Ab Initio Quantum Mechanical and Density Functional Theory Calculations on Nucleophile- and Nucleophile and Acid-Catalyzed Opening of an Epoxide Ring: A Model for the Covalent Binding of Epoxyalkyl Inhibitors to the Active Site of Glycosidases

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A model system consisting of methyloxirane, formate, and formic acid was used to study the nucleophile-catalyzed and nucleophile and acid-catalyzed opening of an epoxide ring using ab initio quantum mechanical (up to the MP4(SDQ)/6-31+G**//MP2/6-31+G** level) and density functional theory calculations (Becke3LYP/6-31+G**). This system serves as a model for the covalent binding of the epoxide inhibitor to the active site of glycosidase. The effects of solvation on reaction energies were estimated using the isodensity surface polarized continuum model. The opening of the oxirane ring was calculated to preferably take place between the epoxide oxygen and the less-substituted carbon. In agreement with the earlier experimental inferences, the results indicate that both the nucleophile and the acid/base catalyst are needed for the ring opening reaction to take place efficiently. The implications of the results for the enzyme-catalyzed opening of the epoxide ring were discussed.

Introduction

Most glycosidases employ a pair of carboxylic acid residues, aspartate or glutamate, as catalytic groups.[1] One of these functions as a general acid/base catalyst while the other acts as a nucleophile/base. Hydrolysis reactions catalyzed by glycosidases occur via two major mechanisms resulting either in an overall retention or in an inversion of the anomeric carbon of the carbohydrate ring.[2] In both reaction types the position of the proton donor is within hydrogen-bonding distance of the glycosidic oxygen. In retaining enzymes, the nucleophilic residue is in the close vicinity of the anomeric carbon (∼5 ≈). The corresponding residue is located more distantly (∼10 ≈) in inverting enzymes that must accommodate a water molecule between the nucleophile/base and sugar.

Mechanism-based epoxide inhibitors have been used widely in mechanistic studies of retaining glycosidases. They have been applied in the identification of nucleophilic residue in an active site.[3] The inhibitors usually consist of a substrate-like part, which binds to the active site and is connected through an alkyl group to an epoxide part of the inhibitor.[4−8] Several crystal structures have recently been reported in which structurally different covalently bound epoxide inhibitors have been used to rationalize the binding of carbohydrate inhibitors to the enzyme active sites.[9−11]

Høy et al.[12,5] have proposed a reaction mechanism for the covalent attachment of epoxyalkyl inhibitors to the active site of *Bacillus subtilis* endohydrolase (Figure 1). In this mechanism the epoxide oxygen, which corresponds to the glycosidic oxygen of the substrate, is first protonated by the catalytic acid. Subsequently the carboxylate group attacks the electrophilic carbon, leading to the breaking of the C−O bond of the epoxide and the formation of a covalent bond between the ligand and the enzyme. It is assumed that the epoxide functionality is a finely tuned electrophile and does not yield readily to nucleophilic attack. Especially the reactions with physiological nucleophiles that open the epoxide ring are expected to be extremely slow in the absence of a general acid or Lewis acid catalysis.[13] Furthermore, the rate of inactivation is strongly affected by additional structural elements in the inhibitor, such as the structure of the glycosyl unit, the length of the aglycon chain, and the absolute configuration of the chiral centers of the inhibitor. The sensitivity of the rate of inactivation on structural variables means that it may be possible to design selective inactivators for glycosidases.

