LYP/6–31+G(2d,p) level.

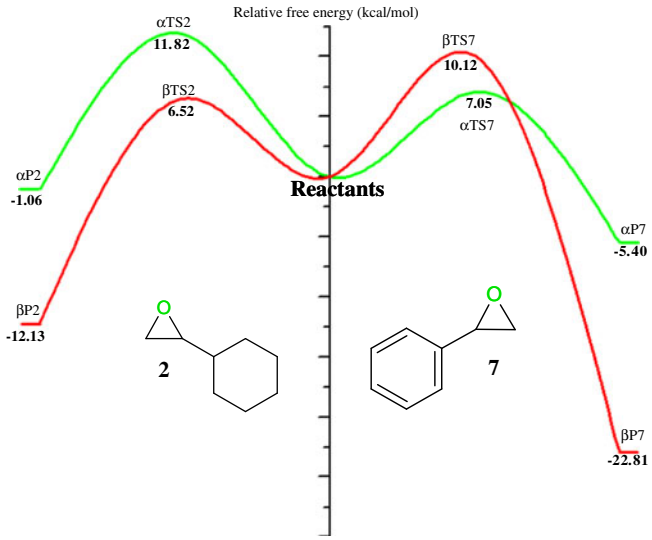


Figure 6. Relative free energies of the uncatalysed azidolysis of **2** (aliphatic) and **7** (aromatic) epoxides at the B3LYP/6–31+G(2d,P) level.

respectively. The azide ion makes a backside attack on C_α or C_β and this further leads to the cleavage of C_α–O or C_β–O bond respectively in a single-step in both catalysed and uncatalysed reactions following S_N2 mechanism.

3.1 *Azidolysis of epoxides in gas phase*

Uncatalysed reactions are numbered as 1–9 and their transition states and products are referred to as αTSn and βTSn and αPn and βPn (n = 1–9) respectively. Table 1 lists the activation and reaction-free energies for all the reactions in gas phase and in solution and $\Delta\Delta G^{\ddagger}$ values.

Activation and reaction-free energies for the reactions 1–6 (table 1) reveal that aliphatic epoxides undergo β cleavage preferentially in good agreement with experiment.[18–22] The β cleavage of aliphatic epoxides is kinetically and thermodynamically favoured than α cleavage. The activation free energies differ by 3.23–6.54 cal/mol and reaction-free energies show a difference in the range –2.43 to –20.34 cal/mol. Aromatic epoxides styrene oxide (7) and *p*-nitro styrene

