Studies of Nearest-Neighbor Interactions between Amino Acids in Gas-Phase Protonated Peptides

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Abstract: The probe of intramolecular interactions in gas-phase biomolecules using the combination of proton transfer reactions, hydrogen/deuterium exchange reactions, and molecular orbital calculations is illustrated by exploring the nearest-neighbor interactions in protonated peptides. The interactions, specifically $-NH_2\cdots H^+\cdots O{=}C$ and $C{=}O\cdots H^+\cdots O{=}C$, are investigated with peptides that model them. The compounds that include β-Ala, β-Ala-Gly, and Gly-β-Ala, Ala-Gly and Gly-Ala are used to evaluate the structural and electronic factors that are involved in the protonation of the terminal amine. Similarly, *N*-acetylglycine and *N*-acetylglycine amide are used to evaluate carbonyl group interactions in the peptide backbone. The β-alanine residue on the terminal amine is found to increase the gas-phase basicity and decrease the H/D exchange reactivity of the protonated compound relative to analogous compounds containing only α-amino acids. A β-alanine residue on the C-terminus produces compounds with similar gas-phase basicity and H/D exchange behavior as those with α-amino acids. The gas-phase basicity and H/D exchange behavior of the acetylglycines point to stronger intramolecular hydrogen bonding in the amide derivative than in the acid. An amide carbonyl has a greater intrinsic basicity than a carboxylic carbonyl. An analysis proposed by Meot-Ner is used to separate electronic effects from structural effects in the two types of protonation sites.

Introduction

The conformational stability of proteins results from the near cancellation of several large opposing intramolecular forces.[1−3] The equilibration of these forces determines the internal architecture of globular proteins and consequently their reactivity. Intramolecular hydrogen bonding is often considered to be a large driving force for protein folding. A survey of protein crystal structures reveals the extent of hydrogen bonding; only 11% of carbonyl groups and 12% of NH groups are not involved in hydrogen bonding.[4,5] The α-helix and β-turn substructures are examples of structural features which are due primarily to hydrogen bonding.

The majority of known hydrogen-bonding interactions in proteins involve neutral hydrogens. However, basic residues of proteins are typically protonated under physiological conditions. Furthermore, the interaction of protons with proteins is critical in some biological functions as illustrated by the classical example of the proton pump. This process ushers protons from the interior of mitochondria to the exterior resulting in a potential gradient.[6] This gradient is then harnessed to provide the energy for ATP synthesis. All hydrogen bonds are believed to be essentially electrostatic in nature.[7−9] The major difference between ionic and neutral hydrogen bonds lies in their relative strength; the ionic hydrogen bond is between 8 and 30 kcal/mol more stable than the analogous neutral hydrogen bond.[10] The effects of the former on protein stability, however, have received relatively little attention.

Efforts in this and other laboratories have focused on developing gas-phase ion/molecule reactions to obtain a better understanding of the interactions of protons in peptides and proteins.[11−18] The solvent-free environment provides thermodynamic information which can later be used in conjunction with other thermodynamic values to obtain, for example, heats of solvation as McIver and co-workers have shown.[19] Furthermore, the hydrophobic cores of globular proteins attain an environment which may be very similar to that in gas-phase peptides. More recently, electrospray ionization studies have attempted to probe relationships between the solution-phase and gas-phase structures of proteins.[20,21]

As in solution phase, gas-phase intramolecular interactions are expected to be composed of local (neighboring group) and nonlocal interactions. For peptides containing amino acid residues with only alkyl side chains, two types of interactions predominate: the interaction of the protonated terminal amine

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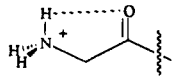
with the neighboring carbonyl group producing a N—H$^+$···O=C interaction (**1**) and the interaction of two adjacent carbonyl groups via a bridging hydrogen bond, C=O···H$^+$···O=C (**2**). Protonation on the amide nitrogen, while in principle possible, yields a structure considerably higher in energy than either **1** or **2**, which is probably not accessible at ambient temperature.[17]



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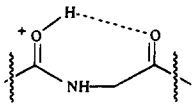

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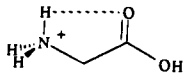
In this study, we investigated both N—H$^+$···O=C and C=O···H$^+$···O=C interactions with an appropriate selection of amino acids and peptide derivatives in order to determine the factors that influence the binding in both types of coordination. The interactions were probed using the combination of gas-phase basicity (GB) measurements, hydrogen/deuterium (H/D) exchange, and molecular orbital (MO) calculations. Gas-phase basicities are affected by the specific sites of protonation, as well as the intramolecular interactions associated with those sites.[17,22] Molecular orbital calculations, mainly the semiempirical AM1[23-25] approach, have been used to support experimental results and have provided additional insights into the types of interactions present in protonated species.[17,22] More recently, H/D exchange studies have indicated that H/D exchange behavior is governed by the site of protonation and intramolecular interactions in the protonated peptide/deuterated reagent complex.[26]