Although the epoxide inhibitors have been used to identify the putative nucleophiles of glycosidases, it is not ruled out that, in contrast to the generally accepted mechanism, the covalent bond is formed between the epoxide carbon and the catalytic acid/base of the enzyme

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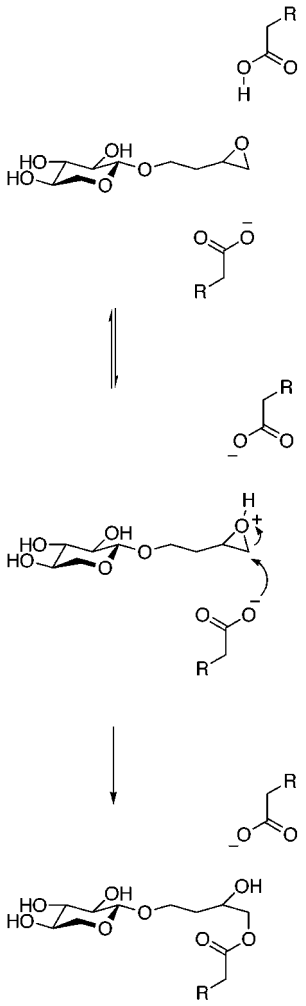

Figure 1. Likely reaction scheme for inhibitory mechanism.

active site. This was confirmed recently when three covalently bound enzyme−inhibitor crystal structures were determined by our group.[14] The complex structures determined showed that, as expected, the 4,5-epoxypentyl-β-D-xyloside and 2,3-epoxypropyl-β-D-xyloside form a covalent bond with the putative nucleophile Glu86 of *endo*-1,4-β-Xylanase II (XYNII) enzyme from *Trichoderma reesei*. However, 3,4-epoxybutyl-β-D-xyloside was found to bind to the putative acid/base catalyst Glu177.

In this study we have used ab initio quantum mechanical (QM) and density functional theory (DFT) calculations and a model system approach to investigate the ring opening of the epoxide inhibitors catalyzed by the catalytic elements of the glycosidases, the nucleophile, and the acid/base catalyst. The aim of the study was to provide a detailed theoretical understanding of the mechanism of the epoxide ring opening and in this way help to explain the experimental observations.

Computational Details

Geometry optimizations were performed at the HF/6-31+G**, MP2/6-31+G**, and Becke3LYP/6-31+G** levels. The effects of the larger basis set (aug-cc-pVDZ) and higher levels of electronic correlation correction (MP4(SDQ)) were done by performing single-point energy calculations using the MP2/6-31+G** geometries. Vibrational frequencies were calculated at the HF/6-31+G** level in order to confirm the nature of stationary points and to achieve the zero-point vibrational energies (ZPE). Zero-point energies were scaled by 0.89. All the energies reported include zero-point energies. The effect of solvation on the reaction energies was estimated using the isodensity surface polarized continuum model (IPCM-method) as implemented in Gaussian94.[15,16] In the solvent calculations the relative permittivity (ϵ) was set to 78.5 (water) and a value of 0.0004 e au^{-3} for the charge density was used in the determination of the solute cavity boundary (unless otherwise noted). This value of the charge density has been found to provide solvation energies which, when combined with the gas-phase energies, provide reaction energies in reasonable agreement with the experiments.[17,18] All the calculations were done using the Gaussian 94 program.[19]

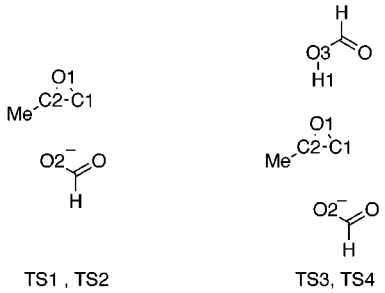

Figure 2. Atomic numbering of model systems.

Results and Discussion

Formate-Catalyzed Opening of the Methyl Oxirane Ring. The nucleophile-assisted opening of the C−O bond between the oxygen and the less- (O1−C1 bond) and more-substituted (O1−C2 bond) carbon was studied first (for the numbering see Figure 2) by using methyloxirane and formate as model reactants. The calculations were done in order to study (i) the regiochemistry of the reaction, and to help estimate the catalytic role of (ii) the nucleophile and (iii) the nucleophile and the acid catalyst in the ring opening. In addition, the methyloxirane−formate system was used to evaluate the reliability of the density functional theory (B3LYP) calculations as compared to the MP2 and MP4-(SDQ) calculations and to study the effects of the larger basis sets (aug-cc-pVDZ). B3LYP method was chosen because of the earlier promising results with similar systems to those studied here.[20−23]