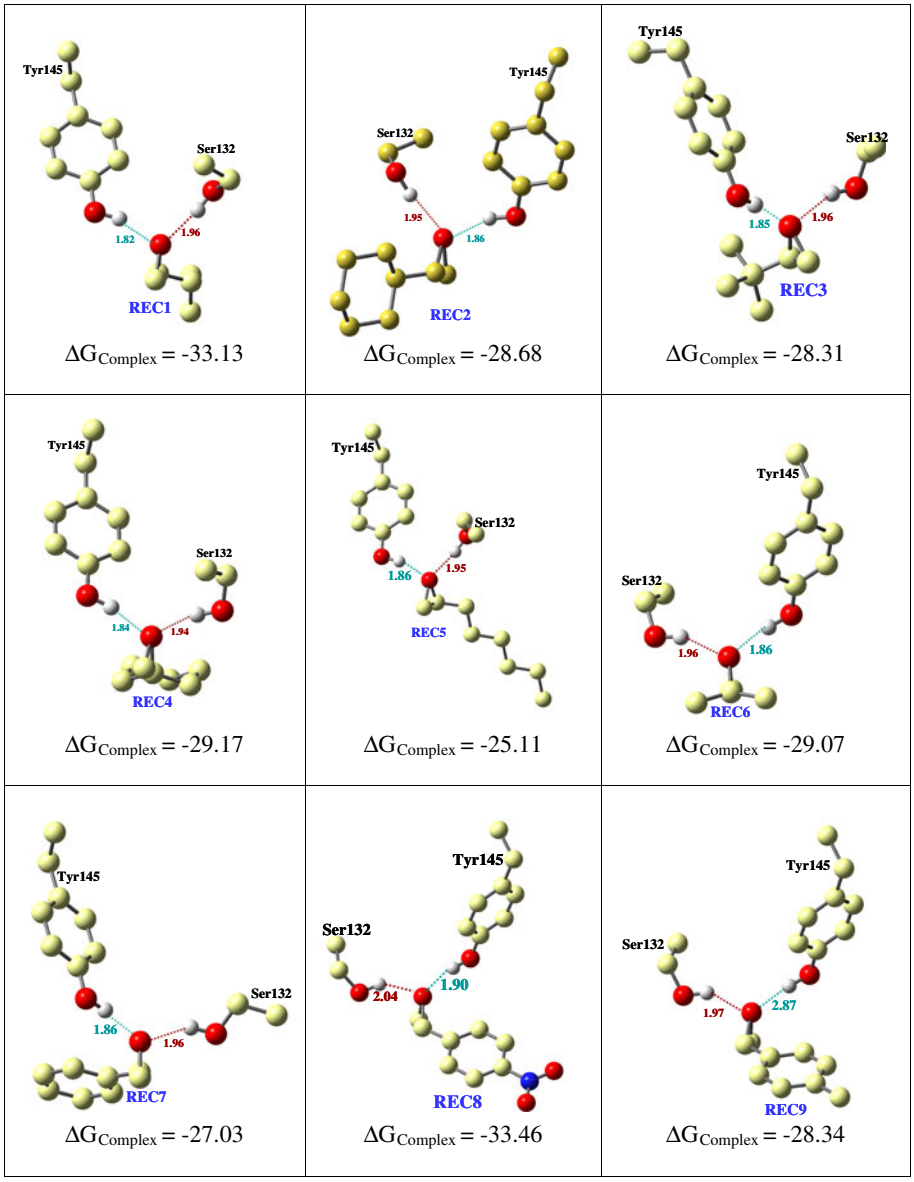


Figure 7. B3LYP/6–31+G (2d,p) optimized reactant enzyme complex (REC) for the reactions E1–E9 and free energies of complexation $\Delta G_{Complex}$ (in kcal/mol; the hydrogen atoms are omitted for clarity except interacting hydrogen atoms).

