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Modelling the hydrolysis of succinimide: formation of aspartate and reversible isomerization of aspartic acid *via* succinimide

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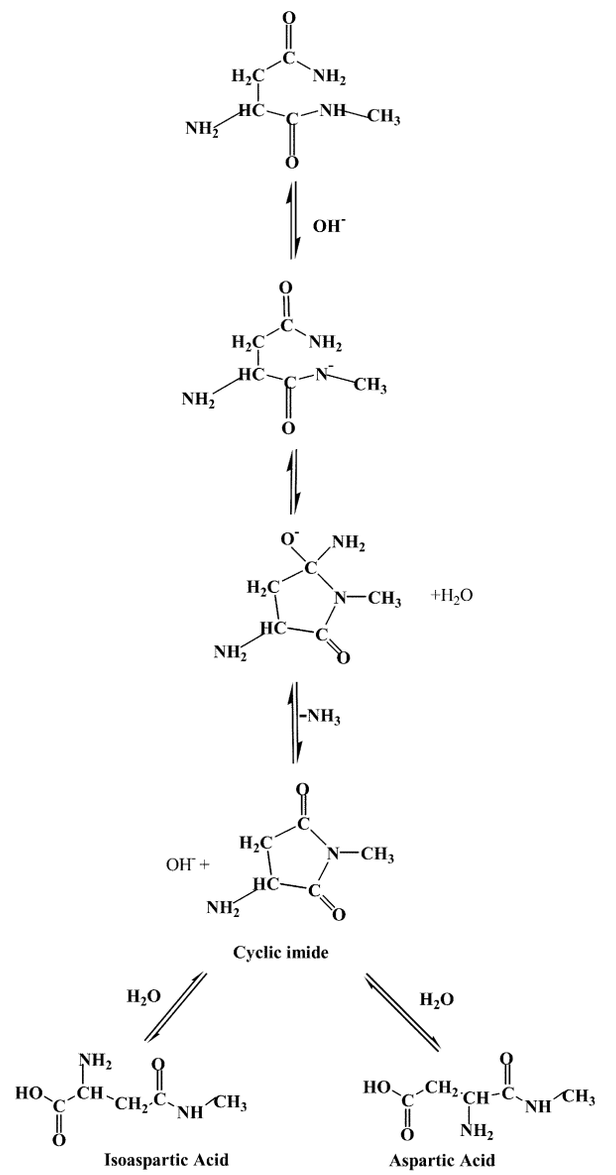
In the present study, we have modelled the nucleophilic attack of water and a hydroxyl anion on the carbonyl carbon of a succinimide derivative leading to aspartate and aspartic acid. Calculations have been carried out at the B3LYP/6-31+G* level in a vacuum. The IEF–PCM methodology has been used to carry out single point calculations in solution. In neutral medium, hydrolysis is facilitated by the presence of a polar continuum, whereas in basic medium the polar environment hinders the hydrolysis of succinimide. The ΔH° and ΔS° values for the cyclization reactions of aspartic acid yielding succinimide are 29.2 kJ mol^{-1} and 133.5 kJ mol^{-1} K^{-1} respectively in accordance with the experimental results on the isomerization of the Ac–Asp–Gly–NHMe dipeptide unit. In a neutral medium, the isoaspartate : aspartate is found to be 2.2 : 1 in a vacuum and 3.4 : 1 in solution, in line with the experimental findings based on the hydrolysis of a tetrapeptide (Ac–Gly–Asn–Gly–Gly–NHMe) and a hexapeptide (Val–Tyr–Pro–Asn–Gly–Ala) where this ratio was found to be 3.1 : 1.

Introduction

The deamidation reactions of asparagine (Asn) residues, the hydrolysis reaction of amides and the reversible isomerization of aspartic acid (Asp) residues have proven to be among the most common chemical modifications resulting in covalent damage of polypeptide chains.[1–3] These reactions have received much attention through the years because of their fundamental importance in enzyme-catalyzed reactions. It is well known that both the deamidation and the isomerization reactions occur *via* an aminosuccinyl residue arising from intramolecular nucleophilic attack on the β-carbonyl carbon of Asp or Asn residues by the amido NH group of the next residue, with the elimination of water and ammonia respectively (Scheme 1).[4–6] For the succinimide ring, 35.6 kJ mol^{-1} strain energy is reported in the literature due to a loss of internal rotational entropy that ends up with an increase of strain energy.[7] In solution, the succinimide ring is labile and its hydrolysis may occur on either side of the imide nitrogen, yielding a mixture of α- and β-linked aspartyl peptides as shown in Scheme 1.[5,6,8,9] In the absence of a bulky side chain or the residue next to Asn or Asp, both reactions are accelerated.[7] It is known that, the ratio of isopeptide to normal peptide formed at 37 °C from L-imide hydrolysis is 3.5 : 1 while that from D-imide hydrolysis is 3.1 : 1 with no or little pH dependence, as shown by Clarke and Gieger[5] and Capasso *et al.*[6] for a hexapeptide (Val–Tyr–Pro–Asn–Gly–Ala) and a tetrapeptide (Ac–Gly–Asn–Gly–Gly–NHMe) respectively. In the presence of a lysine (Lys) residue next to Asn, from pH 7 to 8, the ratio of the amount of IsoAsp to Asp varied from 1.5 to 0.7. It has been found that the transition state for cyclization is stabilized by the ε-amino group of the lysine (Lys) residue *via* an interaction between the carbonyl oxygen of the ring and the positively charged Lys side chain hence increasing the deamidation rate for Asp with respect to Isoasp.[10] However, the stabilization is found to be higher for the process yielding the aspartate product rather than the isoaspartate.[10] In the isomerization reaction, the peptide backbone is transferred from the α-carboxyl of an aspartic acid residue to the side chain β-carboxyl. Although succinimide formation from aspartic acid is thought to occur by a similar mechanism to that of asparagine, the rate of Asp–succinimide formation was measured at 37 °C and pH 7.4 in peptides and found to be 13–35 times less than that for the corresponding Asn–peptide.[5,11] At pH 7.4, the Asp side chain has only 1 in every



Scheme 1

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3000 carboxy groups protonated and thus is available for succinimide formation.[11] The rate of succinimide formation for a protonated Asp would therefore approach that of an ester and would be much more rapid than the amide.[11] Thus not only the conformation but also the relative degree of protonation will affect Asp succinimide formation from aspartic acid residues.

