Theoretical Study on the Alkaline and Neutral Hydrolysis of Succinimide Derivatives in Deamidation Reactions

F. Aylin Konuklar,[†] Viktorya Aviyente,*[,†] and Manuel F. Ruiz Lopez[‡]

Department of Chemistry, Boğaziçi University, 80815 Bebek-Istanbul, Turkey, and Laboratoire de Chimie Théorique, Université Henri Poincaré-Nancy I, UMR CNRS-UHP No. 7565, 54506 Vandoeuvre-les-Nancy, France

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The hydrolysis of a succinimide derivative in alkali and neutral media has been studied at the B3LYP/6-31+G* level. Stepwise and concerted mechanisms have been considered in a vacuum. Both mechanisms have been also studied in solution by means of integral equation formalism-polarizable continuum model with a single point calculation. In basic medium, the stepwise mechanism consists of a bond cleavage and a subsequent rotation of the hydroxyl group that require 8.6 and 3.0 kcal/mol, respectively. For the concerted hydrolysis in the same medium, the barrier is 9.8 kcal/mol. In neutral medium, the hydrolysis is facilitated by the presence of a polar continuum, whereas in basic medium, the polar environment hinders hydrolysis of succinimide.

Introduction

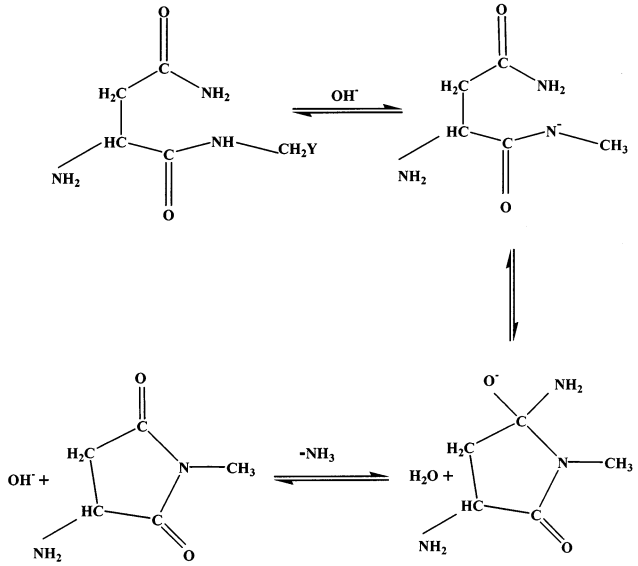
The deamidation reaction of proteins is a well-known phenomenon that may occur under physiological conditions.[1−3] The enzyme should contain an asparagine (Asn) residue followed by a glycine (Gly) residue in order for the deamidation to occur. Furthermore, a proper conformational arrangement of the dipeptide unit is necessary in order to initiate the deamidation reaction.[4] The latter takes place via acylation of the amino group of the Gly residue by the β-carbonyl group of the Asn residue producing a five membered succinimide ring. In aqueous solution, the cyclic imide is unstable, and its hydrolysis can occur from either side of the imide nitrogen to give L-isoaspartic acid (IsoAsp) and L-aspartic acid (Asp) with a ratio of 3:1 and no or little pH dependence, as shown by Clarke et al.[5] and Capasso et al.[6] In addition, small amounts of D-aspartic acid and D-isoaspartic acid were observed, indicating slow racemization of the cyclic imide. From moderately acidic to basic pH, the reaction involves the cyclization reaction for the formation of a succinimide ring (Scheme 1) and a subsequent the release of ammonia.[7]

A marked dependence of the relative yield on the pH has also been reported with a study on the enzyme bovine pancreatic ribonuclease A (RNAseA).[8] From pH 8 to 7, the ratio of IsoAsp to Asp varied from 1.5 to 0.7. The only explanation for the unusual pH dependence of the relative yield of the deamidation is the presence of positevely charged lysine residue that may stabilize the transition states.

The two consecutive reactions, deamidation and hydrolysis, terminate the catalytic activity of many enzymes such as in triosephosphate isomerase (TIM).[4] TIM is a dimeric enzyme that is active in the glycolitic pathway. It catalyzes the interconversion of glyceraldehyde-3-phosphate (GAP) to dihydroxy acetonephosphate (DHAP). TIM from mammals undergoes a specific deamidation that is followed by hydrolysis at the asparagine−glycine residues on each subunit interface of the enzyme. The two consecutive reactions lead to the destabilization of the enzyme and to its degradation. A relationship between the catalytic activity of the enzyme TIM and its deamidation is known to exist.[4,9,10] Yuksel et al. have assesed the rate of the deamidation to increase as a function of the number of catalytic turnovers.[10] They have proposed the deamidation to be a consequence of the number of times that the substrate induces conformational change. This conformational change causes the critical Asn−Gly dipeptide unit to be exposed to the solvent or assume a conformation more likely to deamidate [i.e., molecular wear and tear].[10]

The clarification of the deamidation and hydrolysis mechanisms is expected to shed light on the initial steps of the degradation of TIM and other proteins. The deamidation of asparagine residues via succinimide intermediates has been

SCHEME 1: Reaction Mechanism Proposed for the Cyclization and Deamidation Reactions



* To whom correspondence should be addressed.
[†] Boğaziçi University.
[‡] Université Henri Poincaré-Nancy I.

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investigated in our previous study.[11] In this paper, we focus on the hydrolysis mechanism of the succinimide ring in neutral and basic media.

The hydrolysis reaction of amidic linkages is a widespread studied reaction.[12−35] It has been used as a model for the enzymatic cleavage of the peptide bonds. Even though acids can promote amide hydrolysis, base catalysis has been found to be more efficient.[12,20,21] A theoretical study regarding the formation of formamide from ammonia and formic acid molecules has been performed by Oie et al.[12] In their study, both concerted and stepwise mechanisms have been examined at various levels of theory. In 1992, Krug et al. studied the neutral, base-promoted, and nitrogen- and oxygen-protonated acid-catalyzed hydrolysis of formamide at the MP2(FULL)/6-31G**//4-31G level.[21] They found that the nucleophilic addition of hydroxyl to the carbonyl carbon is barrierless in a vacuum. The intermediate formed is 46.0 kcal/mol lower in energy relative to the reactants at infinite separation. The rate determining step for the stepwise path in alkali medium has a barrier of 19.0 kcal/mol. In neutral medium for the concerted path, the transition state has an energy 44.0 kcal/mol higher than the reactants, whereas in the stepwise path, the first step requires 42.0 kcal/mol and the next one 35.0 kcal/mol.