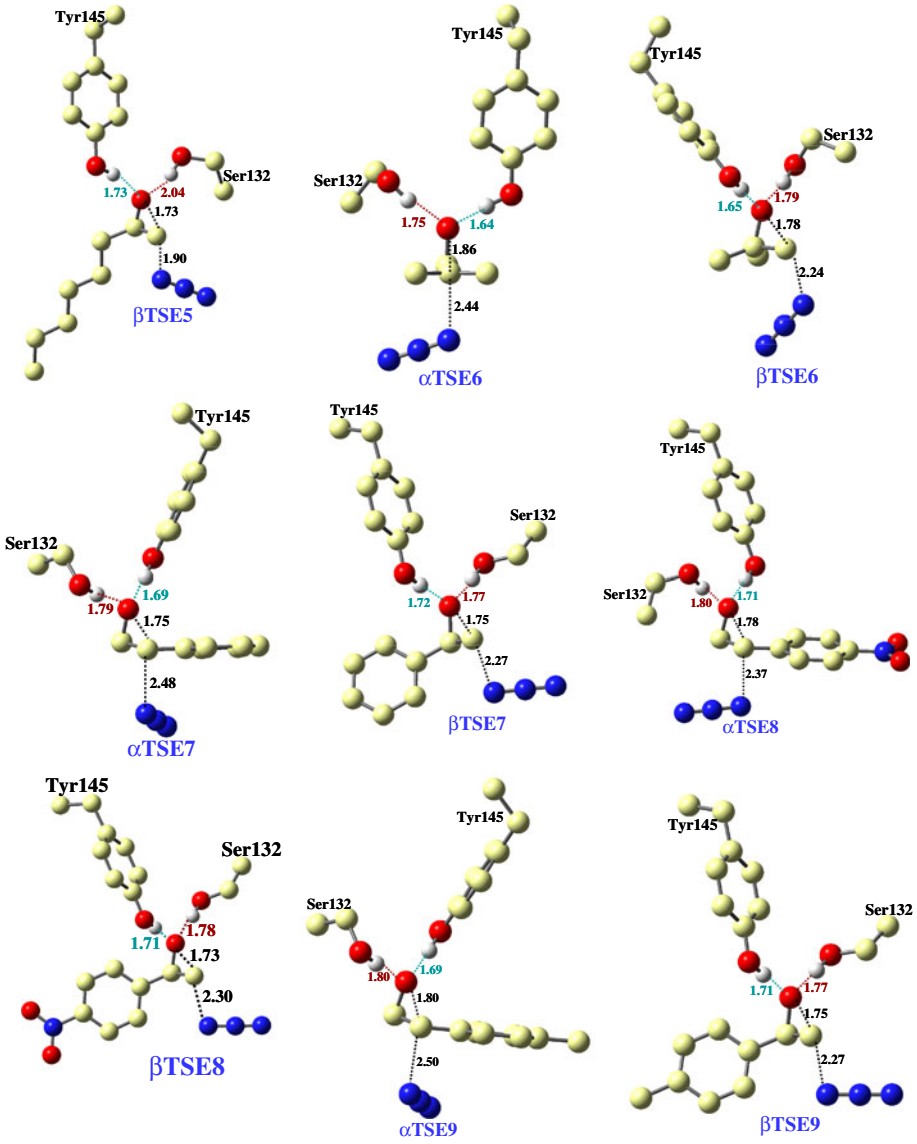


Figure 7. (continued)

oxide (8) show α selectivity while other epoxide (9) show β selectivity. Activation free energies for the reactions 7–9 (table 1) clearly predict this selectivity trend in total agreement with experiment.[14] These activation free energies and ΔG_r values for 7–9 show that these are kinetically controlled reactions.

Activation free energy data further reveal that aromatic epoxides undergo azidolysis much faster than that of aliphatic epoxides and this is due to the reason that aromatic substituents easily polarize the C_α–O or C_β–O bond by charge stabilization at the incipient carbon atom than aliphatic epoxides. Aliphatic epoxides and aromatic epoxides barring 7 and 8 prefer to cleave the C_β–O bond for steric reasons. But in styrene oxide (7) and *p*-nitro styrene oxide (8) the charge stabilization at C_α by the aromatic ring and electron withdrawing group alter the direction of nucleophilic attack. Free energy profile for the gas phase reaction 8 and 9 (figure 5) and reaction 2 and 7 (figure 6) further support this conclusion.

3.2 *Azidolysis of epoxides in aqueous solution*

PCM calculations show that inclusion of water as solvent (table 1) has significantly increased the barrier and made the reaction endothermic. This is due to the extensive solvation of azide anion and oxido anionic product formed compared to solvation of transition states. This observation agrees clearly with available theoretical and experimental results.[17,23]

3.3 *HheC catalysed azidolysis*

Azidolysis of epoxides (E1–E9) in presence of HheC follow two-step mechanism. The residues of HheC (Tyr145 and Ser132) make hydrogen bonds with epoxide reactant and this leads to the formation of reactant enzyme complex (RECn; n = 1–9). Then the azide anion attacks C_α or C_β from behind and opens the ring. This results in the formation of oxido anion and this subsequently undergoes hydrolysis resulting a formation of diol.