The geometries of the bimolecular reactant and product complexes and the transition states (TS1 and TS2,

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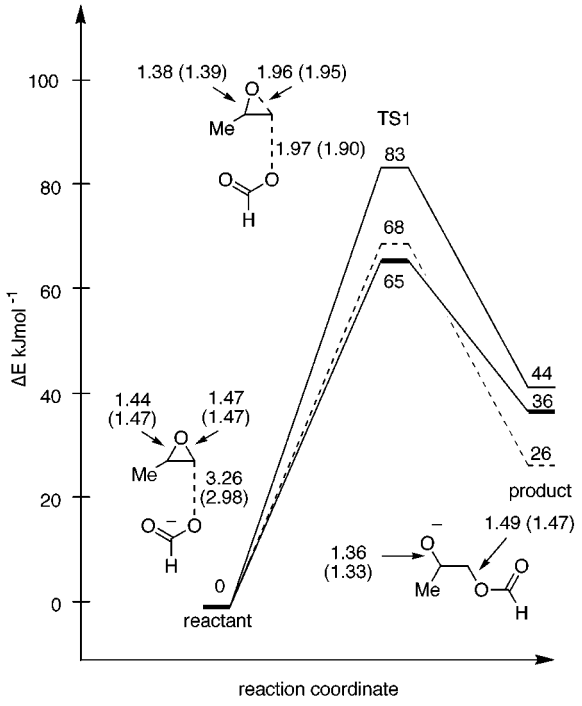

Figure 3. Relative energies MP2/6-31+G** (—, thin marks) and B3LYP/6-31+G** (—, thick marks) for the formate-assisted ring opening reaction between the less-crowded C1 carbon and the O1 oxygen in the gas-phase and solvent B3LYP/6-31+G** + HF(IPCM)/6−31+G** (water, $\epsilon = 78.3$, dashed line). Selected B3LYP/6-31+G** (MP2/6−31+G**) bond parameters are included.

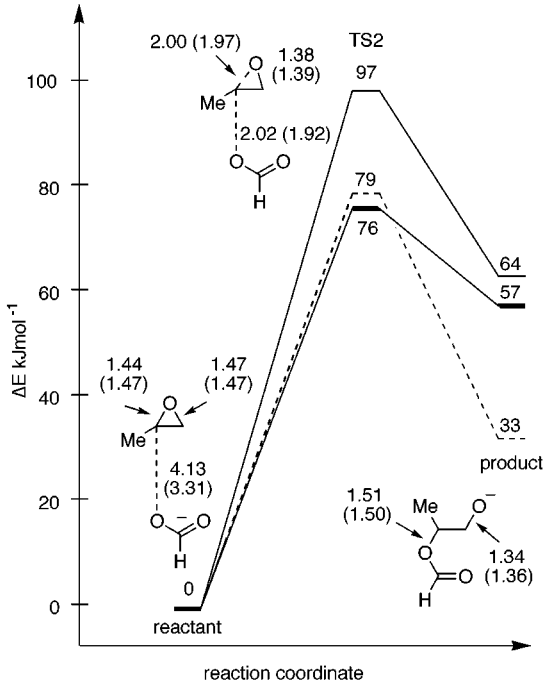

Figure 4. Relative energies MP2/6-31+G** (—, thin marks) and B3LYP/6-31+G** (—, thick marks) for the formate-assisted ring opening reaction between the more-crowded C2 carbon and the O1 oxygen in the gas-phase and solvent (IPCM-HF/6−31+G**) (water, $\epsilon = 78.3$, dashed line). Selected B3LYP/6-31+G** (MP2/6−31+G**) bond parameters are included.