Quantum mechanical calculations on neutral and alkaline hydrolysis of β-lactam antibiotics have been carried out by Pitarch et al.[15,16] at the HF and MP2 levels by using the 3-21G*, 6-31G*, and 6-31+G* basis sets. They have proposed concerted and stepwise reaction mechanisms for both alkali and neutral media. In the neutral hydrolysis of the *N*-methyl azetidione ring, Pitarch et al.[15,16] have found the stepwise mechanism to be preferred by 2.0 kcal/mol at the HF/6-31G* level. When a single-point energy calculation is performed at the MP2/6-31G* level, the preference is reversed by 4 kcal/mol.[15,16] In alkali medium, they have found the concerted mechanism to be always preferred. The preference of the concerted mechanism by 7.0 kcal/mol over the stepwise has also been verified with an ab initio study of the basic hydrolysis of the pyrrolidione ring.[17]

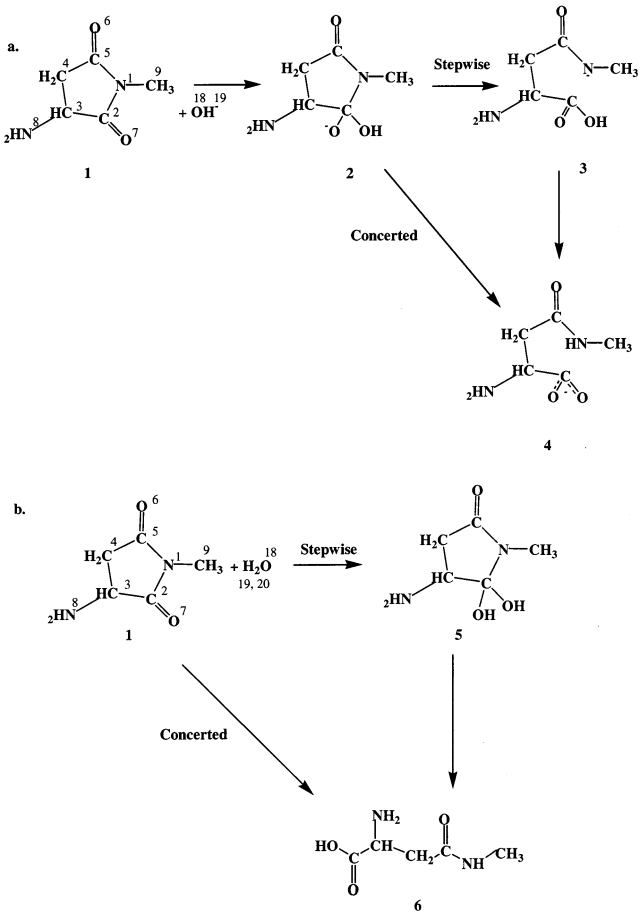
Coll et al. have also studied the alkaline hydrolysis of an oxo-β-lactam and bicyclic aza-β-lactam structures from a theoretical point of view.[18,19] The catalytic effect of water has been investigated for the hydrolysis of amides both in neutral and acidic media.[22,23] The stepwise hydrolysis of the azetidin-2-one ring in basic medium has been found to require 20 kcal/mol with semiempirical methods.[29]

In this study, we first present the methodology employed in the computations. Afterward, the reaction mechanisms for succinimide hydrolysis in neutral and basic media are described. Concerted and stepwise mechanisms have been considered, and they are compared to previous reported results in the literature for related systems. Finally, the results for single point calculations in solution will be reported.

Methodology

Preliminary examination of the potential energy surface for the succinimide hydrolysis has been carried out at a semiempirical level (PM3)[36] by using the Spartan program package.[37] The transition states were located by using the linear synchronous transit (LST) option in the Spartan program. Further geometry optimization of the stationary points has been done using the density functional method (DFT)[38] at the B3LYP/6-31+G* level where both diffuse and polarization functions on heavy atoms have been included. The utilization of diffuse functions is especially necessary in the optimization of anionic

SCHEME 2: (a) Reaction Mechanism Proposed for the Hydrolysis Reaction in Alkali Medium and (b) Reaction Mechanism Proposed for the Hydrolysis Reaction in Neutral Medium.



systems.[39] The latter computations have been carried out using the Gaussian 98 program package.[40] All of the stationary points are characterized by a frequency analysis which was also used to provide the zero-point energy (ZPE) and the thermal corrections. Energies used along the discussion include ZPE values. The intrinsic reaction coordinate (IRC) approach[41] has been used to determine the species reached by each transition structure. The charges of the stationary structures have been calculated by using the ChelpG methodology.[42] Natural bond orbital (NBO) analysis has been used in order to verify and quantify the importance of stereo-electronic effects on the stability of intermediates.[44−46] The effect of a polar environment on the reaction path has been taken into account by calculating single-point energies in water (ϵ= 78.5). The integral equation formalism polarized continuum model (IEF−PCM), which defines the cavity as the union of a series of interlocking atomic spheres, has been employed for the solvated reaction paths.[46,47] In this model, the effect of polarization of the solvent continuum is computed by numerical integration.

Results and Discussion

Scheme 2 displays the reaction mechanisms considered for the hydrolysis of succinimide in alkaline and neutral media, respectively. The numbering system used throughout the discussion is also shown in Scheme 2. In an alkaline medium, the nucleophile, in our case hydroxyl, can attack the carbonyl carbon C2 or C5 of the succinimide ring (**1**) leading to isoaspartate or aspartate, respectively. As mentioned by Clarke et al.[5] and

TABLE 1: Energies (au) (B3LYP/6-31+G*) for the Compounds Formed during the Hydrolysis of Succinimide Ring in Alkali Medium[a]

	E_{el} + ZPE	E_{el} + E_{th} + ZPE	G_{rel}
1+OH^-	−530.993284	−531.049195	0
2a	−531.064990	−531.099947	−31.8
2b	−531.064873	−531.099895	−31.8
3a	−531.054430	−531.091200	−24.9
3b	−531.050484	−531.087674	−24.1
4a	−531.096860	−531.133945	−56.2
4b	−531.098226	−531.135479	−54.1
TS(2a-3a)	−531.051354	−531.086592	−23.5
TS(2b-3b)	−531.050402	−531.085928	−23.0
TS(3a-4a)	−531.049648	−531.086279	−23.3
TS(3b-4b)	−531.046971	−531.083971	−21.8
TS(2a-4a)	−531.049640	−531.085049	−22.5
TS(2b-4b)	−531.049669	−531.084828	−22.4

[a] The energies in the first column include the zero-point corrections at 25 °C (E_{el} + ZPE). The second column displays the sum of electronic and thermal free energies (E_{el} + E_{th} + ZPE). The third column shows the relative free energies (G_{rel}).

TABLE 2: Energies (au) (B3LYP/6-31+G*) for the Compounds Formed during the Hydrolysis of Succinimide Ring in Neutral Medium[a]

	E_{el} + ZPE	E_{el} + E_{th} + ZPE	G_{rel}
1+H_2O	−531.606421	−531.663722	0
5a	−531.595969	−531.631218	+20.4
5b	−531.595969	−531.631218	+20.4
6a	−531.615861	−531.654397	+5.8
6b	−531.612991	−531.652190	+7.2
TS(1−5a)	−531.549691	−531.585425	+49.1
TS(1−5b)	−531.549691	−531.585425	+49.1
TS(5a-6a)	−531.530991	−531.566260	+61.2
TS(5b-6b)	−531.534005	−531.568904	+59.5
TS(1−6a)	−531.535264	−531.570493	+58.5
TS(1−6b)	−531.533192	−531.568574	+59.7

[a] The energies in the first column include the zero-point corrections at 25 °C (E_{el} + ZPE). The second column displays the sum of electronic and thermal free energies (E_{el} + E_{th} + ZPE). The third column shows the relative free energies (G_{rel}).

