The Interaction of Protonated Diglycine with Ammonia: A Density Functional Theory Model Study

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The interaction of protonated diglycine, GLY_2H^+, with ammonia has been studied through density functional theory (DFT) calculations of structures and energetics at the B3LYP/6-31+G**// B3LYP/6-31+G** level. Five GLY_2H^+/NH_3 complexes were located which can be categorized as hydrogen-bonded ion/dipole complexes: one at the N-terminus, two at the C-terminus, and two at the amide bond. Two GLY_2/NH_4^+ complexes were located in which the proton had shifted from diglycine to ammonia: one at the N-terminus and one at the amide bond. Potential energy profiles including transition states were constructed. The profiles for complexation at the N-terminus and at the C-terminus demonstrate fairly deep wells (21 and 18 kcal/mol, respectively). The profile for complexation at the amide bond has a relatively shallow well (14 kcal/mol). The profiles for complexation at the N-terminus and at the amide bond are quite flat with very low intermediate barriers between the complexes. The computational results are discussed in the light of previously proposed mechanisms for H/D exchange between ND_3 and protonated peptides, in particular protonated diglycine. Exchange takes place at the N-terminus via the "onium" mechanism. The salt-bridge structure suggested as part of the H/D exchange mechanism at the C-terminus, in which the NH_4^+ ion stabilizes a zwitterion structure of the peptide, is observed but only as a transition-state structure along the reaction profile. Exchange of the amide hydrogen takes place via a tautomerized peptide structure with a partial salt-bridge character. The relatively deep wells (∼20 kcal/mol) on the one hand and the shallow well (∼14 kcal/mol) on the other are in agreement with the previous observation of at least two chemically activated collision complexes with quite different lifetimes.

I. Introduction

There has been an increasing interest in recent years in anhydrous protein and peptide ions.[1] Evidence has been presented that the conformational properties of biomolecules in solution are preserved during the process of electrospray ionization (ESI) that is used in mass spectrometry (MS) to introduce these biomolecules into the gas phase. Hydrogen−deuterium (H/D) exchange has been used as a probe of gas-phase ion conformation. The generally held idea has been that compact structures protect some labile hydrogen atoms from H/D exchange in the gas phase. As a result, open conformers are expected to reach higher levels of exchange than compact ones. H/D exchange by MS has complemented NMR to give insight into the populations and structures of folding intermediates.[2] ESI was combined with Fourier transform ion cyclotron resonance (FTICR) spectrometry and correlations were drawn between specific H/D exchange levels observed in the gas phase and conformations that had been characterized in solution.[3] H/D exchange was studied for shape-resolved cytochrome *c* conformers preselected through their drift velocities in ion-mobility experiments.[4] Many sites that rapidly exchange in solution seemed to be restricted for exchange in the gas phase, even for very open conformers. In addition, the level of H/D exchange, observed for seemingly identical conformers, was considerably lower in the drift tube[4] than in the FTICR experiment.[3,5] It was concluded that any structural assignments made from H/D exchange alone must be interpreted with caution.

The proper interpretation of H/D exchange experiments requires a detailed knowledge of the reaction mechanisms involved, and studies of glycine oligomers as model systems have been reported by Beauchamp and co-workers.[6−8] Several other groups have studied H/D exchange between protonated peptides and deuterated solvent molecules.[9−18] ND_3 was the most efficient reagent studied for promoting H/D exchange of protonated glycine oligomers. Other reagents studied include D_2O, CD_3OD, DI, and CD_3CO_2D. On the basis of these studies, a general classification was proposed for possible H/D exchange mechanisms. In Type I, the labile proton at the charge site is intimately involved. For example, exchange of the three hydrogens on the protonated N-terminus amino group of glycine oligomers with ND_3 as the reagent gas proceeds via an "onium" mechanism in which the endothermicity of proton transfer to ammonia is compensated by intermolecular solvation of the resultant ammonium ion. In Type II, protons at the charge site are not directly involved. An example is the salt-bridge mechanism in which proton transfer to a basic exchange reagent leads to a charge-separated ion pair that can be stabilized by favorable interaction with a proximal charge (e.g., the protonated N-terminus). Exchange of carboxylic acid and amide hydrogens in glycine oligomers may proceed via a Type II salt-bridge mechanism.