The hydrolysis reaction of amidic linkages has been used as a model for the enzymatic cleavage of the peptide bonds.[12–36] The hydrolysis reaction can take place both in neutral and alkali media. It is known that the base catalysis is more efficient relative to the acid catalysis.[12,20,21] In a theoretical study by Oie *et al.*, the amide hydrolysis reactions for both the concerted and stepwise mechanisms could compete with each other since both have comparable activation energies.[12] 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]

Quantum mechanical studies on cyclic amide structures have been performed at various levels of theory. Pitarch *et al*. studied the hydrolysis reaction of β-lactams through concerted and stepwise reaction paths for both alkali and neutral media at the HF and MP2 levels by using the 3-21G*, 6-31G* and 6-31+G* basis sets.[15] Kinetically, the concerted mechanism was favored in both alkali and neutral media. The preference of the concerted mechanism by 7 kcal mol^{-1} over the stepwise mechanism has also been verified with an *ab initio* study of basic hydrolysis of the pyrrolidione ring at the MP2/6-31+G*//MP2/6-31+G* level.[17] Coll *et al.* have also studied the alkaline hydrolysis of oxo-*β*-lactam and bicyclic aza-β-lactam structures from a theoretical point of view.[18–19,29–31] Antonczak *et al.* have revisited different reaction mechanisms with various theoretical methods: semiempirical, *ab initio* and density functional and have suggested density functional theory to be adequate for the description of the hydrolysis reactions of amide linkages.[23] Hori *et al*. have found the leaving group to determine the favorable mechanism for alkaline hydrolysis in their *ab initio* study on the amide hydrolysis.[25]

In our previous work, the hydrolysis of the same succinimide derivative was studied in neutral and basic media to yield isoaspartate.[36] We found that in basic medium, the stepwise mechanism consists of bond cleavage and a subsequent rotation of the hydroxyl group which require 8.6 and 3.0 kcal mol^{-1} respectively. The concerted mechanism in basic medium displayed a barrier of 9.8 kcal mol^{-1}. In neutral medium, both mechanisms have higher activation barriers relative to alkali medium: the stepwise mechanism is favored with respect to the concerted mechanism by 7.1 kcal mol^{-1} at the B3LYP/6-31+G* level. The exothermicity of the hydrolysis reaction in alkali medium is higher than in neutral medium. In neutral medium the hydrolysis is facilitated by the presence of a polar continuum, whereas in basic medium the polar environment hinders the hydrolysis of succinimide.[36]

In the present study, we have investigated the attack of the nucleophile (water or hydroxyl) on the carbonyl carbon of the cyclic succinimide ring to form aspartate and aspartic acid. First, the reaction paths in neutral and alkali media are described. Then, concerted and stepwise mechanisms have been considered and compared to previous reported results in the literature for similar systems. Next, the isomerization reaction of aspartic acid has been modelled and compared with the experimental results. The solvent effect on the reaction paths is discussed. Moreover, the isoaspartate : aspartate ratio is calculated and compared with experimental findings.

Computational details

Preliminary studies of the potential energy surface of reactants and products have been carried out at the semiempirical level (PM3)[37] by using the Spartan program package[38]. The geometries corresponding to stationary points have been optimized with the B3LYP density functional hybrid method[39] with the 6-31+G* basis set as implemented in the Gaussian98 program package.[40] It is known that the utilization of diffuse functions is particularly necessary in the optimization of anionic systems.[41] Geometries of stationary points were optimized without any constraints. Local minima and first order saddle points on adiabatic potential energy hypersurfaces were identified by the number of imaginary vibrational frequencies, which were calculated from analytical second order derivatives of the potential energy in the harmonic approximation. The computed harmonic vibrational frequencies are also used to determine the zero-point energy correction (ZPE). Energies used along the discussion include ZPE values. Free energy profiles were calculated after thermal correction of the potential energy using standard formulas of statistical thermodynamics at T = 298 K and P = 1 atm. The reaction pathways connecting a postulated reactant and product over a transition state were verified by calculations of intrinsic reaction coordinates (IRC) with the B3LYP/6-31+G* method.[42] Electron densities of the selected structures on reaction paths were analyzed in terms of possible valence structure presentations by means of localized bond orbitals with the aid of the natural bond orbital (NBO) option in the Gaussian98 program package.[43–45] 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. In order to have a reliable comparison with experimental findings the aspartate : isoapartate ratio has been evaluated for fully optimized structures (B3LYP/6-31G*) in neutral water solution (ϵ = 78.5). Electronic energies including thermal corrections in solution have been considered for the stepwise mechanisms for aspartate and isoaspartate.

The kinetics involved in the isoaspartate : aspartate ratio have been calculated according to the activated complex theory

$$k = \frac{k_b T}{h} \frac{Q^{\#}}{Q_A Q_B} e^{-\Delta Ea / RT}$$

where k_b is the Boltzmann constant, h is the Planck constant, $Q^{\#}$ is the partition function of the transtition state, Q_A and Q_B are the partition functions of the reactants, ΔE_a is the activation barrier and T is the temperature of the reaction

Results and discussion

The hydrolysis of succinimide in alkali and neutral media takes place *via* either stepwise or concerted mechanisms. A nucleophilic attack on the amide carbon of the succinimide ring yields a tetrahedral intermediate with cleavage of either N1–C2 or N1–C5 bonds forming isoaspartate or aspartate respectively (Scheme 1).[15–19] The transition states are denoted as TS (n − n′) where n is the reactant and n′ is the product of the path investigated. The main geometrical parameters of the three dimensional structures and the energy profiles (electronic energies including zero point corrections) are represented in Figs 1a,b and 2a,b. Relative free energies in a vacuum and in water are displayed in Fig. 3. Table 1 gathers the total energies of computations in vacuum and in solution.