Figures 3 and 4) of the ring opening reaction were optimized. The attack of the nucleophilic formate on C1 (leading to TS1) and C2 (TS2) was considered. Gas-phase energies at the MP2/6-31+G** and B3LYP/6-31+G** levels, B3LYP/6-31+G** energies including the solvation energies (IPCM-HF/6-31+G**), and selected geometric parameters of the species studied are shown in Figures 3 and 4. Additional single-point energy calculations were performed for the C1 attack at the MP2/aug-cc-pVDZ//MP2/6-31+G** and MP4(SDQ)/6-31+G**//MP2/6-31+G** level. The comparison of the different computational methods is presented in Table 1. It can be seen that at the MP4(SDQ) and MP2 levels the activation energies are within 1 kJ/mol whereas the B3LYP method gives somewhat lower (19 kJ/mol) activation energy. At the MP2 level the use of large aug-cc-pVDZ basis set decreases the activation energy by 12 kJ/mol. The reaction energies are within 8 kJ/mol with all the computational methods when the 6-31+G** basis set is used. In this case the aug-cc-pVDZ basis set gives the lowest reaction energy, 32.5 kJ/mol. It can be concluded that for the purpose of this study the energies of the ring opening reaction are in reasonable agreement with all the computational levels tested and that the computationally economic density functional theory is reliable enough to warrant its use in studying the ring opening reaction.

Table 1. Summary of the Activation Energies and Reaction Energies (kJ/mol) for the Reaction via TS1 in the Methyloxirane-Formate System

	theory level basis set			
	MP2/ 6-31+G**	MP2/ aug-cc-pVDZ[a]	MP4(SDQ)/ 6-31+G**[a]	B3LYP/ 6-31+G**
E_a	83.3	71.8	83.0	64.6
ΔE_{rxn}	44.5	32.5	39.8	36.4

[a] Single-point calculations on MP2/6-31+G** optimized geometries.

In the reactant complex the covalent bond-forming formate is coordinated at the "back" of the methyloxirane, and the carboxylate oxygens interact with the methyloxirane's methyl and methylene hydrogens (Figures 3 and 4). In the product complex a covalent bond is formed between the carboxylate oxygen and C1 or C2 of the methyloxirane. The energy barrier for the C2 attack was calculated to be 76 kJ/mol, 11 kJ/mol higher than for the attack on the less crowded C1. In the case of the C1 opening the product complex was calculated to be 36 and 44 kJ/mol less stable than the reactant complex at the B3LYP/6-31+G** and MP2/6-31+G** level, respectively. The product of the C2 attack is less stable than that of the C1 attack by 21 (B3LYP/6-31+G**) and 20 kJ/mol (MP2/6-31+G**). The geometries of the transition state TS1 (attack on C1) calculated at the B3LYP/6-31+G** and MP2/6-31+G** levels are similar, excluding the distance of the forming O2−C1 bond, which is slightly longer (1.97 Å vs 1.90 Å) at the B3LYP/6-31+G** level. The geometries of the transition states TS1 and TS2 are similar. The bond distances in the transition states are within 0.05 Å. The imaginary frequency of TS1 and TS2 calculated at the HF/6-31+G** level is visualized in Figure 5 by drawing arrows showing qualitatively the direction and magnitude of the atomic movements of the frequency (transition vector). Figure 5 shows that at TS1 a bond is forming between O2 and C1, and the C1−O1 bond of the oxirane is breaking. The atomic movements for TS2 are similar, except a bond is forming between O2 and C2 while the C2−O1 bond of the oxirane is breaking.

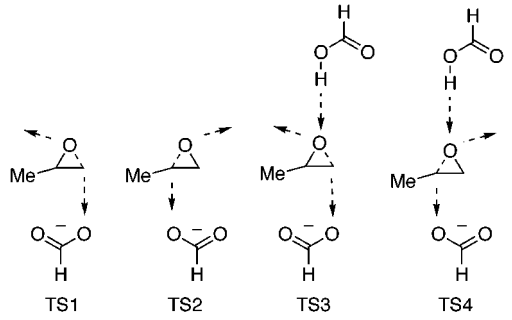

Figure 5. Visualization of vibrational analysis for the enzyme model and the formate-assisted ring opening reaction. Qualitative direction of vibrational movements joined to the negative frequency are represented by arrows.