Capasso et al.,[6] isoaspartate is the product formed with a greater abundance (3:1). Thus, in this study, we have modeled the hydrolysis leading to isoaspartate only. The tetrahedral intermediate (**2**) is formed after the attack of the nucleophile to **1** (Scheme 2, path a). Then, the reaction continues by either stepwise or concerted pathways. On the stepwise path, the C2N1 bond is first cleaved to form the intermediate (**3**), and then the proton transfer is observed to form isoaspartate (**4**). In the concerted path, both the bond cleavage and proton transfer occur synchronously. In a neutral medium, the water molecule can attack succinimide from either side of the ring (Scheme 2, path a). Both possibilities have been considered. In the concerted path, the attack of water and the cleavage of the C−N bond occur synchronously forming the isoaspartic acid (**6**). In the stepwise path, the addition of water to the succinimide ring occurs forming the intermediate (**5**). Then, transfer of the proton to N1 leads to the cleavage of the C2−N1 bond.

The calculated energies in Tables 1 and 2 belong to the compounds presented in Figures 1A,B and 2A,B. In Figures 1 A and 2A, the structures for the global minima are shown. In Figures 1B and 2B, the transition structures that are located between the global minima are displayed. The transition states are denoted as TS (n−n′) where n is the reactant and n′ is the product of the path investigated. The reaction paths which have the lowest electronic energy barriers have been chosen and displayed in Figure 3A−D. The solvent effect on free energy reaction paths for both alkali and neutral media is reproduced in Figure 4 parts A and B. Figure 5 shows the free energy profile for the addition of hydroxyl ion to the succinimide ring.

1. Alkaline Hydrolysis. In this mechanism, the nucleophilic addition of the hydroxyl ion to the succinimide ring (**1**) leads to the formation of a tetrahedral intermediate (**2**) and the subsequent cleaveage of the C2N1 bond forms isoaspartate (**4**). Similar mechanisms have been modeled for the pyrazolidinone ring,[17] β-lactam antibiotics,[15,16] oxo-β-lactam,[18] and bicyclic aza-β-lactam structures.[19]

The attack of the hydroxyl ion to succinimide can yield two possible structures, whose geometrical parameters are shown in Figure 1A. The two groups, $-NH_2$ and $-OH$, are located either on the same side of the succinimide ring, as in conformer **2a**, or on opposite directions, as in conformer **2b**. The hydrogen of the hydroxyl group is oriented toward the carbonyl oxygen in both cases. **2a** has an insignificant stabilization of 0.1 kcal/mol in energy (including ZPE) over **2b**, so that they can be considered as isoenergetic. Before the attack of the hydroxyl ion, the succinimide ring (**1**) is almost planar (∠C2C3C4C5= −11.4°). This planarity is distorted to ∼20° by the addition of the hydroxyl ion in **2a**. We have assumed that hydroxyl ion binds to the succinimide ring without an energy barrier in the gas phase as has been described by previous theoretical and experimental studies on β-lactam antibiotics.[16,17,18,22] In the tetrahedral intermediates (**2a**−**2b**), the nitrogen atom shows an important pyramidalization because of the distortion of planarity of the ring. The C2N1 bond length in the tetrahedral intermediates (**2a**−**2b)** is 0.14 Å longer than in succinimide (**1**). The main differences between the two structures are the shortening in the C2O18 distance from 1.512 (**2a**) to 1.480 Å (**2b**) and the lengthening of the C2C3 bond from 1.570 (**2a**) to 1.576 Å (**2b**). In structure **2a**, there is a long-range stabilizing interaction between one of the hydrogen atoms bonded to N8 and O18 with an H···O distance of 2.357 Å. The same kind of stabilization effect is present in **2b** because of the interaction of the same hydrogen atom with O7 (H···O distance: 2.229 Å). The intermediate structures (**2a**−**2b**) are approximately 45.0 kcal/mol more stable than the separated reactants.

In the stepwise mechanism, the C2N1 bond is cleaved before proton transfer to N1 takes place. An intermediate (**3)** is formed after the cleavage of the C2N1 bond. The most stable structure among the conformers **3a**−**3b** is the one where both $-NH_2$ and $-OH$ groups are on the to same side of the plane of the ring, **3a**. Although, both groups are on the same side in structure **3a**, the long-range interaction between one of the hydrogen atoms on N8 and O7 (2.745 Å) stabilizes the conformer. The N1C2 bond length varies between 2.600 and 2.750 Å indicating that this bond is almost cleaved. In structure **3a** relative to structure **2a**, the N1C5 bond is shortened by about 0.03 Å and the C5O6 bond is lengthened by about 0.045 Å because of delocalization of the charge on the nitrogen atom. This delocalization stabilizes all of the isomers of structure **3** by forming an imine-like structure. Although the single bond between C2 and O7 (1.300 Å) in structure **2** is converted to a double bond (1.220 Å) in structure **3**, the C2O18 bond shortens by 0.2 Å relative to structure **2**. The transition state **TS(2**−**3)**, which is located between **2** and **3**, is characterized by a partially cleaved amide C2N1 bond. The $-NH_2$ and $-OH$ groups are located on the same side of the plane of the ring for **TS(2a**−**3a)**. They are located on the opposite sides of the ring for **TS(2b**−**3b)**. The activation energy barriers for the **TS(2a**−**3a)** and **TS(2b**−**3b)** are 8.6 and 9.3 kcal/mol respectively. In **TS(2a**−**3a)**, the hydrogen atoms on N8 are located 2.400 and 2.765 Å away from O18. These long-range hydrogen bond interactions stabilize