Aspartate formation-alkali medium

The hydrolysis reaction in alkali medium leading to aspartate formation starts with the addition of the nucleophile (hydroxyl group) to the carbonyl carbon β to the carbon bearing the NH_2 group. (Scheme 2a). The structure formed upon addition of the hydroxyl group to the succinimide is denoted as **2a** or **2b**

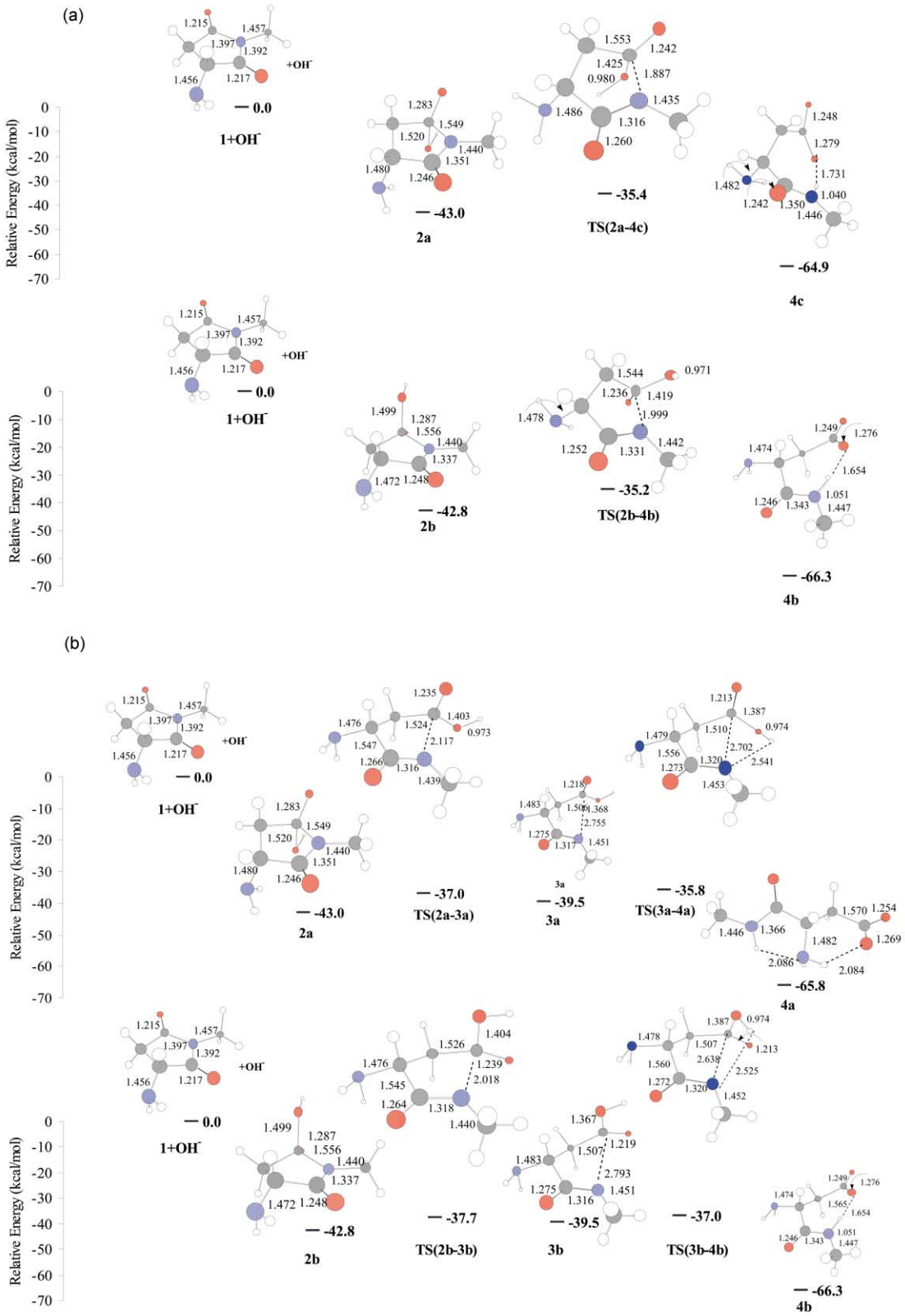


Fig. 1 a. Reaction pathway for the concerted mechanism in alkali medium. (Relative electronic energies (kcal mol^{-1}) include zero point correction) b. Reaction pathway for the stepwise mechanism in neutral medium. (Relative electronic energies (kcal mol^{-1}) include zero point correction).

depending on the orientation of the $–NH_2$, and –OH groups. When the two groups $–NH_2$, and –OH are located on the same side of the succinimide ring, the structure is denoted as **2a**, and as **2b** when the groups are on opposite sides. Structures **2a** and **2b** are isoenergetic. The 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 cyclic *β*-lactams and amides.[13,15,21,36,48] In the tetrahedral intermediate **2a** due to the pyramidal nature of nitrogen, the planarity of the ring is distorted from (<C2C3C4C5) 11.4° in **1** to −27.7° . Also in **2a** note the lengthening of the N1–C5 bond (+0.150 Å) as compared to its homologue in the succinimide ring **1**.

In the concerted path, the cleavage of the N1–C5 bond and the proton transfer to the nitrogen are carried out simultaneously with a four membered transition state, **TS(2–4)**,