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The generally accepted[12(e)] simplified mechanism[19,20] for H/D exchange between a protonated substrate and a deuterated reagent in the gas phase consists of three steps: the initial formation of a loose hydrogen-bonded complex, complete or partial transfer of the proton to the reagent, resulting in isotope scrambling, and dissociation of the complex to yield either the original or the exchanged substrate species. For exchange to be observed, the energy made available by complex formation must be sufficient to overcome the barrier to internal proton transfer. This barrier depends on the proton affinity difference, ΔPA, between the substrate and the reagent. The proton affinities of all the exchange reagents used are much lower than those of the peptide substrates, with that of ND_3 being the highest (204 kcal/mol). The proposed H/D exchange mechanism invokes formation of an intermediate complex with multiple hydrogen bonding. Multiple hydrogen bonding in the complex allows overcoming the proton transfer barrier in the ND_3 case.

Multiply hydrogen-bonded complexes are of great importance in chemical and biological systems. In recent years, our group has studied[21−24] the formation of complexes in the gas phase from proton-bound clusters and protonated crown ethers. Ammonia and methanol, which are well-known isotope exchange reagents for peptides and proteins, are precisely the base molecules that were observed to undergo insertion reactions into proton-bound dimers[21] and protonated 12-crown-4-ether,[24] giving multiply hydrogen-bonded species. We have demonstrated recently[25] that an electrospray ion source combined with a flow-tube reactor can be used to study ion/molecule reactions of protonated diglycine, GLY_2H^+, under carrier gas pressures of several tenths of a Torr. We have furthermore observed the formation of collisionally stabilized complexes of GLY_2H^+ with NH_3, methanol, and a series of amines and studied their formation kinetics. In a study of H/D exchange with ND_3,[26] we monitored for the first time the collision complexes corresponding to the consecutive H/D exchanges of the five labile hydrogens.

Protonated diglycine is the simplest peptide, yet it has most of the characteristic labile protons of larger peptides and proteins: three at the protonated amine N-terminus, one at the amide bond, and one at the carboxyl C-terminus. Its advantage is that it can be calculated with modern quantum chemical methods. That is the topic of our current research effort to be described here. These calculations can then be compared with and discussed in light of the previous experimental data. Previous efforts have involved semiempirical AM1, PM3, and molecular mechanics calculations.[6−8] Protonated glycine oligomers were found to bind the proton preferentially to the N-terminus with the peptide folding up to solvate the charge by forming hydrogen bonds to carbonyl oxygens. Potential energy profiles were calculated for H/D exchange with ND_3 by the onium, tautomer, and salt-bridge (zwitterion) mechanisms. Molecular dynamics calculations were performed[14,15] on polyprotonated peptides to gain insight into conformations. Ab initio calculations were performed by the same group[27,28] to determine proton affinities, gas-phase basicities, and structures of polyglycines and other small peptides. As expected, density functional theory (DFT) calculations of proton affinities of polyglycines gave better agreement with experimental proton affinities than semiempirical (AM1 and PM3) values.[29] Recently, we have successfully applied DFT calculations to proton-bound dimers and to their multiply hydrogen-bonded ammonia complexes,[30] to gaseous glycine and its derivatives,[31] and to the protonated betaine/ammonia complex.[32] Here we describe results of DFT calculations on proton-bound ammonia/diglycine complexes. A major objective is the clarification of the gas-phase H/D exchange mechanism of protonated peptides and proteins. This is expected to lead eventually to conclusions concerning the connection between the efficiency of the exchange and the structural conformation of the protein.

II. Methods

Density functional calculations were performed using the Gaussian 98 package[33] running on a DEC Alpha TurboLaser 8400 computer at the Institute of Chemistry, Hebrew University, on a Cray J932 at the Israeli Inter-University Computation Center and on a DEC Alpha 233/4 and a DEC Alpha 433 at the Chemistry Department, Bogaziçi University. The geometries of the reactants, products, intermediates, and transition states were optimized using the B3LYP (Becke three-parameter Lee−Yang−Parr) exchange-correlation functional[34,35] which combines the hybrid exchange functional of Becke[34] with the gradient-corrected correlation functional of Lee et al.[35] by means of the Berny geometry optimization algorithm. The excellent performance of this method for geometries and harmonic frequencies was noted previously (see ref 30 and references therein). Previous experience[31,36] has taught us that the use of diffuse functions and polarization functions is of vital importance in systems with hydrogen bonds. At least the 6-31+G** basis set had to be used in conjunction with the B3LYP method for small hydrogen-bonded dimers.[37] This is the basis set used in the present relatively large system of the proton-bound ammonia/diglycine complexes. It has been shown in the case of monoglycine[31] to yield structures that are in reasonable agreement with those obtained at higher levels of theory. Vibrational frequencies were calculated at the B3LYP/6-31+G** level for all the stationary geometries. Real vibrational frequencies confirmed the presence of minima on the potential energy surface; one imaginary frequency for the bond of interest indicated the existence of a transition state. An intrinsic reaction coordinate (IRC) calculation[38,39] was performed at the B3LYP level with mass-weighted internal coordinates to ascertain that a transition state belongs to the reaction coordinate of interest. The IRC path was computed using the same basis set that was used for the stationary point optimization. The single-point energy calculations for the optimized geometries were performed by B3LYP/6-31+G**. Taking into account correlation energy through second-order Møller−Plesset perturbation theory (MP2),[40] the relative energies of one set of low-lying transition states were recalculated. The calculated vibrational frequencies were used for zero-point energy (ZPE) corrections. The charge distributions were derived from Mulliken population analyses[40] to assess the importance of salt-bridge complexes.