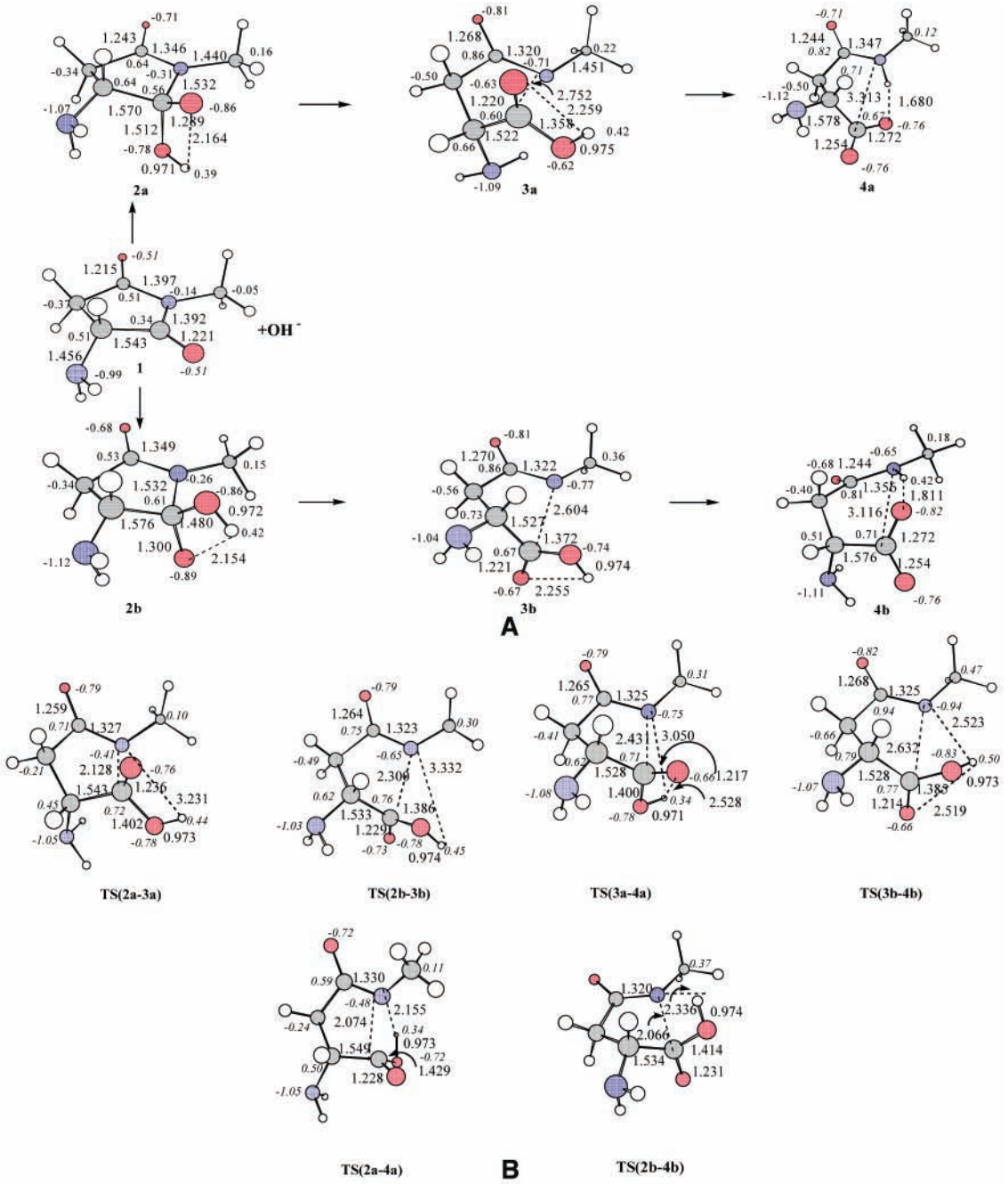


Figure 1. A. Structures corresponding to the global minima in alkali hydrolysis. B. Structures corresponding to the transition state geometries in alkali hydrolysis.

TS(2a−3a) more than **TS(2b−3b)**. In all of these structures, the hydrogen on O8 is tightly bound to it (0.97 Å). The five-membered succinimide ring is no longer planar. The bond cleavage reaction is endothermic, and the transition state resembles compound **3** as expected based on the Hammond's postulate.

In the stepwise mechanism, the proton transfer from structure **3** to **4** occurs via a rotational transition state **TS(3−4)**. The transition state is located via a rotation around the C2O18 bond with the hydrogen (H19) atom tightly bound to the oxygen (O18) (0.974 Å). The N1H19 distance is considerably reduced with respect to its value in **3a** and **3b** by means of rotation around the C2O18 bond. The energy of activation for this transition state is 3.0 and 2.2 kcal/mol depending on the orientation of $-NH_2$ and $-OH$ groups with respect to each other. The whole reaction mechanism terminates with the formation of isoaspartate, compound **4**.

In the concerted pathway, compound **2** rearranges into a four membered transition state, **TS(2−4)**, the hydrogen is transferred to the nitrogen, and the C2N1 bond is cleaved simultaneously. In **TS(2a−4a)** and **TS(2b−4b)**, the C2N1 bond length varies between 2.066 and 2.074 Å. The N1H19 bond length is 2.154 Å in **TS(2a−4a)** in which the $-NH_2$ and $-OH$ groups are on the same side of the succinimide ring and 2.336 Å in **TS(2b−4b)** in which the $-NH_2$ and $-OH$ groups are on the opposite side of the succinimide ring. The reaction is highly exothermic. The activation energy barrier for the formation of four membered transition state is 9.6 or 9.8 kcal/mol (Figure 3B) depending on the position of $-NH_2$ group relative to the $-OH$ group.