Solvation energies (IPCM-HF/6-31+G**) increase the activation barrier TS1 by 3 kJ/mol and stabilize the product complex by 10 kJ/mol. The increase in the activation barrier is 3 kJ/mol for TS2, and the stabilization of the product is 24 kJ/mol. The products are stabilized the most because of the localized negative charge on the oxyanion formed in the reaction. Stabilization is larger for the C2 attack because the oxyanion is located in a more accessible terminal position.

Formate and Formic Acid-Catalyzed Opening of the Methyloxirane Ring. Formate- and formic acid-catalyzed opening of the methyloxirane ring was calculated at the B3LYP/6-31+G** and HF/6-31+G** levels. In this reaction the formate acts as a nucleophile and formic acid as an acid which delivers proton to the anionic oxygen of the epoxide ring. The gas-phase (B3LYP/6-31+G** and MP2/6-31+G**//HF/6-31+G**) and aqueous phase energies (B3LYP/6-31+G** + IPCM-HF/6-31+G**) and the selected geometric parameters of the ring opening reactions are presented in Figures 6 and 7. In the optimized reactant complexes the nucleophilic formate is located in such a way that it is ready to attack C1 or C2 of the methyloxirane. In the complexes formic acid constructs a hydrogen bond with the O1 of the epoxide ring and is suitably positioned to donate proton to the oxygen. In the product the covalent bond is formed between the oxirane's C1 or C2 and the formate's O2, and the formic acid has donated a proton to O1. At TS3 the distance between the nucleophilic oxygen O2 and C1 of the oxirane is 2.17 Å (B3LYP/6-31+G**). At TS4 the corresponding distance between O2 and C2 is 2.24 Å. The O1−C1 distance of the oxirane has elongated to 0.32 Å at TS3 (0.35 Å at the TS4) as compared to the corresponding distance in the reactant complex. In the transition state optimized at the B3LYP/6-31+G** level the hydrogen H1 of the catalytic acid, which in the reaction is transferred from the acid to O1 of the oxirane, is between O3 and O1. At TS3 the H1−O3 distance is 1.13 Å (1.15 Å at the TS4) and the H1−O1 distance 1.44 Å (1.28 at the TS4). The imaginary frequency, which is visualized in Figure 5, confirms that at TS3 and TS4 the C1−O2/C2−O2 bond is forming, the C1−O1/C2−O1 bond is breaking, and the H1 proton is in flight from O3 to O1. The geometry of TS3 optimized at the HF/6-31+G** level differs from that optimized at the B3LYP/6-31+G** level by the location of the transferred proton. At the HF/6-31+G** level the proton is fully transferred from O3 to O1, while at the B3LYP/6-31+G** level the proton is still bonded to O3 of the formic acid. At the transition states the O−C1−C2 (C1 attack) and O−C2−C1 (C2 attack) angles are 113.8−105.2°. These values are similar to the proposed most favorable attack angles of nucleophiles in the epoxide ring opening reactions.[24]