III. Results

Protonated diglycine, GLY_2H^+, was observed to form seven complexes upon interaction with ammonia, among which five, I, III, IV, V, and VI, are ion/dipole complexes, GLY_2H^+/NH_3. In two of the complexes, II and VII, which can be presented schematically as GLY_2/NH_4^+, the proton shifted from the diglycine to ammonia. Potential energy profiles for the reaction paths in which these complexes are involved are presented in Figures 1, 2, and 3. Structures of complexes I and II and of transition state (TS) 1 connecting them are included in Figure 1. Structures of complexes III and IV and of TS2 are included in Figure 2. The structures of complexes V, VI, and VII, and of TS3 and TS4 are shown in Figures 4 and 5, respectively. The energies for all the species under consideration are summarized in Table 1.

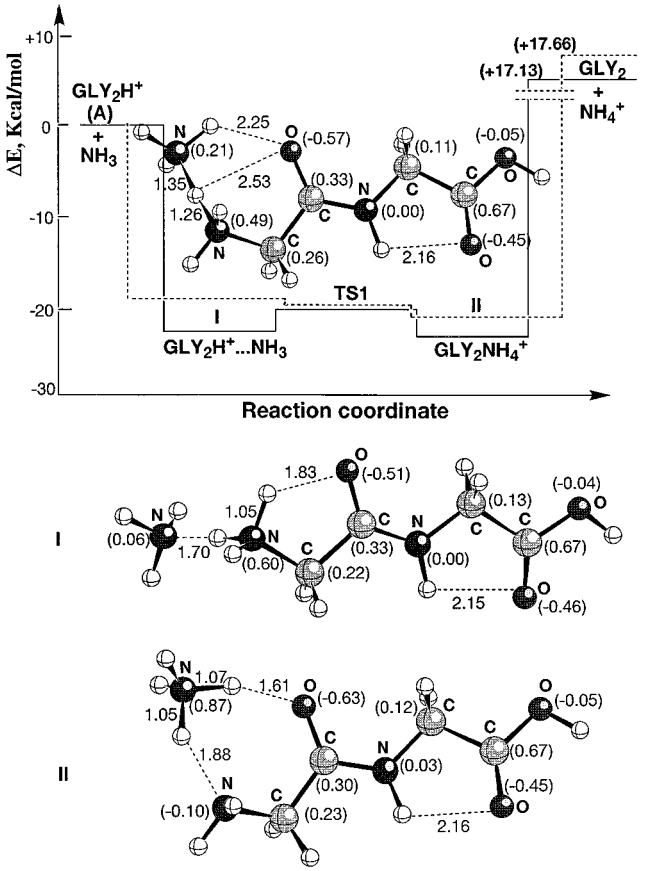

Figure 1. Computed potential energy profile (at the B3LYP/6-31+G**//B3LYP/6-31+G** level of theory) for the interaction of ammonia with protonated diglycine at the N-terminus. Continuous line, without ZPE; dashed line, with ZPE. The computed structures for complexes I, II, and TS1 are presented. Selected bond lengths (in Å) and local charges (in parentheses), based on the Mulliken population analysis, are included.