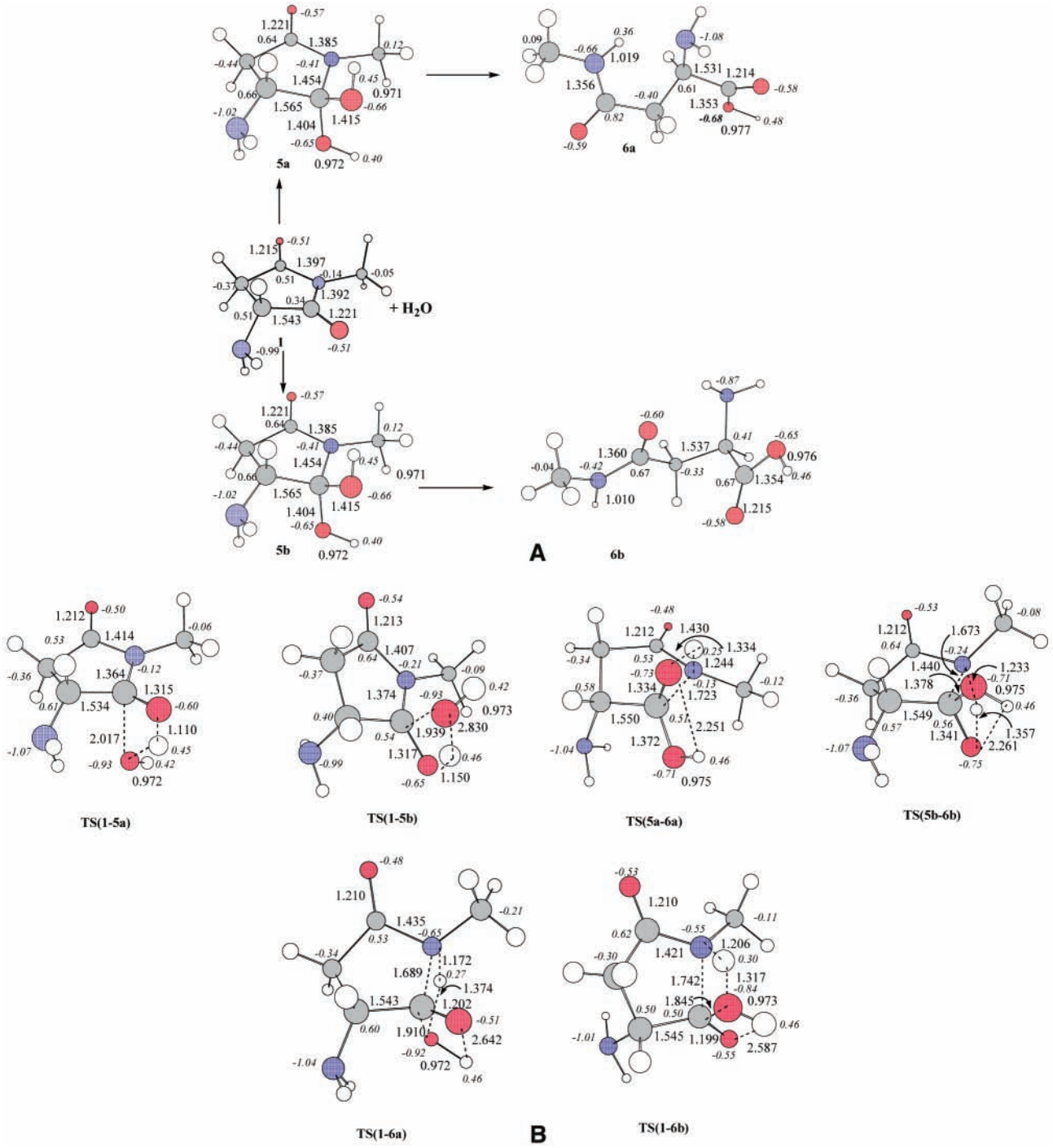
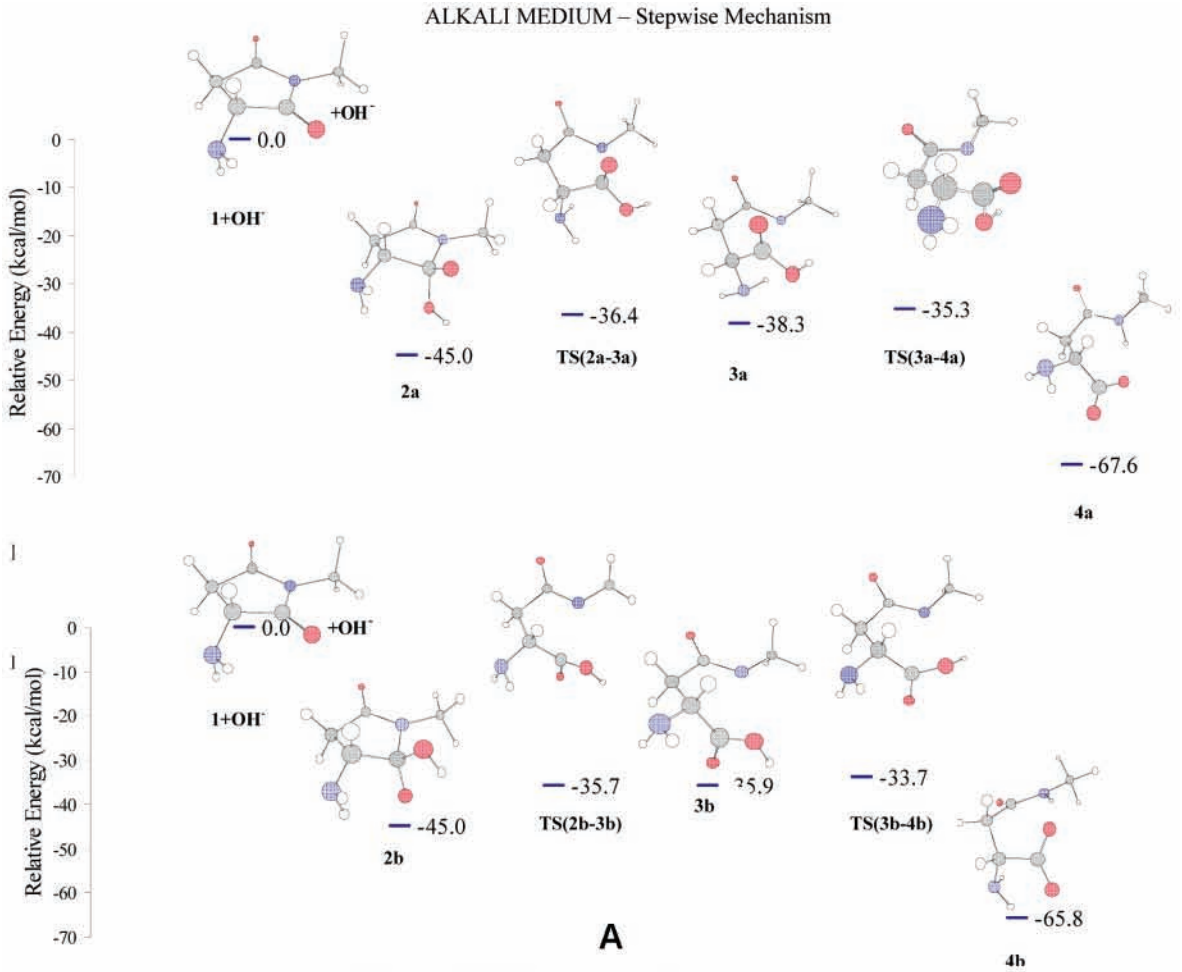
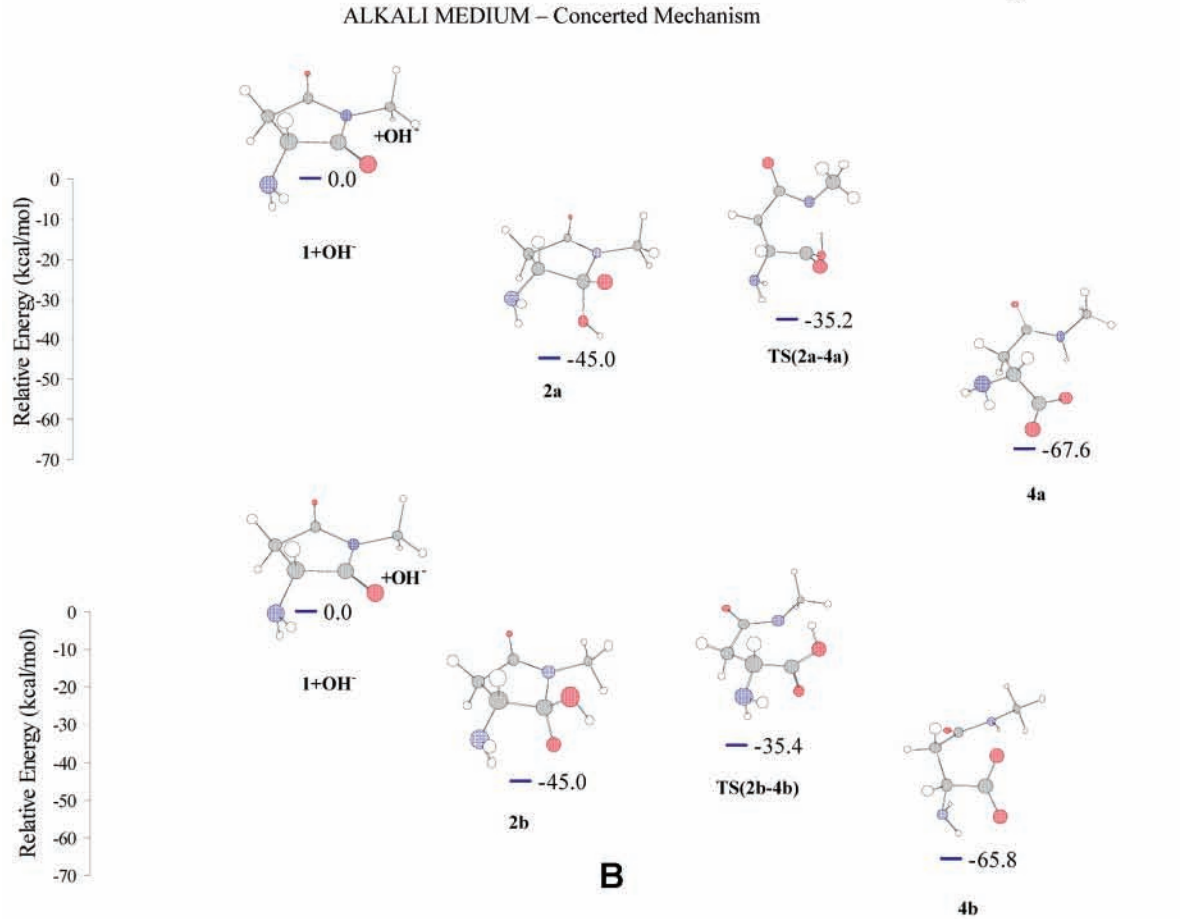


Figure 2. A. Structures corresponding to the global minima in neutral hydrolysis. B.Structures corresponding to the transition state geometries in neutral hydrolysis.