Computed activation and reaction-free energies for E1–E9 are presented in table 2. B3LYP/6−31+G (2d,p) geometries of reactant complexes and transition states are presented in figures 7 and 8 respectively. Figures 9 and 10 present the free energy profile for reactions E2 and E7 and E8 and E9 respectively. In figure 11 the relative free energy profile for reactions 7 and E7 are given side by side for comparison. Free energies of complexation presented in figure 7 indicate that the enzyme reactant complexes are substantially stabilized by hydrogen bonding interactions. Computed $\Delta G^{\ddagger}$ and

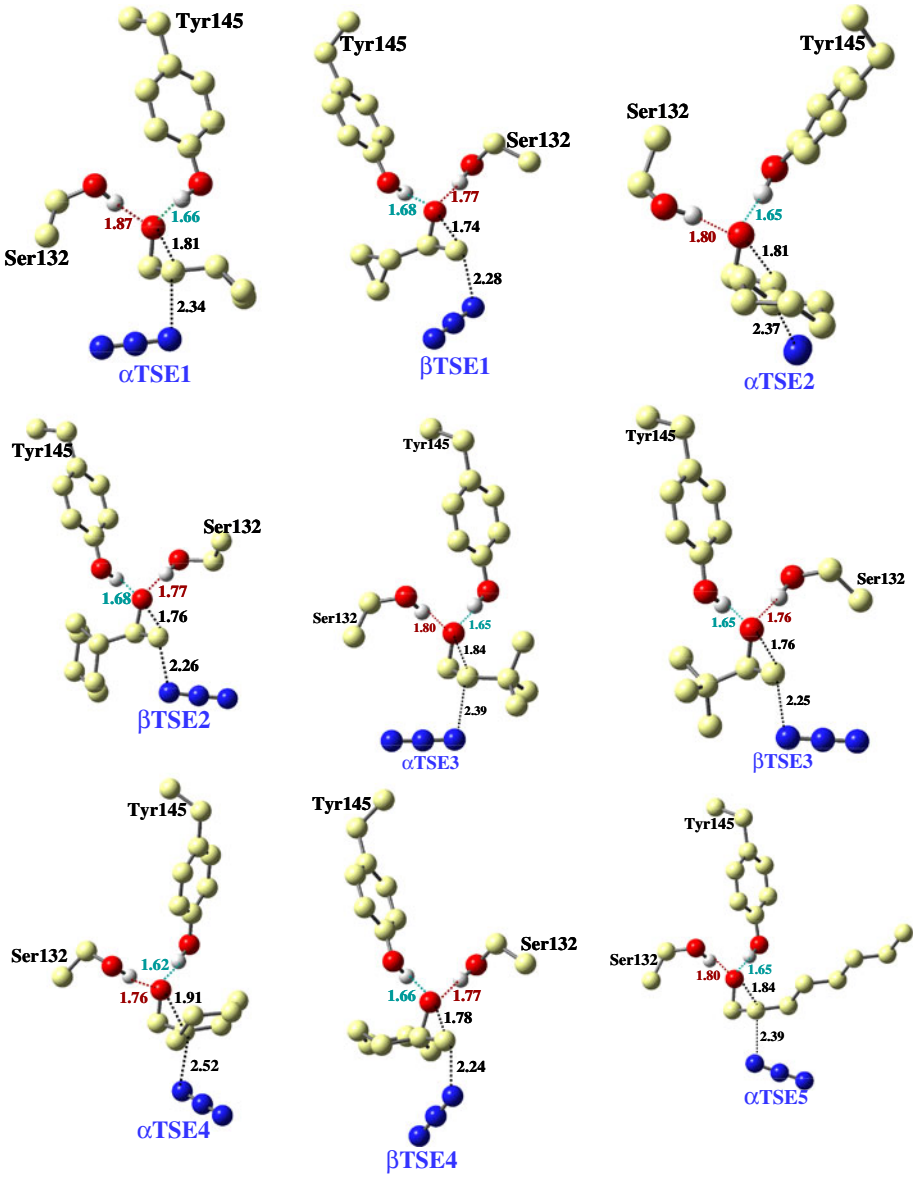


Figure 8. B3LYP/6–31+G (2d,p) level optimized transition state structures for the reactions E1–E9 (hydrogen atoms are omitted for clarity except interacting hydrogen atoms).