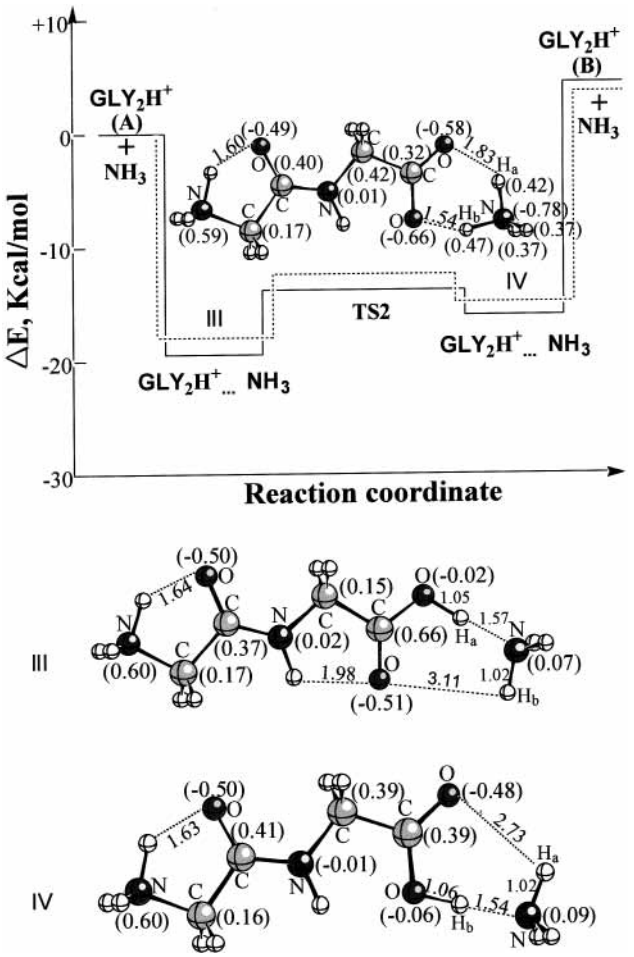

Figure 2. Computed potential energy profile (at the B3LYP/6-31+G**//B3LYP/6-31+G** level of theory) for the interaction of ammonia with protonated diglycine at the C-terminus. Continuous line, without ZPE; dashed line, with ZPE. The computed structures for complexes III, IV, and TS2 are presented. Selected bond lengths (in Å) and local charges (in parentheses), based on the Mulliken population analysis, are included.

IV. Discussion

The discussion is planned along two major lines. First, the H/D exchange mechanisms suggested by Beauchamp and coworkers[7] invoked an "onium" complex at the N-terminus, a salt-bridge complex at the C-terminus, and formation of a tautomer of the peptide for exchange of the amide hydrogens of the peptides. The complexes and transition structures found in the present calculations are discussed in the light of these suggested mechanisms. Diglycine contains all the complexing sites of higher peptides: the N-terminus, the C-terminus, and the amide bond. The experiments and semiempirical calculations of Beauchamp's group involved triglycine, because triglycine was considered to be a better small representative of higher oligomers, but triglycine would have been too difficult a task for the present higher level calculations. Second, the kinetics of complex formation and of high pressure (0.1−0.4 Torr) H/D exchange in the electrospray−flow tube experiments[25,26] are discussed in relation to the new computational findings.

Both complexes I and II (Figure 1) involve the interaction of ammonia with the amine end of diglycine. Complex I is an ion/dipole complex between ammonia and protonated diglycine. The ammonia nitrogen is hydrogen-bonded to one of the protons of the $-NH_3^+$ group of GLY_2H^+. Further stabilization of the structure is achieved through hydrogen bonding between a second proton of the end $-NH_3^+$ and the adjacent carbonyl group. The charge distribution based on the Mulliken population analysis demonstrates that most of the positive charge resides on the end $-NH_3^+$ group of diglycine. The hydrogen bond between ammonia and the amine end of GLY_2H^+ is rather strong, in excess of 20 kcal/mol. Complex II is an ammonium ion solvated by diglycine. Our density function calculations show that proton transfer to ammonia occurs in the complex, although the proton affinity of ammonia is lower than that of diglycine, because it is assisted by simultaneous solvation of the ammonium ion by the carbonyl oxygens of the peptide. The solvated ammonium ion carries a large fraction of the positive charge and is hydrogen bonded to the amine end of diglycine and to the adjacent carbonyl. Complexes I and II are the ones invoked in the so-called "onium" ion mechanism for H/D exchange at the amine end of the peptide.[7] The corresponding potential energy profile for triglycine was calculated[7] semiempirically at the PM3 level. The PM3 calculation for triglycine gave the ammonium ion complex solvated by the peptide considerable stability in excess of that of the ion/dipole complex. The present results, at the much higher B3LYP/6-31+G** level for diglycine, give only a slight advantage to the onium complex over the ion/dipole complex. Nevertheless, this complex pair and the corresponding transition state, TS1, are in accord with the onium complex mechanism for H/D exchange. If protonated diglycine is allowed to interact with ND_3, complex I will be formed with ND_3, this will rearrange to complex II via TS1, which involves transfer of H^+ to ND_3. The ND_3H^+ group in the onium complex can rotate and back-transfer a D^+ via TS1