The hydrolysis reaction in alkali medium starts with the nucleophilic addition of the hydroxyl group to the carbonyl carbon. It was found that this reaction occurs spontaneously without an energy barrier in the gas phase.[13,15−17,21,48,49] Therefore, we have only considered the reaction taking place after the addition of the hydroxyl group. In the stepwise mechanism, the rate determining step is the cleavage of the five membered succinimide ring after the addition of the hydroxyl ion. When the **TS(2a−4a)** is compared with the corresponding stepwise transition state **TS(2a−3a)**, one sees that the stepwise mechanism is energetically favored by 1.2 kcal/mol. This result contradicts the previous studies for β-lactams[15,16] and pyrazolidinone[17] ring, for which the concerted pathway was reported to be favored over the stepwise pathway. In a vacuum, for the β-lactams in alkali medium, the concerted mechanism has a preference over the stepwise mechanism by 5.7 kcal/mol at the HF/6-31+G* level.[15] In the stepwise mechanism, the first step is an endothermic reaction (6.7 kcal/mol), whereas in the concerted path, it is an exothermic (−22.6 kcal/mol) reaction. The hydrolysis reaction of amide and peptide linkages has been found to favor a concerted path both thermodynamically and kinetically.[15−17]

In the stepwise path, the nitrogen (N1) is negatively charged after the clevage of the N1C2 bond. In previous calculations, the structures under investigation did not have the capability of delocalizing the negative charge on nitrogen.[15−17] In the case of succinimide, the presence of two carbonyl groups next to the nitrogen alter the preferred pathway. The charge on nitrogen


ALKALI MEDIUM – Stepwise Mechanism
Relative Energy (kcal/mol)
0
-10
-20
-30
-40
-50
-60
-70
+OH-
0.0
1+OH-
-45.0
2a
-36.4
TS(2a-3a)
-38.3
3a
-35.3
TS(3a-4a)
-67.6
4a
0.0
+OH-
1+OH-
-45.0
2b
-35.7
TS(2b-3b)
3b
-33.7
TS(3b-4b)
-65.8
4b
A


ALKALI MEDIUM – Concerted Mechanism
Relative Energy (kcal/mol)
0
-10
-20
-30
-40
-50
-60
-70
+OH-
0.0
1+OH-
-45.0
2a
-35.2
TS(2a-4a)
-67.6
4a
+OH-
0.0
1+OH-
-45.0
2b
-35.4
TS(2b-4b)
-65.8
4b
B

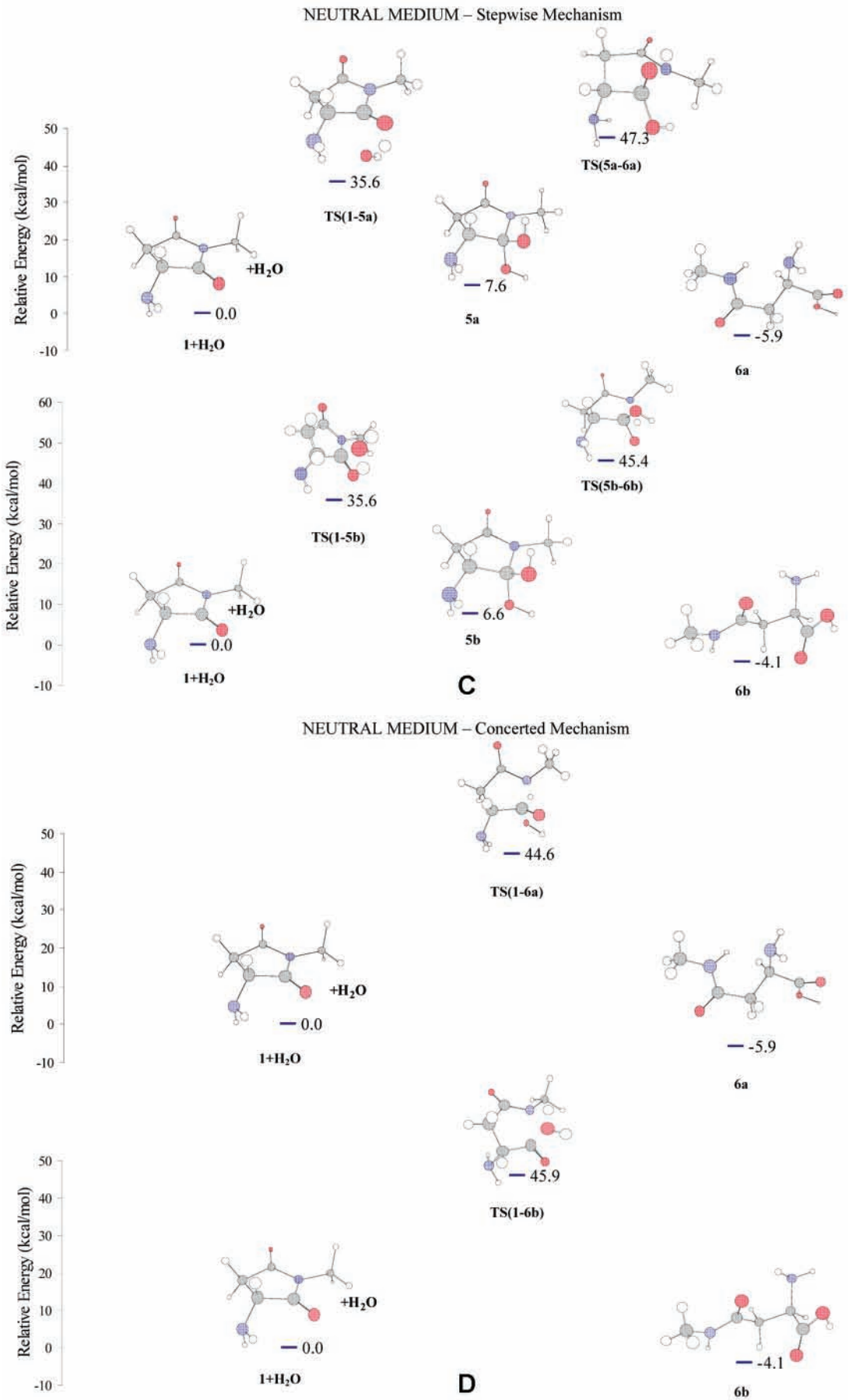


Figure 3. A. Relative energy (E_{el} + ZPE) profile for the stepwise mechanisms in alkali hydrolysis. B. Relative energy (E_{el} + ZPE) for the concerted mechanisms in alkali hydrolysis. C. Relative energy (E_{el} + ZPE) for the stepwise mechanisms in neutral hydrolysis. D. Relative energy (E_{el} + ZPE) for the concerted mechanisms in neutral hydrolysis.