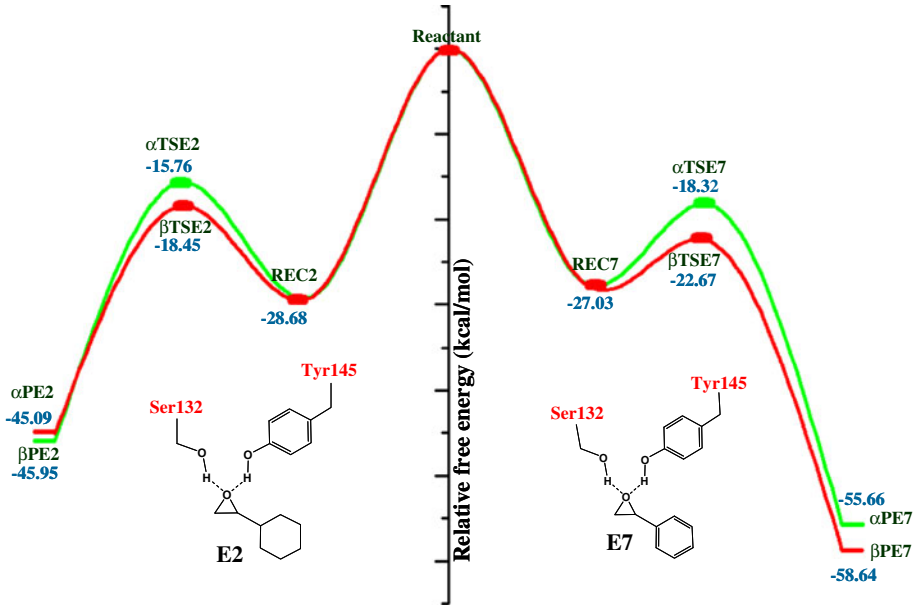


Figure 9. Relative free energies of the HheC catalysed azidolysis of E2 (aliphatic) and E7 (aromatic) epoxides at the B3LYP/6–31+G(2d,p) level.

ΔG_r for reaction E1–E9 (table 2) clearly indicate that all the epoxides prefer β cleavage and all the reactions are highly exothermic.

In most cases C_α–N bond length in α transition states are higher than C_β–N in β-transition states indicating that β-transition states are more fovaurable transition states. Bond lengths noted in the transition state structures (figure 8) also indicate that invariably N_3^- form a slightly stronger bond with C_β than with C_α in all the cases in the TSs. Both of these observations justify lower activation energies for β cleavage in E1–E9. Free energy profiles presented in figures 9 and 10 clearly show that the β cleavage paths are lying lower than α cleavage path and hence β cleavage is favoured kinetically and thermodynamically than α cleavage and this is total agreement with experiment.