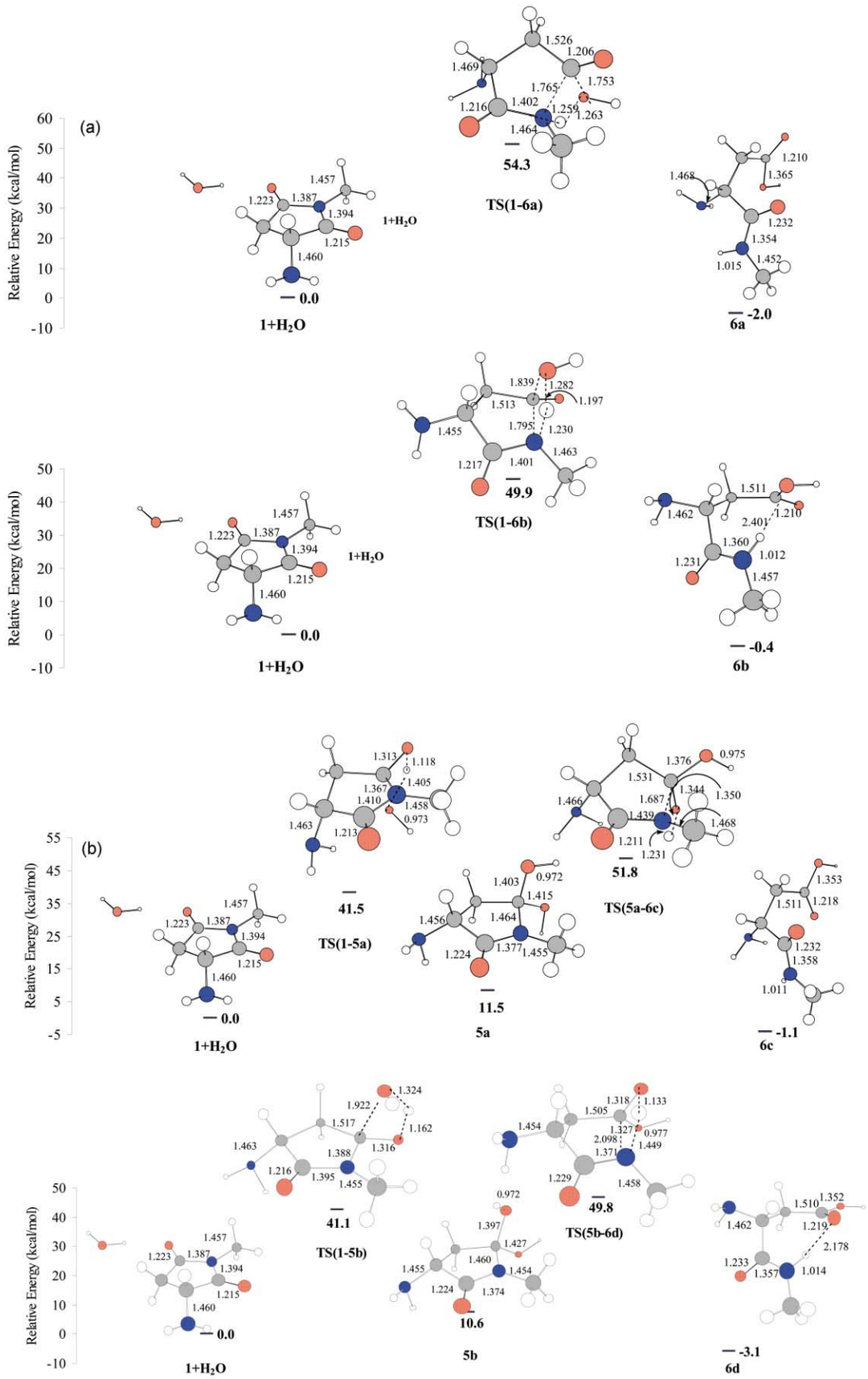


Fig. 2 a. Reaction pathway for the concerted mechanism in alkali medium. (Relative electronic energies (kcal mol^{-1}) include zero point correction) b. Reaction pathway for the stepwise mechanism in neutral medium. (Relative electronic energies (kcal mol^{-1}) include zero point correction).

where the proton rotates towards the lone pairs of N1. The ring is almost closed and the N1–C5 bond length is 1.887 Å. The reaction barrier is 7.6 kcal mol^{-1} and the reaction is exothermic by 64.9 kcal mol^{-1} (Fig. 1a).

In the stepwise mechanism, the rate determining step is the cleavage of the five membered succinimide. An intermediate (**3**) is formed followed by the cleavage of the N1–C5 bond. The N1–C5 bond length varies between 2.750 Å and 2.800 Å indi-

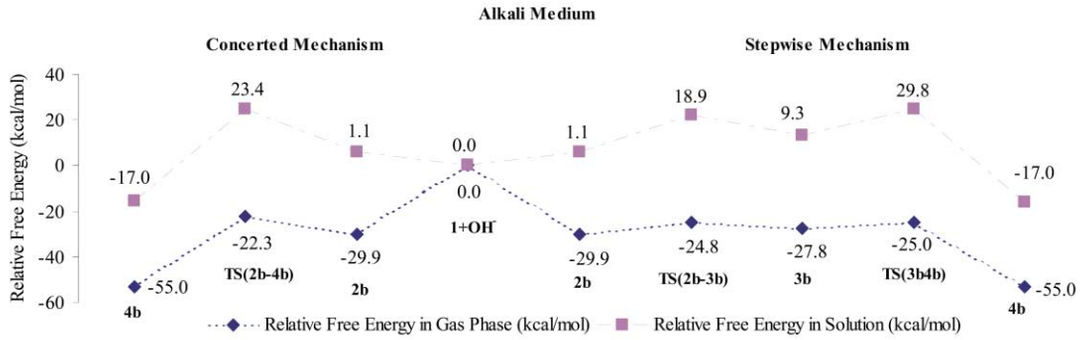


Fig. 3 Comparison of the relative free energies (kcal mol^{-1}) in the gas phase and in solution for both the concerted and the stepwise mechanisms in alkali medium.

Table 1 Energetics (B3LYP/6-31+G*) in a vacuum and in solution

	1 [a]	**2** [b]	**3** [c]
1 + OH−	−530.993284	−531.049195	−531.326664
2a	−531.061826	−531.096612	−531.323460
2b	−531.061556	−531.096845	−531.324929
3a	−531.056192	−531.093545	−531.310598
3b	−531.056243	−531.093440	−531.311779
4a	−531.096678	−531.133971	−531.353020
4b	−531.098911	−531.136884	−531.353805
4c	−531.098910	−531.136878	−531.353805
TS(2a–3a)	−531.052285	−531.088146	−531.296206
TS(2b–3b)	−531.053302	−531.088780	−531.296593
TS(3a–4a)	−531.050324	−531.086943	−531.292373
TS(3b–4b)	−531.052288	−531.088991	−531.293489
TS(2a–4c)	−531.049664	−531.084265	−531.289188
TS(2b–4b)	−531.049912	−531.084686	−531.289422
1+H_2O	−531.611528	−531.663715	−531.804760
5a	−531.593217	−531.628646	−531.786393
5b	−531.594582	−531.630061	−531.785438
6a	−531.614656	−531.653071	−531.804170
6b	−531.612130	−531.650869	−531.802058
6c	−531.613263	−531.651721	−531.805093
6d	−531.616451	−531.654628	−531.806475
TS(1–5a)	−531.545383	−531.581174	−531.723750
TS(1–5b)	−531.546060	−531.582382	−531.721272
TS(5a–6c)	−531.528958	−531.563756	−531.711814
TS(5b–6d)	−531.532094	−531.568164	−531.715778
TS(1–6a)	−531.524983	−531.560898	−531.706782
TS(1–6b)	−531.531931	−531.567731	−531.710441

[a] **1**. Total electronic energies including zero point energies (Hartrees) for optimized gas phase geometries. [b] **2**. Total free energies (Hartrees) for optimized gas phase geometries. [c] **3**. Total free energies of solvation (Hartrees) for single point calculations in solution.