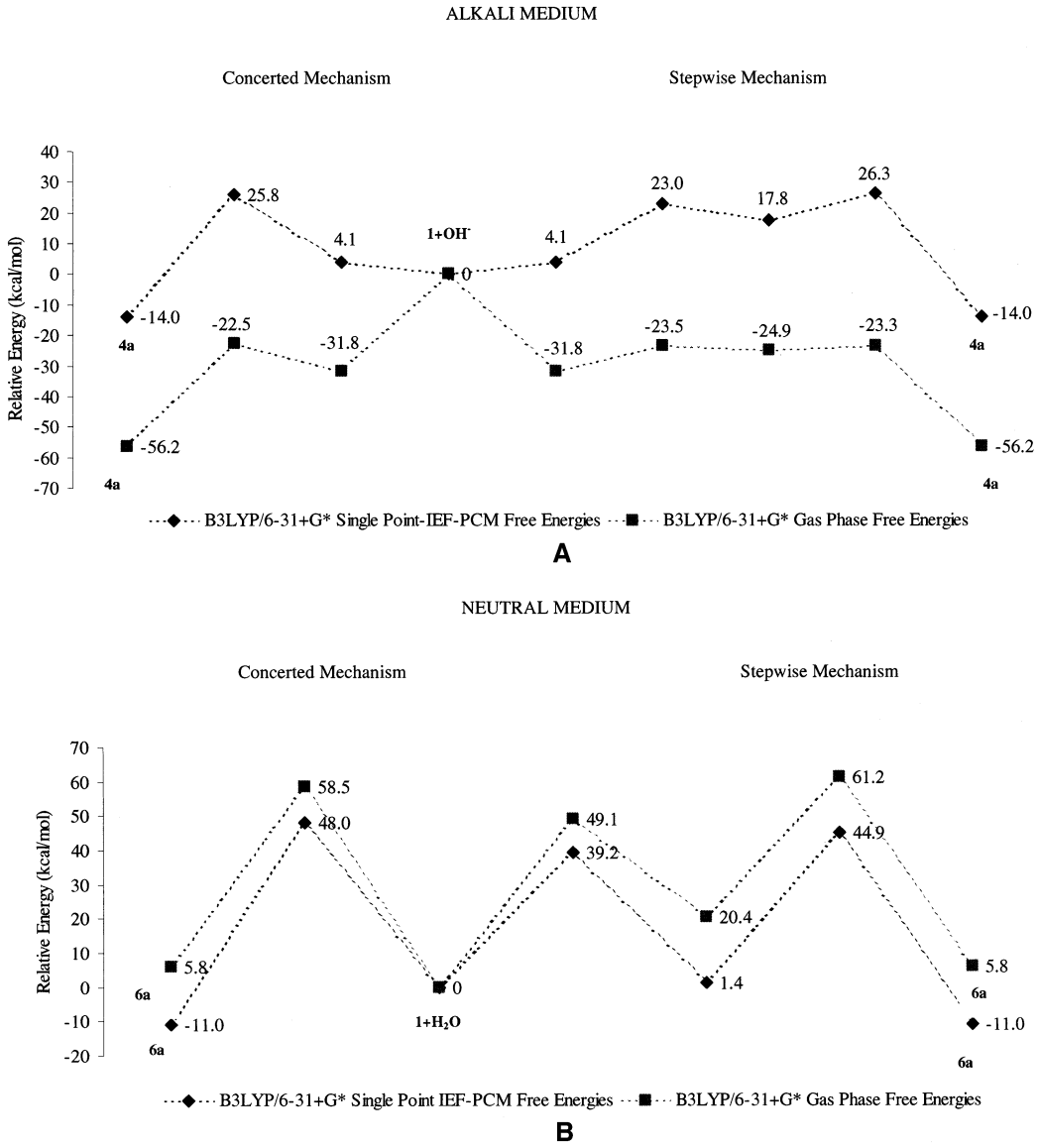


Figure 4. A. Comparison of free energies of reaction pathways in alkali medium. B. Comparison of free energies of reaction pathways in neutral medium.

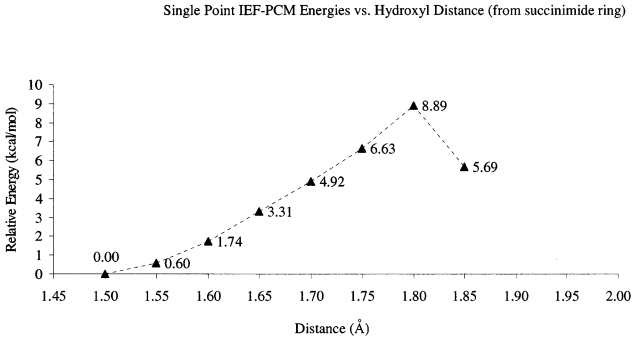


Figure 5. Single-point IEF−PCM energy profile for the addition of hydroxyl ion to succinimide ring

is delocalized along the NC and CO bonds forming a stable imine-like structure. The variation in bond distances can be considered to verify this hypothesis. In structure **2a**, the N1C5 bond length is 1.346 Å, whereas in **3a**, it decreases to 1.320 Å. An increase in the C5O6 and N1C9 bond lengths by 0.025 Å and 0.011 Å from **2a** to **3a** support the idea. The charges located on the relevant atoms have also been used to confirm these findings (Figure 1A). An increase in the negative charge on

TABLE 3: Second Order Perturbation Analysis of Fock Matrix Energies in NBO Basis (kcal/mol)

	intermediate structures	
NBO interaction	**2a**	**3a**
LP(1) N1 →σ* C4C5		1.66
LP(1) N1 →σ* C5O6	77.96	15.04
LP(1) N1 →σ* C5O6	0.53	
LP(1) N1 →σ* C2O18	11.33	0.72
LP(1) O6 →σ* C4C5	2.57	2.02
LP(1) O6 →σ* N1C5	2.28	3.79
LP(1) O6 →σ* C4C5	19.25	18.83
LP(2) O6 →σ* N1C5	23.05	17.57
LP(3) O6 →σ* N1C5		100.58

O6 from −0.71 in **2a** to −0.81 in **3a** and an increase in the positive charge on C5 from +0.64 in **2a** to +0.86 in **3a** show that the charge is more delocalized in **3a** in comparison to **2a**. The combination of the variations in bond lengths and charges confirm the relative stability of the structure **3a**. The NBO analysis results are displayed in Table 3. In **2a**, there is a strong n→σ* donation from lone pairs of nitrogen (N1) to the C5O6 antibonding orbital. However, in **3a**, there is a stronger n→σ* donation from lone pairs of oxygen atom (O6) to the respective

N1C5 antibonding orbital. Overall the stabilizing effect created by the delocalization of electrons from the lone pairs of N1 and O6 is greater in **3a**. Thus, the stabilization of **3a** decreases the activation barrier from **2a** to **TS(2a−3a)**.

It is known that the potential energy surfaces of hydrolysis reactions especially those involving charged species were altered when the effect of the solvent is considered.[19,20] In this paper, free energies in solution have been calculated on gas-phase geometries by employing the IEF−PCM methodology.