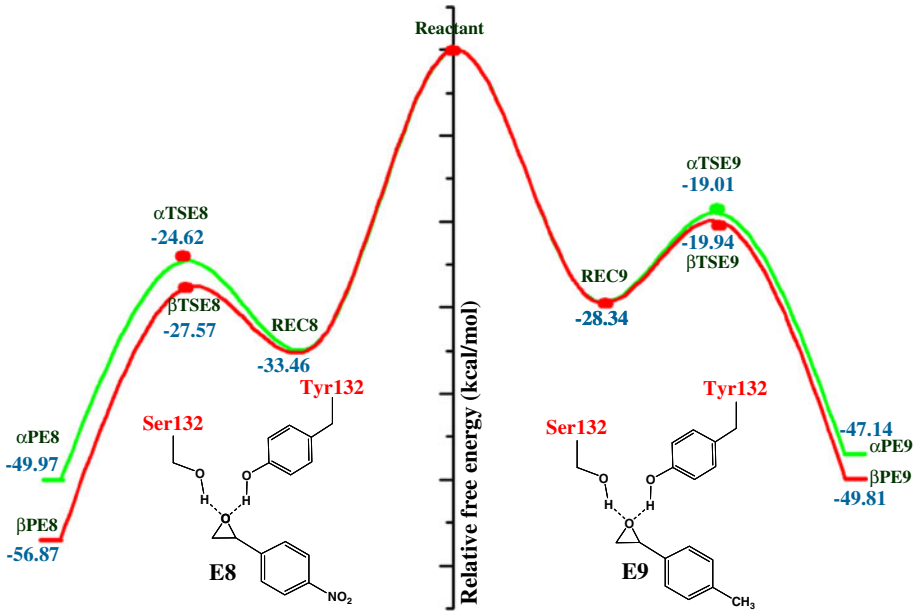


Figure 10. Relative free energies of the HheC catalysed azidolysis of epoxides E8 and E9 at the B3LYP/6–31+G(2d,p) level.

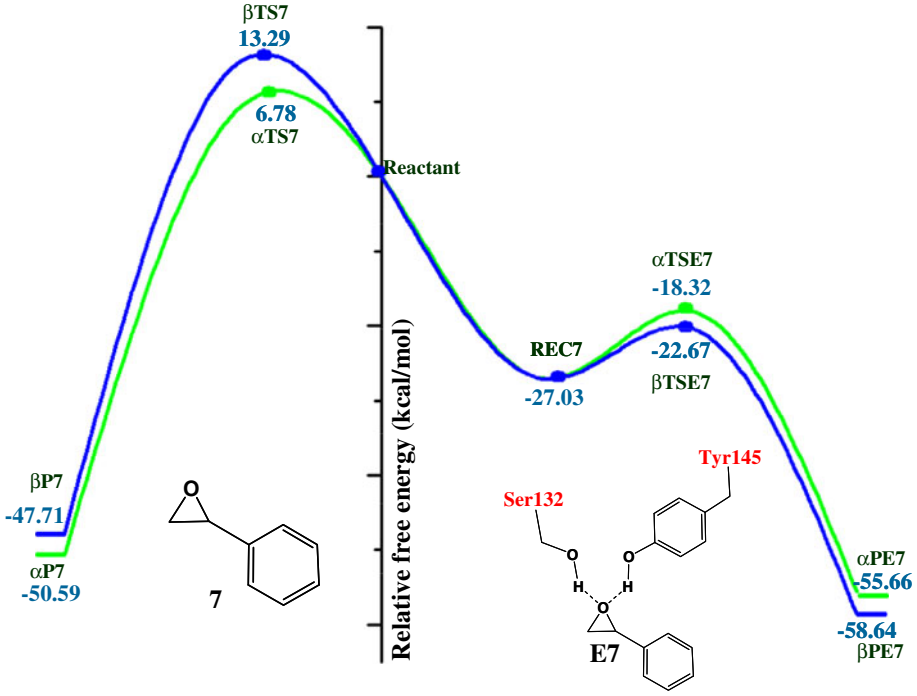


Figure 11. Relative free energies of the uncatalysed (**7**) and HheC Catalysed (E**7**) azidolysis of styrene oxide at B3LYP/6–31+g(2d,p) level.

The relative free energy profile for E7 and 7 have been presented in figure 11. Free energy profile for the uncatalysed reaction 7 and HheC catalysed reaction E7 clearly show (figure 11) the reversal of α to β selectivity in the presence of HheC. This gives clear proof that HheC catalysed epoxide ring cleavage occur with 100% β-selectivity and the type of enzyme epoxide interaction is discussed in detail in the following section.