cating that it is almost cleaved. In structure **3b** the N1–C2 bond is shortened from 1.337 Å to 1.316 Å relative to structure **2b**, and a lengthening in the C2–O7 bond from 1.248 Å to 1.275 Å is observed due to delocalization of the charge on the nitrogen atom. This delocalization stabilizes all the isomers of structure **3** by forming an imine-like structure. The transition state **TS(2–3)**, which is located between structures **2** and **3**, is characterized by a partially cleaved amide N1–C5 bond. The activation energy barriers of **TS(2a–3a)** and **TS(2b–3b)** are 6.0 kcal mol^{-1} and 5.1 kcal mol^{-1} respectively. The five membered succinimide ring is no longer planar. The first step is endothermic, the transition state resembles compound **3** more than compound **2** as expected based on the Hammond's postulate. Ring opening precedes the proton transfer from structure **3** to **4**. The proton transfer from O18 to N1 occurs through a rotational transition state, **TS(3–4)** with a rotation around the C5–O18 bond. The hydrogen (H19) atom is still tightly bound to the oxygen (O18) (0.974 Å). The N1–H19 distance is considerably reduced with respect to its value in **3a** and **3b**. The energy of activation for this transition state is 3.7 or 2.5 kcal mol^{-1} depending on the orientation of –NH_2 and –OH groups with respect to each other. The overall exothermic reaction, is terminated with the formation of aspartate (**4**) (Fig. 1b).

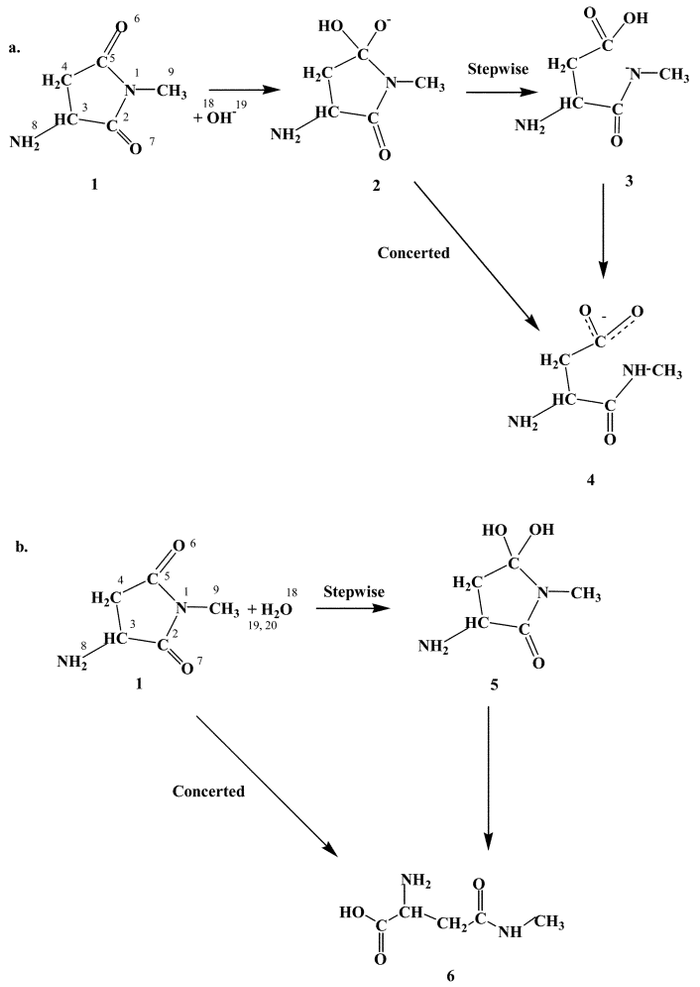
In their theoretical study, Weiner *et al*. studied a gas phase hydrolysis reaction of formamide with a hydroxide anion.[13] They analyzed the nucleophilic addition of hydroxide ion for various distances between the hydroxyl and the carbonyl carbon. They located an optimized tetrahedral anionic intermediate without any barrier. The energy of this intermediate is 47.0 kcal mol^{-1} lower relative to the reactants at an infinite separation distance.[13] There are other studies based on the fact that hydroxide anion addition occurs barrierless for gas phase reactions.[15,19,36] In this study, a tetrahedral anionic intermediate more stable by 29.9 kcal mol^{-1} relative to the reactants on the free energy surface has been located in vacuum (Fig. 3). In contrast to gas phase results, theoretical studies in solution have shown that when the reaction occurs in water, a barrier exists for the addition of a hydroxyl anion to the amide linkages. In a study on hydrolysis reactions of β-lactams, Pitarch *et al*. observed a 2.2 kcal mol^{-1} barrier at 1.933 Å distance from the carbonyl carbon.[15] Similarly, Kollman *et al.* have found a barrier along the potential energy surface for the hydrolysis reaction of formamide in basic medium.[13] In this work, in order to simulate the reaction profile for the addition of hydroxyl, the reaction has been considered with single point free energy calculations in solution. The distance of OH^- to the carbonyl carbon is varied from 1.500 Å to 1.900 Å. A transition structure where OH^- is at a distance of 1.850 Å from C5 is found to be 20.0 kcal mol^{-1} higher in energy than the tetrahedral complex **2a** as shown in Fig. 4. The presence of the solvent has altered the thermochemistry and the kinetics of the reactions.

Aspartatic acid formation-neutral medium

In agreement with the previous studies on cyclic β-lactams, the succinimide ring has a planar structure (C2C3C4C5 −11.4°).[15] According to the side of the attack of the water, either from the upper or lower side of the ring, two isomers are formed due to the presence of –NH_2 on the chiral C3 atom.

The concerted mechanism proceeds through either the transition structure **TS(1–6a)** or **TS(1–6b)** depending on the side of the nucleophilic attack. The planarity of the ring is distorted following the pyramidal geometry of the nitrogen, as indicated by the change of the value of the dihedral angle C9N1C2C5 from 178.9° in structure **1** to −131.7° in **TS(1–6a)**. The presence of stabilizing long range interactions between N8 and H20 (2.296 Å) and a delocalization of electrons along the C5–O6 bond in **TS(1–6b)** (1.197 Å *vs* 1.206 Å) makes this structure energetically favorable relative to **TS(1–6a)**. Structures **6a** and **6b** are 1.1 and 2.7 kcal mol^{-1} higher in energy than the global minimum **6d** that is stabilized through an intramolecular hydrogen bond between O18 and H19 (2.178 Å).