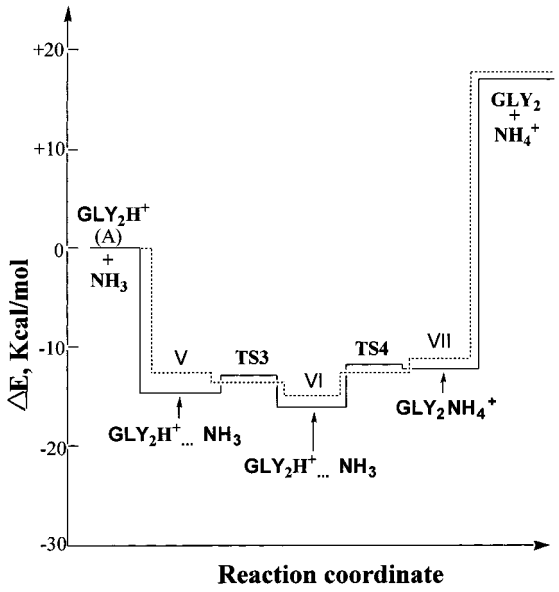

Figure 3. Computed potential energy profile (at the B3LYP/6-31+G**//B3LYP/6-31+G** level of theory) for the interaction of ammonia with protonated diglycine at the hydrogen of the amide bond. Continuous line, without ZPE; dashed line, with ZPE. (See text and Figures 4 and 5.)

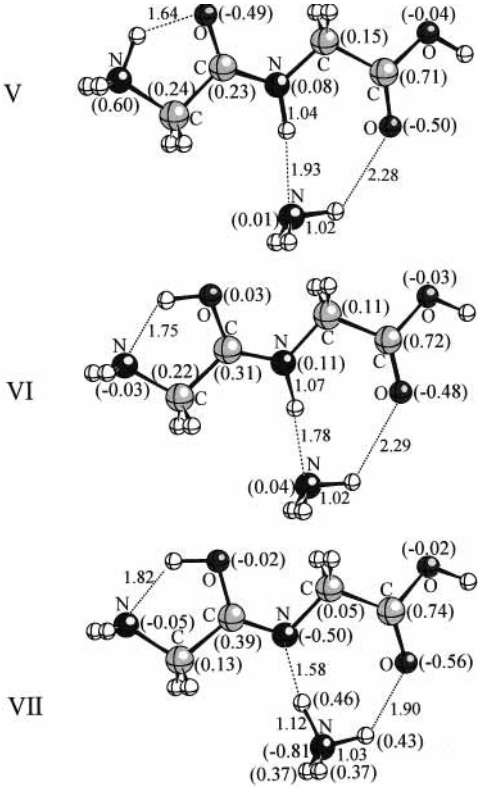

Figure 4. Structures of complexes V, VI, and VII formed upon interaction of ammonia with protonated diglycine at the hydrogen of the amide bond. Selected bond lengths (in Å) and local charges (in parentheses), based on the Mulliken population analysis, are included.

leading to isotopically exchanged GLY_2D^+ and ND_2H. Coming back to the unlabeled analogue, given enough energy, complex II can dissociate in principle to the NH_4^+/diglycine pair. The difference in proton affinities between ammonia and diglycine calculated here, 17.66 kcal/mol, when combined with the proton affinity of ammonia, 204 kcal/mol,[41] places the proton affinity of diglycine at $PA(GLY_2) = 221.7$ kcal/mol in very good agreement with previous B3LYP calculations.[29]

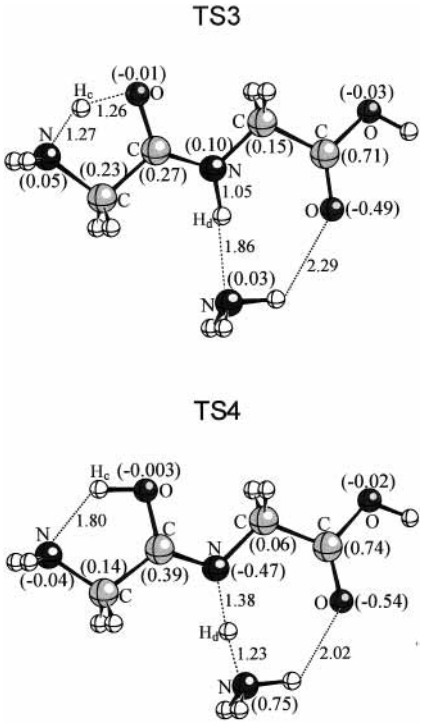

Figure 5. Structures of TS3 and TS4 formed upon interaction of ammonia with protonated diglycine at the hydrogen of the amide bond. Selected bond lengths (in Å) and local charges (in parentheses), based on the Mulliken population analysis, are included.