3.4 *Role of catalytic Ser132-Tyr145 duo*

The NBO calculations have been performed to understand further the role of Tyr145 and Ser132 in the catalysis and also in the regioselectivity of HheC catalysed azidolysis. The computed second order perturbation interaction energies of $O(n_\sigma)$ and $O(n_\pi)$ with σ^* orbital of hydroxyl group of Tyr145 and Ser132 residues of all HheC epoxide complexes and HheC

Table 3. B3LYP/6−31+G(2d,p) level computed second order perturbation energy for reactant enzyme complexes and transition states of HheC catalysed azidolysis of epoxides.

Reaction entry	RECn		αTSn			βTSn		
	$O(n_\sigma)$ to Ser132$-\sigma^*_{O-H}$	$O(n_\pi)$ to Tyr145$-\sigma^*_{O-H}$	$O(n_\sigma)$ to Ser132$-\sigma^*_{O-H}$	$O(n_\pi)$ to Tyr145$-\sigma^*_{O-H}$	N_3^- to σ_c^*	$O(n_\sigma)$ to Ser132$-\sigma^*_{O-H}$	O (n_π) to Tyr145$-\sigma^*_{O-H}$	N_3^- to σ_c^*
E1	2.34	11.34	13.24	22.82	41.50	12.05	24.15	45.20
E2	2.74	10.54	11.94	28.62	37.25	13.11	29.12	45.85
E3	5.76	6.86	12.98	18.99	39.60	11.67	20.48	45.83
E4	5.55	10.37	14.52	27.18	35.08	14.45	26.99	53.38
E5	6.73	10.47	13.76	22.38	34.99	12.67	24.09	39.78
E6	5.05	10.11	14.68	26.31	36.90	13.97	27.42	51.72
E7	5.74	7.28	10.85	18.68	05.67	11.03	20.97	343.20
E8	4.37	6.64	10.41	15.18	35.59	10.99	17.22	394.12
E9	5.57	7.40	11.09	19.28	04.58	11.09	20.13	341.21

Table 4. Deletion energies of $O(n_\pi)-\sigma^*_{O-H}$ Tyr145 and $O(n_\pi)-\sigma^*_{O-H}$ Ser132 computed at the B3LYP/6−31+G(2d,p) level for reactant enzyme complex (REC) and transition states of HheC catalysed azidolysis of epoxides.

Reaction entry	Deletion energy(in kcal/mol)					
	RECn		α TSn		βTSn	
	$O(n_\sigma)-$ Ser132$-\sigma^*_{O-H}$	$O(n_\pi)-$ Tyr145$-\sigma^*_{O-H}$	$O(n_\sigma)-$ Ser132$-\sigma^*_{O-H}$	$O(n_\pi)-$ Tyr145$-\sigma^*_{O-H}$	$O(n_\sigma)-$ Ser132$-\sigma^*_{O-H}$	$O(n_\pi)-$ Tyr145$-\sigma^*_{O-H}$
E1	8.90	13.12	16.74	27.08	14.96	28.82
E2	8.63	12.15	15.22	33.74	16.60	36.84
E3	6.65	10.01	16.26	20.98	14.47	23.56
E4	6.93	11.83	18.16	30.68	18.67	31.49
E5	8.54	12.07	17.25	25.37	17.98	26.98
E6	6.32	11.54	18.32	29.89	18.11	32.05
E7	6.69	9.08	13.53	21.14	13.70	22.18
E8	5.08	8.05	12.92	16.98	13.72	18.48
E9	6.45	9.21	13.83	21.83	13.78	23.58

catalysed transition states are presented in table 3. These interaction energies show that the hydrogen-bonding interaction of the Tyr145 and Ser132 to the epoxide determine the regioselectivity by lowering the activation barrier for the β-attack compared to α-attack. The higher interaction energy of $O(n_\pi)$ with σ^* of Tyr145 hydroxyl group compared with interaction of $O(n_\sigma)$ with of Ser132 hydroxyl group clearly explain that Tyr145 makes stronger hydrogen bond with epoxide oxygen lone pair $O(n_\pi)$ compared to Ser132 hydrogen bonding with epoxide oxygen lone pair $O(n_\sigma)$.