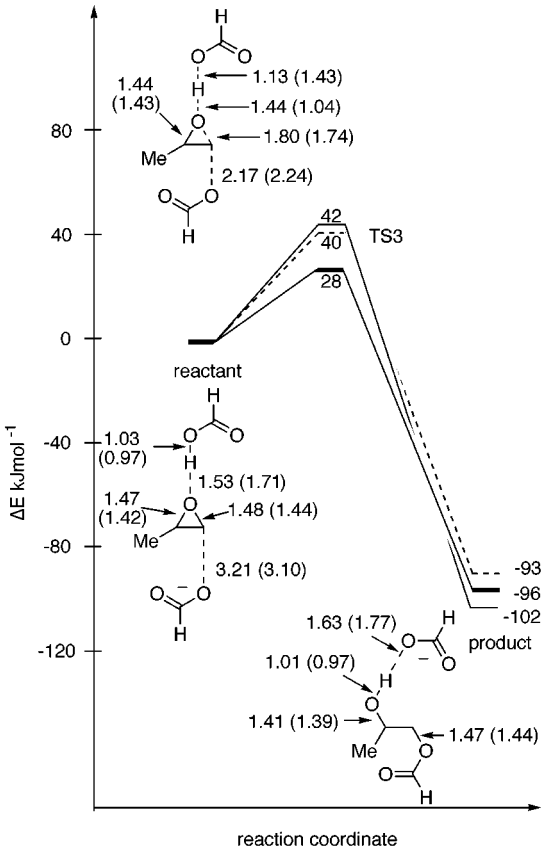

Figure 6. Relative energies MP2/6-31+G**//HF/6-31+G** (—, thin marks) and B3LYP/6-31+G** (—, thick marks) for the enzyme model reaction to the less-crowded C1 carbon in the gas-phase and solvent (IPCM-HF/6−31+G**) (water, $\epsilon = 78.3$, dashed line). Selected B3LYP/6-31+G** (HF/6−31+G**) bond parameters are included.

In the methyloxirane−formate−formic acid system the product of the C1 attack is 96 kJ/mol (81 kJ/mol for C2 attack) more stable than the reactant complex in the gas phase at the B3LYP/6-31+G** level and 102 kJ/mol (89 kJ/mol for C2 attack) more stable at the MP2/6-31+G**//HF/6-31+G** level. Solvation destabilizes the products by 3 kJ/mol (5 kJ/mol) as compared to the reactants. The high exothermicity of the ring opening reaction when both the nucleophile (formate) and the acid (formic acid) are present indicates that O-protonation is important in facilitating the reaction. This observation is in agreement with earlier studies on acid-catalyzed opening of the epoxide ring.[25−27] At the B3LYP/6-31+G** level the gas-phase energy of TS3 was calculated to be 28 kJ/mol (36 kJ/mol for TS4). When solvation energies were included, the energy of TS3 was 40 kJ/mol (56 kJ/mol). Thus the addition of the catalytic acid lowers the gas-phase barrier by 37 kJ/mol (40 kJ/mol) and the solution barrier by 28 kJ/mol (23 kJ/mol). In addition, in both

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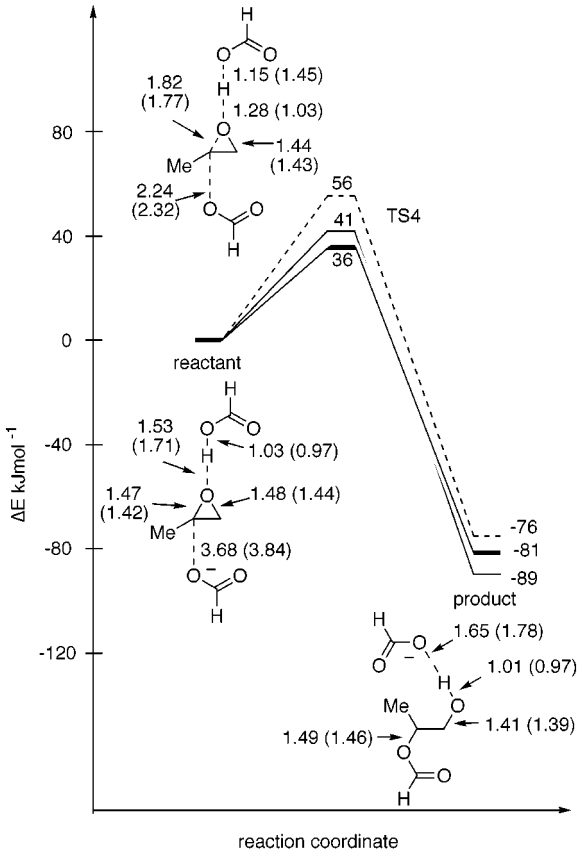