TABLE 1: Overview of Stationary Points for Species Relevant to the Reaction of Protonated Diglycine, GLY_2H^+, with NH_3 at the B3LYP/6-31+G Level**

species	E_{elec} (Hartree)	ZPE[a] (kcal/mol)	relative energies[b] $E_{elec+ZPE}$ (kcal/mol)
NH_3	−56.566985	21.15	
NH_4^+	−56.9061282	30.56	
GLY_2H^+(A)	−492.8489448	92.06	
GLY_2H^+(B)	−492.8418967	91.91	
GLY_2	−492.4824978	83.17	
$GLY_2H^+...NH_3$ (I)	−549.4514417	115.17	−20.32
$GLY_2NH_4^+$ (II)	−549.4532103	115.53	−21.07
$GLY_2H^+...NH_3$ (III)	−549.4465443	114.60	−17.82
$GLY_2H^+...NH_3$ (IV)	−549.4406523	114.50	−14.22
$GLY_2H^+...NH_3$ (V)	−549.4384003	114.91	−12.40
$GLY_2H^+...NH_3$ (VI)	−549.4403286	114.45	−14.06
$GLY_2NH_4^+$ (VII)	−549.4349201	114.31	−10.81
TS1	−549.4486966	113.24	−20.53
TS2	−549.4373573	114.55	−12.10
TS3	−549.4360747	112.74	−13.10
TS4	−549.4345132	112.48	−12.39
GLY_2H^+(A) + NH_3	−549.4159298	113.21	0
GLY_2H^+(B) + NH_3	−549.4088817	113.05	+4.26
$GLY_2 + NH_4^+$	−549.3886260	113.73	+17.66

[a] Zero point energy (scaled by 0.9804). [b] With inclusion of ZPE.

Proton transfer from GLY_2H^+(A) to NH_3 within complex I has a barrier of 1.7 kcal/mol when only electronic energies are considered. On the other hand, when the zero point energies are added, the energy exceeds the barrier and TS1 becomes more stable than complex I by −0.2 kcal/mol. This negative number has no quantitative meaning but indicates that the two minima are physically indistinguishable. The sequence, complex I, TS1, and complex II illustrates the motion of the proton oscillating back and forth between diglycine and ammonia. A similar situation has been found for the interaction of protonated monoglycine and ammonia.[31]

Complexes III and IV (Figure 2) are both ion/dipole complexes in which the ammonia is hydrogen bonded to the

OH of the carboxyl end of the peptide. They are basically ammonia complexes of two different conformers of protonated diglycine, GLY_2H^+(A) and GLY_2H^+(B), respectively, which are protonated at the amine end of the peptide. They are separated by TS2. This structure is the lowest lying transition state. Rotating the carboxylate group around the C−C bond leads to another (rotational) transition state that lies 3.11 kcal/mol higher than TS2 and that does not contribute to H/D exchange. The structure of TS2 is that of a salt-bridge complex in which two of the positively charged protons of the NH_4 group are hydrogen bonded to the two negatively charged oxygens of the CO_2 group at the C-terminus. The net charge on the NH_4 group is +0.85, whereas the net charge on the CO_2 group is −0.92 and the rest of the complex carries a net charge of +1.1. The situation corresponding to a salt-bridge structure with charges +1, −1, +1, namely a zwitterion stabilized by an adjacent positive ion is nearly ideally met. A salt-bridge mechanism has been suggested[7] for H/D exchange at the carboxyl end of the peptide. PM3 calculations[7] for the GLY_3H^+/NH_3 pair have placed the salt-bridge complex in a minimum along the potential energy profile, whereas the present calculations find only a transition structure. Nonetheless this transition-state salt-bridge complex provides the mechanism for H/D exchange at the C-terminus. This is possible if the dissociated pair, GLY_2H^+(B) + NH_3, involves the isomerized conformer of protonated diglycine, which lies somewhat higher in energy (4.26 kcal/mol, see Table 1 and Figure 2). Interestingly enough we have recently located[31] a salt-bridge complex in the protonated amino acid glycine, GLY/NH_4^+, which resides in a minimum along the potential energy profile. The salt-bridge complex for monoglycine is stabilized by hydrogen bonding between the protonated amine group at the N-terminus and one of the oxygens of the carboxyl group, in addition to hydrogen bonding of the carboxyl oxygens to the NH_4^+ group. Complexes III and IV are less stable than complexes I and II. In fact complex II is the most stable of all the complexes located for the interaction of ammonia with diglycine, as originally anticipated[7] on the basis of PM3 calculations for triglycine.