Takumi Oshima *et al*. recently reported[24] that for acid catalysed electron donating interaction with vacant oxirane Walsh orbital plays a prominent role in the epoxide ring cleavage. This reaction generally proceeds through regioselective ring opening via a S_N2-type anti-nucleophilic displacement. In the present case the HheC residues Tyr145 and Ser132 act as acid catalysts and they are located in the direction of epoxide oxygen lone pair as evident from PDB crystal structure.[16] The Tyr145 makes strong hydrogen bond with epoxide oxygen lone pair which is located in anti-periplanar orientation to the β-carbon (figure 2). This weakens the C_β–O bond in the HheC catalysed azidolysis of epoxides. As seen above in the reactant enzyme complex and transition state, the lone pairs of epoxide oxygen are donated to the Ser132 and Tyr145 orbitals and this hydrogen bonding interaction (table 3) is stabilizing the transition states of the azidolysis reactions. To prove this, NBO deletion calculations have been performed for all HheC catalysed transition states species and its reactant enzyme complex. The computed orbital deletion energies for HheC catalysed transition state and reactant enzyme complex is shown in table 4. Deletion of σ^*_{o-H} of Tyr145 greatly destabilize the transition states compared to σ^*_{o-H} of Ser132. This reveals that $O(n_\pi) - \sigma^*_{o-H}$ Tyr145 interaction in the enzyme reactant complex as well as in TSs are stronger than the $O(n_\sigma) - \sigma^*_{o-H}$ Ser132 interaction.

4. Conclusions

Azidolysis of nine unsymmetrical epoxides in gas phase and in aqueous solution and in the presence of enzyme HheC have been modelled at B3LYP/6−31+G(2d,p) and the reaction path analysis reveals that the reaction goes through S_N2 path way in all the cases. The azide anion attacks the α or β carbon from behind. Computed activation and reaction-free energies indicate that all aliphatic epoxides prefer β cleavage. In the three aromatic epoxides, styrene oxide and *p*-nitro styrene oxide undergo α cleavage while other prefer β cleavage in the uncatalysed azidolysis. All these predictions are in good agreement with the experimental reports. Generally the azide ion attack on α carbon is hindered by steric crowding. But in the two epoxides 7 and 8 α carbon attack is preferred due to the stabilization of positive charge on α-Carbon atom by charge delocalization into the aromatic ring. This seems to offset the steric factor. The computed relative free energies also predict the same. Aromatic epoxides undergo azidolysis much faster than aliphatic epoxides due to stabilization of charge in the former. PCM calculations indicate that preferential solvation of azide ion and oxido anionic species increase the activation barrier as observed experimentally.

HheC enzyme catalyses the azidolysis of epoxides with 100% β regioselectivity. The regioselectivity of the azidolysis of styrene oxide and *p*-nitro styrene

oxide changes from α to β in the presence of enzyme. The active site has been chosen based on the PDB X-ray structure. The prediction of the regioselectivity in the enzyme catalysed reaction is in good agreement with available experimental results. Ser132 and Tyr145 make reactant enzyme complex through weak hydrogen bond with the oxygen atom of the epoxide and they loosen the C_α–O and C_β–O bonds in the HheC catalysed reaction.

NBO calculations performed on the reactant enzyme complex and transition states of the enzyme catalysed reactions show that the Tyr145 and Ser132 make hydrogen bonded reactant enzyme complex through their O–H protons and lone pairs on epoxide oxygen and between the two Tyr145 make stronger hydrogen bond with epoxide oxygen compared to Set132. Second order perturbation stabilization energy and orbital deletion energies support the above conclusion. Due to the hydrogen bond thus formed C_β–O bond is weakened to a greater extent than C_α–O and this facilitates β-cleavage in the presence of enzyme.

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