Figure 7. Relative energies MP2/6-31+G**//HF/6-31+G** (—, thin marks) and B3LYP/6-31+G** (—, thick marks) for the enzyme model reaction to the more-crowded C2 carbon in the gas-phase and solvent (IPCM-HF/6−31+G**), (water, $\epsilon = 78.3$, dashed line). Selected B3LYP/6-31+G** (HF/6−31+G**) bond parameters are included.

the formate-catalyzed and formate- and formic acid-catalyzed reaction the attack of the nucleophile on C1 leads to the more favorable reaction.

Comparison of Formate/Formic Acid and Acetate/Acetic Acid as Nucleophile and Acid. Since formate/formic acid is a rather simple model for the carboxylic group in aspartate and glutamate, and carboxylic acids in general, we compared formate/formic acid ($pK_a = 3.71$) and acetate/acetic acid ($pK_a = 4.76$) as catalyst in the ring opening reaction. This was done by calculating reaction energies (MP2/6-31+G**//HF/6-31+G**) for the nucleophile-assisted and the acid and nucleophile-assisted ring opening reactions for the two systems. Since some of the solvation energy calculations of acetate species did not converge when charge density of 0.0004 e au^{-3} was used, a value of 0.0002 e au^{-3} was used instead in the comparison of formate and acetate species. The bigger cavity size was estimated to change the solvation energy difference between the reactants and products less than ±6 kJ/mol. The reaction energies for the formate and acetate species in the gas phase and with the solvation energies included are presented in Table 2.

The reaction energy of the acetate-catalyzed ring opening was calculated to be 10.1 and 7.7 kJ/mol more favorable for the C1 and C2 attack, respectively, than the energy of the formate-catalyzed reaction. The inclusion of solvation energies stabilized the acetate species further by 18.7 and 18.3 kJ/mol. In the nucleophile and acid-catalyzed reactions acetate/acetic acid stabilizes the

Table 2. Comparison of the Reaction Energies (MP2/6-31+G//HF6-31+G**, kJ/mol) for the Reactions on C1 and C2 Carbons of the Methyloxirane−Formate/Acetate and Methyloxirane−Formate/Acetate−Formic/Acetic Acid Systems in the Gas Phase (ΔE(gas)) and in the Solution (IPCM-HF/6-31+G**) (ΔE(aq) = ΔE(gas) + ΔG(solv)**

		ΔE(gas)		ΔE(aq)	
		C1	C2	C1	C2
nucleophile	ΔE_{rxn}/formate	40.6	60.6	25.5	42.6
catalyzed	ΔE_{rxn}/acetate	30.5	52.9	6.8	24.3
nucleophile and	ΔE_{rxn}/formate	−101.5	−89.0	−103.0	−86.2
acid catalyzed	ΔE_{rxn}/acetate	−105.2	−94.3	−111.6	−97.0

products less than in the nucleophile-catalyzed reactions. In the gas phase the stabilization is 3.7 kJ/mol for the C1-product and 5.3 kJ/mol for the C2-product. In this case the solvation effects were 8.6 and 10.8 kJ/mol. These results are in line with the expectations based on the higher pK_a of acetic acid (4.76) than formic acid (3.71). As the pK_a difference suggests acetate, the conjugate base of acetic acid, is more nucleophilic than formate. In consequence, the nucleophile-catalyzed reaction is more favorable for acetate. However, in the case of the nucleophile and acid-catalyzed reaction the higher acidity of formic acid compensates for the higher nucleophilicity of acetate. This results in similar calculated reaction energies for the two species in the nucleophile and acid-catalyzed reaction. Thus, the increased nucleophilicity of the carboxylate group (higher pK_a of the acid) and the increased acidity of the carboxylic group (lower pK_a) lower the activation energy of the ring opening reaction. Similar changes in the pK_as of the catalytic amino acids most probably allow the enzymatic reactions of glycosidases to occur more efficiently.