Complexes V, VI, and VII (Figures 3 and 4) are involved in H/D exchange of the amide hydrogen and are particularly important because most of the hydrogens that are exchanged in large peptides and proteins are necessarily those of the peptide bonds, namely amide hydrogens. The mechanism originally proposed[7] for exchanging the amide hydrogen involves proton transfer from the N-terminus to the amide carbonyl in concert with transfer of the amide proton to ammonia to form an ammonium ion solvated by the tautomerized peptide. We do observe in our computations the ammonium ion solvated by the tautomerized peptide (complex VII). Its formation from the ion/dipole complex V, in which the N-terminus is protonated and ammonia is hydrogen bonded to the amide hydrogen and to the adjacent carbonyl of the C-terminus, is seemingly a two-step process and not concerted. In the first step, a proton is transferred from the N-terminus to the amide carbonyl, forming complex VI, which is an ion/dipole complex between ammonia and the tautomerized peptide. This takes place via TS3 (Figures 3 and 5) in which hydrogen atom H_c is nearly midway between the nitrogen atom of the N-terminus and the oxygen atom of the amide carbonyl. The transfer of the amide proton to ammonia takes place in the second step, via TS4 (Figures 3 and 5), in which the hydrogen atom H_d is nearly midway between the two nitrogen atoms. The NH_3 group has already moved closer to the amide hydrogen in complex VI compared with complex V (Figure 4). The potential energy well is not very deep and rather flat (Figure 3) as verified by computations at the MP2/6-31+G**// B3LYP/6-31+G** level reproduced in Table 2. Inclusion of the zero point energies washes out the small energy differences (Table 1 and Figure 3) causing the formation of a single potential well and leading to a concerted mechanism as originally anticipated.[7]

TABLE 2: Comparison of MP2 Single-Point Energy Calculations with B3LYP Data for the Species on the Potential Energy Profile of Figure 3

	E_{MP2} (Hartree)	relative energies[a] (kcal/mol)	
		E_{MP2}	E_{B3LYP}
$GLY_2H^+...NH_3$ (V)	−547.8952982	−16.89	−14.10
$GLY_2H^+...NH_3$ (VI)	−547.8943513	−16.29	−15.31
$GLY_2NH_4^+$ (VII)	−547.8902247	−13.70	−11.92
TS3	−547.8912257	−14.33	−12.64
TS4	−547.8890635	−12.97	−11.66
$GLY_2H^+ + NH_3$	−547.8683886	0	0

[a] Without inclusion of zero point energies.

The ion/dipole complexes V and VI at the amide site are both less stable than the ion/dipole complexes I and III at the N-terminus and C-terminus, respectively. The complex $GLY_2NH_4^+$ (VII) (Figure 4) has a strong salt-bridge-like character. It has a net positive charge of +0.82 on the NH_4 group, nearly equally negatively charged oxygen and nitrogen atoms, sharing a total negative charge of −1.06, hydrogen bonded to the NH_4^+ group, and a net positive charge of +1.22 on the rest of the zwitterionic part of the molecule.

The reaction of GLY_2H^+ with NH_3 was studied recently[25] in a flow-tube experiment for pressures ranging from 0.1 to 0.4 Torr. The major primary reaction product is the complex formed between the ionic and neutral reagents in a ternary association reaction involving the helium carrier gas and oxygen or nitrogen molecules as collisionally stabilizing species. The semilogarithmic plot of the decay of primary ions as a function of the neutral flow rate demonstrated two slopes. Effective binary rate constants, k_{eff}, were deduced from these slopes and each was observed to be pressure-dependent, as expected for a two-step mechanism:

$$GLY_2H^+ + NH_3 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (GLY_2NH_4^+)^* \qquad (1a)$$

$$(GLY_2NH_4^+)^* + M \xrightarrow{k_2} GLY_2NH_4^+ + M \qquad (1b)$$

with M = He, N_2, or O_2.

The two-step reaction mechanism involves formation and unimolecular back-reaction of a chemically activated collision complex in step 1a and collisional stabilization of the complex in step 1b. This leads to an effective binary rate constant:

$$k_{eff} = k_1 \frac{k_2[M]}{k_{-1} + k_2[M]} \qquad (2)$$

The two effective binary rate constants deduced were caused by two very different unimolecular rate constants for the back-reaction: $k_{-1,slow} = (3-7) \times 10^6\ s^{-1}$ (from the fast k_{eff} decay component of the reactant ion) and $k_{-1,fast} = (2-5) \times 10^8\ s^{-1}$ (from the slow k_{eff} decay component of the reactant ion). These two unimolecular rate constants were ascribed to two different lifetimes of the chemically activated complexes because of formation of two (or more) complexes between protonated diglycine and ammonia with different stabilities, that is, different well depths. At the time[25] it was unclear which pairs of