Along the stepwise mechanism water adds to the carbonyl carbon of the succinimide ring to form the diol intermediate **5a** (**5b**) then the N1–C5 bond cleaves and the transfer of H19 to N1 forms aspartate. In **TS(1–5a)** the distance of the transferred



Scheme 2

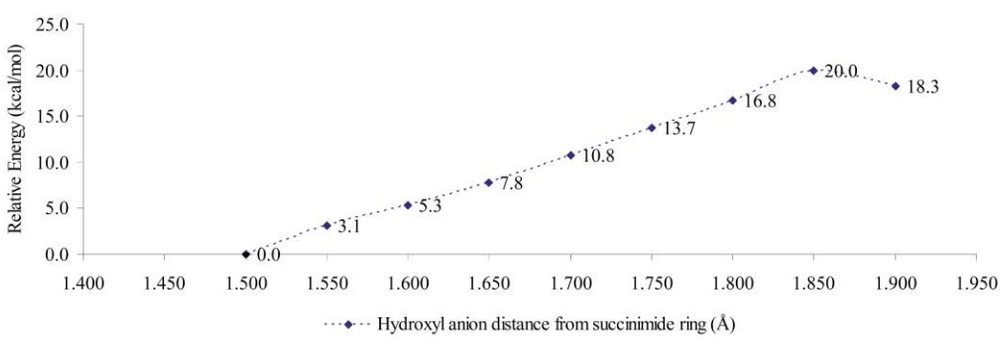


Fig. 4 Free energy path for the addition of the hydroxyl anion to the succinimide ring in solution.

proton (H19) to the water oxygen is 1.405 Å and is larger than the distance to the carbonyl oxygen (O7) (1.118 Å).

Structures **5a** and **5b** are intermediate structures where the diol is formed. The diol intermediate undergoes a ring opening and a proton transfer synchronously. **TS(5a–6c)** and **TS(5b–6d)** represent the transition structures in which the proton transfers from the lower or upper sides of the ring respectively. In **TS(5a–6c)** the proton transfer is more advanced relative to **TS(5b–6d)** (1.231 Å *vs* 1.449 Å). However, in **TS(5b–6d)**, the ring opening is more prone to occur relative to **TS(5a–6c)** (2.098 Å *vs* 1.697 Å). In **TS(5b–6d)**, the N1–C2 distance is shortened to 1.371 Å as compared to the same distance (1.439 Å) in **TS(5a–6c)**. Therefore an imine-like structure is only observed for **TS(5b–6d)**. The geometrical features of the species in this path are different from the ones in the concerted path. The product obtained by IRC calculation, **6d** is more stable than **6b**.

It is known that the neutral hydrolysis of amide linkages has higher energy barriers in both stepwise and concerted paths.[15–16] Although both mechanisms possess high barriers, the stepwise mechanism is energetically favorable. In the stepwise mechanism, the first step is endothermic but the overall process is exothermic. In the concerted path, the reaction is exothermic. When the entropy factor is added, both reactions turn out to be endoergic. It is an experimental observation that amides do not undergo hydrolysis in neutral medium at low temperatures.[15–16] At high temperatures, the process is slow. The barrier for the rate limiting step, including ZPE corrections, is 49.9 kcal mol^{-1} for the concerted reaction and 39.2 kcal mol^{-1} for the stepwise path respectively (Fig. 2a,b).

The energy barriers accompanying the hydrolysis reaction of the succinimide in alkali medium are considerably lower than the ones in neutral medium at 25 °C and 1 atm. The hydrolysis

Table 2 Energetics in a vacuum (B3LYP/6-31+G*) and in solution (B3LYP/6-31G*)

		1+H_2O	5b	TS(1–5b)	TS(5b–6d)
Aspartate	**1**[a]	−531.599741	−531.584141	−531.548042	−531.521591
	2[b]	−531.620653	−531.552388	−531.621428	−531.552695
Isoaspartate	**1**[a]	−531.599741	−531.585782	−531.539217	−531.523962
	2[b]	−531.620653	−531.553237	−531.605903	−531.538331

[a] **1**. Total electronic energies including thermal corrections (Hartrees) in a vacuum (full optimization). [b] **2**. Total electronic energies including thermal corrections (Hartrees) in water (full optimization).

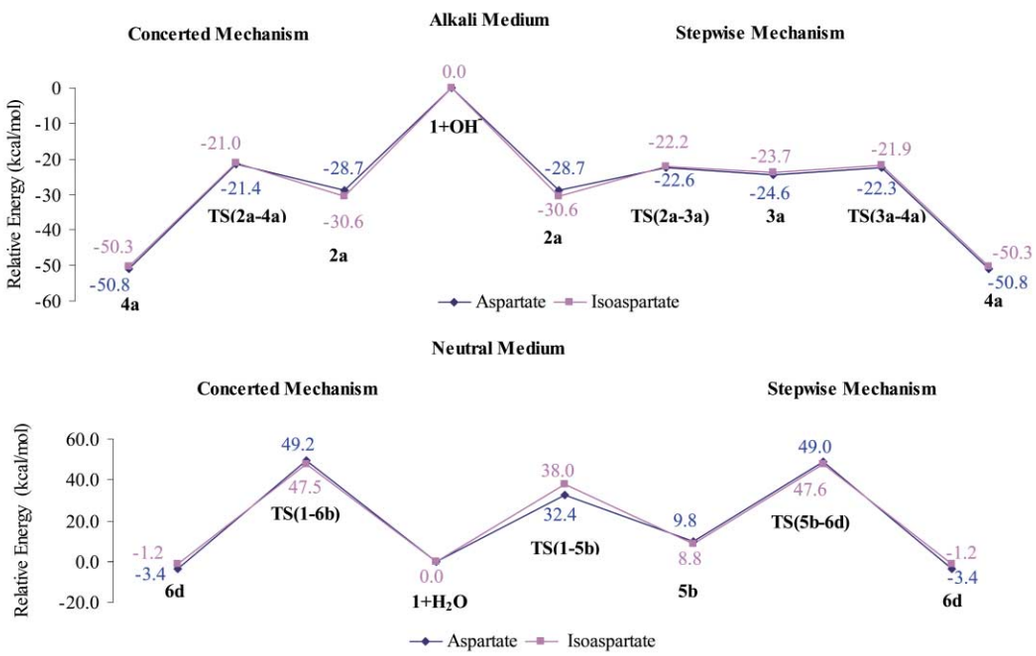


Fig. 5 a. Energetics of the isoaspartate and aspartate formation in alkali medium *via* concerted and stepwise mechanisms. (Relative electronic energies (kcal mol^{-1}) include thermal corrections). b. Energetics of the isoaspartate and aspartate formation in neutral medium *via* concerted and stepwise mechanisms. (Relative electronic energies (kcal mol^{-1}) include thermal corrections).

in alkali medium is preferable to the same reaction in neutral medium both kinetically and thermodynamically.