Implications for Covalent Binding of Epoxide Alkyl Inhibitors to the Active Site of Glycosidases. Glycosyl epoxide derivatives have been extensively used for the identification of the active site residues of glycosidases.[3] Epoxide derivatives bind covalently to the carboxylate group of the catalytic aspartate or glutamate. The binding mechanism involves a nucleophilic attack on the epoxide carbon and protonation of the epoxide oxygen.[12] Therefore, a covalent bond is usually formed between the catalytic nucleophile and the inhibitor, and the acid/base catalyst most probably delivers the proton. The rate of inactivation of glycosidases has been demonstrated to be sensitive to the structural variables of the glycosyl epoxide derivatives. In the case of glycosyl epoxyalkyl derivatives, a group of much investigated inhibitors, the structure and length of the glycosyl part, the linkage position, the length of the alkyl chain, and the stereochemistry of the epoxide functional group have been found to affect the rate of inactivation.[5,11,12]

The calculations of this work showed that the epoxide ring opening and covalent bond formation are a concerted reaction. This means that for the reaction to take place efficiently both the nucleophile and acid are required to be located exactly in the positions where they are close to the epoxide group and ready to function as a nucleophile and an acid. Experimental data support the idea that at least two ionizable groups with different pK_a values are involved in the inactivation reaction. This conclusion comes from the bell-shaped pH dependence of the inactivation rate of *B. subtilisis* endohydrolase.[12] Further, it is well demonstrated that the barrier for the proton transfer, which in the epoxide ring opening takes

place between the epoxide oxygen and the acid, is sensitive to the distance between the proton donor and acceptor.[28] All the points mentioned above suggest that efficient covalent binding of epoxide inhibitor places strict demands on the correct geometry of the nucleophile, acid catalyst, and the epoxide group. Thus, this explains at least partly the high sensitivity of the rate of inactivation of glycosidase by epoxyalkyl inhibitors. If the structural requirements for the efficient ring opening are not fulfilled, the opening reaction takes place slowly or not at all or, in rare cases, the role of the nucleophile and acid/base catalyst can be reversed. It is not ruled out that in the inhibition mechanism the proton, which protonates the oxygen of the epoxide in the ring opening, could be delivered by a water molecule instead of an acidic functionality of the active site. However, in this case the inactivation rate would be greatly suppressed due to the high pK_a value of water (15.7 in water) as compared to the typical pK_a values of the catalytic acids of glycosidases that are 4.0−6.0.

Conclusions

Formate (acetate)- and formate (acetate) and formic (acetic) acid-catalyzed openings of the epoxide ring were calculated to take place preferably between the epoxide oxygen and the less-substituted carbon. In the model system the addition of the catalytic acid was calculated to lower the activation energy of the ring opening in solution by 23−28 kJ/mol. In the formate- and formic acid-catalyzed reaction the activation energy is 40 kJ/mol and the reaction energy −93 kJ/mol in solution at the B3LYP/6-31+G** level. Solvation effects were calculated to increase the activation energies by 12 kJ/mol and the reaction energy by 3 kJ/mol. Acetate was calculated to act as a better nucleophile in the ring opening reaction than formate stabilizing the products in solution by 19 (C1-product) and 18 kJ/mol (C2-product). However, in the nucleophile and acid-catalyzed reaction the higher acidity of formate is almost completely compensated for by the better nucleophilicity of acetate. The calculations of this study indicated that a suitable geometric arrangement of both the nucleophilic and acid catalyst is needed for the ring opening reaction to take place efficiently. This explains why the rate of inactivation of glycosidases is sensitive to the structural variables of the epoxide inhibitors.

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Supporting Information Available: Cartesian coordinates for structures in Figures 7, 8, 10, and 11 (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS; see any current masthead page for ordering information and Internet access instructions.

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