complexes were involved. Now, according to the DFT calculations, the pair cannot be the N-terminus ion/dipole complex and the onium complex because their well depths are nearly equal. Furthermore, even the C-terminus complexes have stability similar to the N-terminus complexes. The big difference in stability is between the ammonia complexes of the amide NH protons and those of the N-terminus complexes. We thus ascribe the fast k_{eff} decay component of GLY_2H^+ to complexation at the N-terminus with a minor contribution from the C-terminus and the slow k_{eff} component to complexation at the amide bond. If this conclusion is correct, it indicates that most H/D exchanges in peptides should take place relatively slowly. However, the shallow flat well of the amide/ammonia complex ensures fast exchange within the well and efficient back-reaction, which are crucial for H/D exchange to take place. On the other hand, complexation at the N-terminus and C-terminus leads to capture in deep potential wells and efficient collisional stabilization, which is not conducive to exchange under drift-tube conditions.

H/D exchange rates found[26] for diglycine are high, $k = (6.3 \pm 2)\ 10^{-10}$ cm^3 molecule^{-1} s^{-1}. The H/D exchange experiments[26] demonstrated that all five labile hydrogens of protonated diglycine can be exchanged; however, the reaction efficiency for the exchange of the first three is higher than for the last two, as judged by competition with collisional stabilization of the corresponding precursor complexes. In agreement with previous results[7] it was demonstrated[26] that multiple exchanges can result within a single complex. What is of particular significance is that the first two exchanges have contributions from at least two complexes with different lifetimes corresponding to the ranges of fast and slow decay of the reactant ion, respectively. On the other hand, the third, and particularly the fourth and fifth exchanges, take place in a range of neutral flow rates corresponding to the slow decay of the reactant ion, that is, efficient back-reaction of the chemically activated collision complex. The exchanges involve, at least in part, complexation at the amide bond, as judged from the shallow well calculated by DFT. Is H/D exchange at the C-terminus slow? The colliding protonated diglycine and ammonia pair is trapped in a fairly deep potential well. However, isotope exchange cannot take place by back-reaction to the original conformer, and forming the exchanged isomerized conformer is uphill in energy. It thus seems that the amide hydrogen and the carboxyl hydrogen exchange slowly, but for different reasons.

V. Conclusions

The major results of DFT calculations are in agreement with conclusions of the former semiempirical calculations:[7]

1. H/D exchange takes place at the N-terminus of diglycine via an "onium" mechanism. Endothermic proton transfer to ammonia takes place within the complex formed by virtue of stabilization via hydrogen bonding to the adjacent carbonyl group. Whereas the semiempirical calculations found that the ammonium ion complex is considerably more stable than the ion/dipole complex initially formed between ammonia and protonated diglycine, DFT calculations find the two complexes to be of nearly equal stability.

2. The salt-bridge structure suggested as part of the H/D exchange mechanism at the C-terminus is observed but only as a transition-state structure along the reaction profile.

3. Exchange of the amide hydrogen takes place via a tautomerized peptide structure having a partial salt-bridge character.

The profiles for complexation at the N-terminus and at the C-terminus demonstrate fairly deep wells (~20 kcal/mol), whereas the profile at the amide bond is more shallow (~14 kcal/mol). These results are in agreement with our previous experimental observations[25] of at least two chemically activated collision complexes with quite different lifetimes. Furthermore, the first and possibly also the second H/D exchange, among the five possible ones of protonated diglycine, were demonstrated[26] to have contributions from more than one collision complex, again in agreement with the observation of complexes at different sites with different well depths.

Many of the original ideas concerning H/D exchange[6,7,10−12] are upheld by the present DFT calculations. Nevertheless, much more work is required, both experimentally and computationally, particularly on larger peptides. This is anticipated in view of the study of anhydrous protein and peptide ions, which led to a renaissance in mass spectrometry.[1]

Note Added in Proof. We have recently carried out additional calculations on the ammonia complexes at the carboxyl end of protonated diglycine. Using the MP2/6-31+G** level we found a salt-bridge complex that resides in a minimum. It is 4.7 kcal/mol less stable than complex III. Complex III and the newly found salt-bridge complex are separated by a TS that is only 0.7 kcal/mol less stable than the salt-bridge complex. The zero point energy exceeds the barrier between the salt bridge complex and complex III. These computational results open up the salt-bridge H/D exchange mechanism as originally envisaged[7] without necessitating the intervention of an endothermic exit channel.

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