The reaction profile for the addition of the hydroxyl ion (OH^-) to succinimide has been considered with single-point free energy calculations in water. The distance of OH^- to the carbonyl carbon is varied from 1.50 to 1.85 Å. This transition structure would be 8.9 kcal/mol higher in energy than the tetrahedral complex **2a**. In the computational study for β-lactam antibiotics,[15] the barrier for OH^- attack at 1.933 Å from the carbonyl carbon was found to be 2.2 kcal/mol higher than the tetrahedral complex at the MP2//HF/6-31G* level. Similarly, Kollman et al.[13] have found a barrier along the potential energy surface for the hydrolysis of formamide in basic medium. Furhermore, the reaction profile in solution (Figure 4A) shows that a polar environment inhibits the whole reaction without changing the preference for a stepwise mechanism. The barriers in solution are higher than the ones in a vacuum.

2. Neutral Hydrolysis. In our previous study, we modeled the deamidation and consequent hydrolysis reaction of asparagine−glycine dipeptide unit in neutral medium at the B3LYP/6-31G*[11] level only for the concerted path. For the sake of completeness in this study, we have modeled both the concerted and stepwise mechanisms at the same computational level (B3LYP/6-31+G*) (Scheme 2b).

In the stepwise mechanism, the first step consists of the addition of water to the C2 atom of the carbonyl bond on succinimide (**1**) to produce the diol intermediate (**5**). Conformer **5a** is the lowest energy conformer among the diol intermediates. Conformer **5b**, which is adopted from the optimized IRC calculation, has exactly the same geometrical parameters as **5a**. The stabilizing factor for these conformers stem from the presence of long-range hydrogen bond interactions between the hydrogen on N8 and O18 (2.457 Å) and H20 and N1 (2.347 Å).

As opposed to alkali medium, the additon of a water molecule is not barrierless and constitutes the first step of the stepwise mechanism. Although the rate determining step is the second step where both proton transfer and bond clevage take place simultaneously, the addition of water to succinimide has also a high activation energy barrier (35.6 kcal/mol). The reaction is endothermic by 6.6 kcal/mol. The geometrical parameters of **TS(1−5a)** are different than the ones of the transition structures for the hydration of formaldehyde.[50] In **TS(1−5a)**, the water proton (H20) is almost transferred to O7 (1.110 Å) and is located 2.879 Å away from water oxygen (O18). In the hydration of formaldehyde, the transition state is rather an early one. Nevertheless, the geometrical parameters for the transition state structure **TS(1−5a)**, are in line with those on a theoretical study on β-lactam antibiotics[15] which has a late transition state like **TS(1−5a)**.

In the second step, the cleavage of the N1C2 bond and the proton transfer to N1 occur simultaneously producing structure **6**. Structure **6a** is more stable than **6b**. The absence of long-range hydrogen bond interactions in **6b** makes it less stable relative to conformer **6a**. The transition structure **TS(5−6)** is quasi-four-membered. In **TS(5a−6a)**, the transferred proton is located under the plane of the ring, whereas in **TS(5b−6b)**, it is located over the plane of the ring. The transition structure **TS(5a−6a)** is 2 kcal/mol higher in energy relative to **TS(5b−6b)**. The energy barriers for the second step are 39.7 and 38.8 kcal/mol, respectively. This path is exothermic and is the rate-limiting path of the whole reaction.

In the concerted pathway, as in the case of alkaline medium, two intermediates may take place depending on the way the water molecule approaches the succinimide ring (either from the bottom (**TS1−6a**) or from the top (**TS1−6b**)). The water molecule approaches succinimide to cleave either one of the N1C2 or N1C5 bonds to form isoaspartate or aspartate. The transition state structure **TS(1−6)** has been located for the formation of isoaspartic acid. In **TS(1−6)**, a four membered ring is formed between breaking N1−C2 and O18−H20 and forming O18−C2 and N1−H20 bonds. The N1−C2 bond length increases from 1.392 Å in succinimide to 1.712 Å in **TS(1−6b)**. The proton of water (H20) is almost transferred to N1 as shown by the N1H20 (1.206 Å) and O18H20 (1.317 Å) distances in **TS(1−6b)**. The transition structures **TS(1−6a)** and **TS(1−6b)** are not planar; the dihedral angle is ∼30° (∠C5C4C3C2). The energy barrier for the concerted mechanism is about 45 kcal/mol. The reaction is exothermic (−4.1 and −5.9 kcal/mol). The concerted mechanism has a higher activation energy barrier in neutral medium than in basic medium. In neutral medium, the stepwise mechanism has a lower energy barrier relative to the concerted mechanism.

The polar medium facilitates the hydrolysis by lowering the activation barriers for the additon of water to succinimide. The reaction path in neutral medium is not affected as severely by the solvent as in the case of the alkali medium. The activation energy barrier of the concerted mechanism decreases by 10 kcal/mol. The same effect is observed for the first step of the stepwise path where the water attacks the succinimide ring. Later, the ring cleavage is not influenced by the solvent.

Conclusions

In this work, we have presented a theoretical study of alkaline and neutral hydrolysis of succinimide leading to isoaspartate as a model for the hydrolysis in the enzyme triosephosphate isomerase. For both neutral and alkaline media, two possible reaction mechanisms (concerted and stepwise) have been analyzed. In the alkaline hydrolysis, a tetrahedral intermediate arising from the hydroxyl binding to the carbonyl toward the amine group is found to be more stable than the separated reactants. Two possible reaction pathways have been followed from the tetrahedral complex. In the concerted one, the N−C bond cleavage and proton transfer take place simultaneously through a single transition structure. In the stepwise mechanism, the ring opens producing in an intermediate in which the proton transfer occurs.

Although in alkaline hydrolysis of amidic linkages the concerted mechanism is preferred over the stepwise mechanism,[12,14] in this study, the concerted and stepwise mechanisms are competitive. In previous studies related to amidic linkages, the structures that had been taken into consideration had only one carbonyl group adjacent to the nitrogen atom. In this study, the presence of a second carbonyl group bonded to a nitrogen, through electron delocalization, has led to the formation of an imine-like structure along the stepwise path. This stabilizing factor lowers the energy barrier in the first part of the stepwise mechanism.

For the hydrolysis in neutral medium two possible transition structures depending on the orientation of the water molecule can be reached from the reactants. In the concerted path, a water

hydrogen atom is already pointing toward the nitrogen atom, and ring opening and proton transfer occur simultaneously. In the stepwise path, the product is reached after passing through two barriers; the first one is the addition of water, and the next one is the cleavage of the N−C bond and simultaneous proton transfer. Both mechanisms have high energy barriers. The stepwise mechanism is favored with respect to the concerted one by 4 kcal/mol at the B3LYP/6-31+G* level.

The hydrolysis reaction in alkaline medium is more exothermic and has a lower energy barrier with respect to the same reaction in neutral medium both for the concerted and stepwise mechanisms. The hydrolysis in alkali medium is preferable to the same reaction in neutral medium both kinetically and thermodynamically. Our result is in accordance with the findings in the literature where the hydrolysis in alkali medium is preferred to the neutral medium.[12,13,20]

The solvent effect has been taken into account by carrying out single-point calculations using the IEF−PCM methodology. In alkali medium, the barriers in solution increase with respect to the ones in gas phase. The binding of the hydroxyl anion to the succinimide ring is found to overcome a barrier. In neutral medium, the hydrolysis reaction is facilitated in a polar environment.

This study stresses the importance of a basic medium in the hydrolysis of amidic linkages. Bulk solvent effects have allowed a more realistic consideration of this reaction both in neutral and basic media. The attack of the nucleophile to C5 that results with the formation of aspartate will be elucidated in our next manuscript.

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