Comparison of isoaspartate/aspartate formation from succinimide

In basic medium, the activation barriers for both the concerted and the stepwise paths for aspartate formation are smaller than the ones for isoaspartate (Fig. 5a). These results have been rationalized by considering the intermediates located in both processes. The intermediate formed after the attack of OH^- is different in the two cases in terms of the vicinity of the NH_2 group to the reactive site. In the case of isoaspartate the NH_2 group stabilizes the reactive site through long range interactions. The transition structures in aspartate and isoaspartate cases are similar to each other. In **TS(2–3)** although long range stabilizing interactions are present in isoaspartate, the formation of imine-like structure has not started yet. In the case of aspartate, for **TS(2–3)**, the charge on nitrogen is more delocalized than in isoaspartate. The elongation in C2–O7 (1.246 Å–1.266 Å) and shortening of N1–C2 (1.351 Å to 1.316 Å) bonds as well as natural bond orbital studies confirm the delocalization of the electrons on N1. Therefore when the activation barriers are compared it is found that the barrier for the isoaspartate formation is higher than the one for aspartate formation.

According to the experimental studies at neutral pH, it is found that the relative abundance of isoaspartate : aspartate is 3.1 : 1 for a hexapeptide (Val–Tyr–Pro–Asn–Gly–Ala) in a study by Clarke and Gieger.[5] and a tetrapeptide (Ac–Gly–Asn–Gly–Gly–NHMe) in a study by Capasso *et al*.[6] In order to mimic the experimental conditions, the relative abundance of the two species has been calculated based on the energy barriers obtained in neutral medium by the addition of a water molecule to the succinimide ring. The activation barriers based on the rate-determining step (**TS(5b-6d)**) with thermal corrections both in the gaseous state and in solution have been used for the evaluation of the rate constants (Table 2). The rate equation based on the activated complex has yielded the isoasparte : aspartate ratio to be 2.2 : 1 for the dipeptide model in the gas phase. The same ratio has been found to be 3.4 : 1 in solution, confirming the validity of the models and the methodologies used.

Isomerization reaction

The isomerization reaction of aspartate to isoaspartate occurs *via* a succinimide derivative, and is found to be much slower than deamidation at neutral and basic pH.[49] The reaction involves initial deprotonation of the NH group next to aspartate residue followed by attack of the nitrogen atom on the carbonyl carbon of the side chain leading to succinimide (Scheme 1).

Capasso has used the temperature dependence of equilibrium constants to deduce the heat of reaction ($\Delta H°$) and entropy ($\Delta S°$) for the isomerization reaction "aspartate ↔ aminosuccinyl↔isoaspartate".[49] The average values of $\Delta H°$ and $\Delta S°$ for the cyclization reactions of the carboxylic acid form of the Asp and β-Asp side chains of the dipeptides and tetrapeptides are 34.3 kJ mol^{-1} and 131 J mol^{-1} K^{-1} respectively. The cyclization reaction to the succinimide derivative is accompanied by the release of a mole of water or ammonia, thereby involving a substantial gain in translational entropy.[50] Moreover, because of the atoms immobilized in the five

membered ring, the process probably involves a loss of internal rotational entropy, and an increase of strain energy. In his study, Capasso has also found the preference for the isoaspartate formation since under neutral and basic pH the concentrations of aminosuccinyl residues were very low and the isoaspartates were dominant.

In our study, the ΔH° and ΔS° values for the cyclization reactions of the succinimide from the aspartic acid are 29.2 kJ mol^{-1} and 133.5 J mol^{-1} K^{-1} respectively. These values are in line with the measurements of Capasso even though the experimental isomerization reaction was based on a tetrapeptide (Ac–Gly–Asn–Gly–Gly–NHMe) as compared to the dipeptide modelled in this work.

Conclusion

The present study was stimulated by our interest in deamidation and hydrolysis reactions in the enzyme triosephosphate isomerase. The alkaline and neutral hydrolysis of succinimide leading to aspartate and aspartic acid has been studied in the gas phase and in solution respectively. For both neutral and alkaline hydrolysis two possible reaction mechanisms (concerted and stepwise) have been analyzed. The stepwise mechanism is preferred in both alkali and neutral media due to the formation of a stable intermediate in the reaction paths.

In a neutral medium, both the stepwise and the concerted paths have higher energy barriers relative to those in an alkali medium. Furthermore the reaction in an alkali medium is more exothermic. The hydrolysis reaction in an alkali medium is preferable with respect to the reaction in a neutral medium both kinetically and thermodynamically.

The ΔH° and ΔS° values for the cyclization reactions of the succinimide from the aspartic acid are found to be 29.2 kJ mol^{-1} and 133.5 J mol^{-1} K^{-1} respectively which is in line with experimental results.[49]

The isoaspartate : aspartate ratio calculated from the rate determining step of the neutral stepwise reaction path is 2.2 : 1 according to the activation barriers obtained from electronic energies including thermal corrections in line with the experimental findings based on the hydrolysis reaction[5,6] of the tetrapeptide (Ac–Gly–Asn–Gly–Gly–NHMe) and the hexapeptide (Val–Tyr–Pro–Asn–Gly–Ala). A quantitative agreement with the experiment (3.4 : 1) is obtained when electrostatic interactions with the bulk solvent are considered.

The results of the present study indicate that calculations on a model compound in solution can be used to mimic the hydrolysis of a peptide bond in enzymes without considering the whole molecule unless special interactions are present.

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References

1 H. T. Wright, *CRC Crit. Rev. Biochem.*, 1991, **26**, 1.
2 J. J. Harding, in *Adv. Protein Chem.*, eds. C. B. Anfinsen, J. T. Edsalll, and F. M. Richards, vol 37, Academic Press, Orlando, 1985, 247.
3 S. E. Zale and A. M. Klibanov, *Biochemistry*, 1986, **25**, 5432.
4 S. W. Berson, F. R. Cruickshank, D. M. Golden, D. R. Haugen, H. E. O'Neal, A. S. Rodgers, R. Shaw and A. Walsh, *Chem. Rev.*, 1969, **69**, 279.
5 T. Gieger and S. Clarke, *J. Biol. Chem.*, 1987, **262**, 785.
6 S. Capasso, L. Mazzarella, F. Sica and A. Zagon, *Peptide Res.*, 1989, **2**, 195.
7 M. Bodanszky and J. Z. Kwei, *Int. J. Pept. Protein Res.*, 1978, **12**, 69.
8 T. V. Brennan and S. Clarke, *Protein Sci.*, 1993, **2**, 331.
9 C. Oliyai, J. P. Patel, L. Carr and R. T. Brochardt, *Pharm. Res.*, 1994, **11**, 901.
10 S. Capasso, G. Balboni and P. Cerbo Di, *Biopolymers*, 2000, **53**, 213.
11 R. C. Stephenson and S. Clarke, *J. Biol. Chem.*, 1989, **264**, 6164.
12 T. Oie, G. H. Loew, S. Burt, J. S. Binkley and R. D. Mac Elroy, *J. Am. Chem. Soc.*, 1982, **104**, 6174.
13 S. J. Weiner, U. C. Singh and P. A. Kollman, *J. Am. Chem. Soc.*, 1985, **107**, 2219.
14 J. S. Kwiatkowski and J. Leszczynski, *J. Mol. Struct.*, 1993, **297**, 277.
15 J. Pitarch, M. F. Ruiz-Lopez, J. L. Pascual-Ahuir, E. Silla and I. Tunon, *J. Phys. Chem.B.*, 1997, **101**, 3581.
16 J. Pitarch, M. F. Ruiz-Lopez, J. L. Pascual-Ahuir, E. Silla and I. Tunon, *J. Am. Chem. Soc.*, 1998, **120**, 2146.
17 M. Coll, J. Frau, F. Munoz and J. Donoso, *J. Phys. Chem A.*, 1998, **102**, 5915.
18 M. Coll, J. Frau, F. Munoz, B. Vilanova, J. Donoso and F. G. Blanco, *J. Phys. Chem A.*, 1999, **103**, 8879.
19 M. Coll, J. Frau, F. Munoz, B. Vilanova, J. Donoso and F. G. Blanco, *J. Phys. Chem B.*, 2000, **104**, 11389.
20 J. Hine, R. S. King, W. R. Midden and A. Sinha, *J. Org. Chem.*, 1981, **46**, 3186.
21 J. P. Krug, P. L. A. Popelier and R. F. W. Bader, *J. Phys. Chem.*, 1992, **96**, 7604.
22 S. Antonczak, M. F. Ruiz-Lopez and J.-L. Rivail, *J. Am. Chem. Soc.*, 1994, **116**, 3912.
23 S. Antonczak, M. F. Ruiz-Lopez and J.-L. Rivail, *J. Mol. Mod.*, 1997, **3**, 434.
24 K. D. Dobbs and D. A. Dixon, *J. Phys. Chem.*, 1996, **100**, 3965.
25 K. Hori, A. Kamimura, K. Ando, M. Mizumura and Y. Ihara, *Tetrahedron*, 1997, **53**, 4317.
26 Y.-J. Zheng and R. L. J. Ornstein, *J. Mol. Struct. (Theochem)*, 1998, **429**, 41.
27 B. Kallies and R. Mitzner, *J. Mol. Mod.*, 1998, **4**, 183.
28 D. Bakowies and P. A. Kollman, *J. Am. Chem Soc.*, 1999, **121**, 5712.
29 J. Frau, J. Donoso, F. Munoz and F. Garcia Blanco, *J. Comput. Chem.*, 1992, **13**, 681.
30 J. Frau, J. Donoso, F. Munoz and F. Garcia Blanco, *J. Comput. Chem.*, 1993, **14**, 1545.
31 J. Frau, J. Donoso, F. Munoz and F. Garcia Blanco, *J. Mol. Struct. (Theochem)*, 1997, **390**, 247.
32 A. Alex and T. Clark, *J. Comput. Chem.*, 1992, **13**, 704.
33 J. Pitarch, J-L Pascual-Ahuir, E. Silla, I. Tunon and M. F. Ruiz-Lopez, *J. Comput. Chem.*, 1999, **20**, 1401.
34 S. Antonczak, G. Monard, M. F. Ruiz Lopez and J-L Rivail, *J. Am. Chem. Soc.*, 1998, **120**, 8825.
35 S. Antonczak, G. Monard, M. F. Ruiz Lopez and J.-L. Rivail, *J. Mol. Mod.*, 2000, **6**, 527.
36 F. A. Konuklar, V. Aviyente and M. Ruiz-Lopez, *J. Phys. Chem. A*, 2002, **106**, 11205.
37 J. J. P. Stewart, *J. Comput. Chem.*, 1989, **10**, 221.
38 Spartan Version 4.0, Wavefunction, Inc. 18401 Von Karman Ave., #370 Irvine, CA 92715, USA, 1996.
39 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648.
40 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. G. Johnson, W. Chen, M. W. Wong, J. L. Andres, M. Head-Gordon, E. S. Replogle and J. A. Pople, GAUSSIAN 98 (Revision A.7), Gaussian, Inc., Pittsburgh, PA, 1998.
41 J. Madura and W. L. Jorgensen, *J. Am. Chem. Soc.*, 1986, **108**, 2517.
42 K. Fukui, *Acc. Chem. Res.*, 1981, **14**, 363.
43 C. M. Breneman and K. B. Wiberg, *J. Comput. Chem.*, 1990, **11**, 36.
44 A. E. Reed, Weinstock and F. Weinhold, *J. Chem. Phys.*, 1985, **83**, 735.
45 J. P. Foster and F. Weinhold, *J. Am. Chem. Soc.*, 1980, **102**, 7211.
46 A. E. Reed and F. Weinhold, *J. Chem. Phys.*, 1985, **83**, 1736.
47 J. Tomasi and S. Miertus, *Chem. Phys.*, 1982, **65**, 239.
48 S. Wolfe, C. Kim, K. Yang, N. Weinberg and Z. Shi, *J. Am. Chem. Soc.*, 1995, **117**, 4240.
49 S. Capasso, *Thermochim. Acta*, 1996, **286**, 41.
50 M. I. Page, *Chem. Soc. Rev.*, 1973, **2**, 295.