The nature of the interaction of the terminal amine with a neighboring carbonyl group (N—H$^+$···O=C) has been investigated for glycine.[19,27,28] Protonation experiments at various temperatures provided the entropy associated with intramolecular interactions. The results show that the interaction between the protonated amine and the carboxylic acid group is insignificant and not observed. These results are supported by several high-level *ab initio* calculations.[29-31] The lack of interaction is due to a combination of structural and electronic factors, i.e. the small angle associated with N—H$^+$···O=C interaction and the relatively weak basicity of the carbonyl group. This interaction can be increased by increasing the basicity of the carboxyl group. For example, the conversion of amino acids to their corresponding amides increases the GB of the compound.[17,30] We can probe the role of structure by allowing the N—H$^+$···O=C angle to assume a more favorable configuration, i.e. closer to linearity. For this reason, we chose to investigate protonated β-alanine. Protonation of this compound differs from that of α-amino acids as the hydrogen is able to interact more favorably with the adjacent carbonyl group (compare **3** and **4**). Recent investigations with β- and γ-amino acids indicate that the strength of neighboring group interactions changes with the structure of the amino acid.[5] β-Alanine has also been suggested as a possible amino acid substitute to obtain a specific structural feature in unnatural peptides.[5,32] The investigation of β-alanine and dipeptides that contain this amino acid by the combination of methods discussed above allows us to assess the effect of increasing the number of methylene groups between the amine and the carboxylic acid group.



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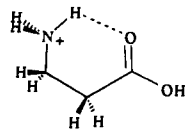

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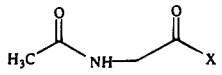
Interactions between two adjacent carbonyl groups (C=O—H$^+$···O=C) in peptides have been postulated.[17] Theoretical calculations, both semiempirical and high-level ab initio, predict that a carbonyl amide has nearly the same basicity as the terminal amine.[17,30] Semiempirical calculations further suggest that migration of the proton in peptides occurs through intermediates involving these interactions.[17] However, low-level *ab initio* calculations suggest that **2** is a transition state and not a stable intermediate.[30] Experimental values that measure the strength of this interaction are not available but are important for evaluating the sites of protonation in peptides. For this reason, the two compounds [*N*-acetylglycine(**5**) and *N*-acetylglycine amide(**6**)] were investigated to model the interactions. H/D exchange provides further evidence for the possibility of exchange between the protonated carbonyl oxygen and the amide hydrogens.



X= OH (**5**), NH_2 (**6**)

Experimental Section

Gas-Phase Experiments. All experiments were performed on an external source Fourier-transform mass spectrometer equipped with a 3 T superconducting magnet and controlled by an Omega data system (Ionspec Corp.). Ions are produced in the external liquid secondary ion mass spectrometry (LSIMS) source with a Cs^+ primary beam. The protonated peptides are transported from the source to the analyzer cell, using a single stage quadrupole rod assembly, where they are trapped for the length of the reaction period. For proton transfer reactions, a background pressure of a reference base (typically between 1×10^{-7} and 3×10^{-7} Torr) was leaked using a precision Varian leak valve. In experiments involving H/D exchange, CH_3OD (1×10^{-7} to 8×10^{-7} Torr) was used as a background gas.[26,33] Pressure was determined by an empirical method using relative ion gauge sensitivities.

All compounds used in the experiments were obtained from commercial sources and were used without further purification. Reference bases and the deuterated methanol were degassed using several (at least three) freeze—thaw cycles. Amino acids and peptides were dissolved in deionized water and applied to a glycerol matrix on a copper-tipped direct inlet probe.

Kinetic analyses for proton transfer and H/D exchange reactions have been discussed in earlier publications.[26] In the H/D exchange experiments, a significant abundance of CH_3OH was present in the background gas. This impurity varied to as much as 30% of the total abundance and its contribution was treated as an additional unknown variable to be optimized during the curve-fitting procedure. The methanol-d_0 is believed to come from H/D exchange reactions occurring

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Table 1. Reaction Efficiencies in the Deprotonation of Peptides by Neutral Reference Compounds[a]

reference (GB in kcal/mol, 300 K)	β-Ala	β-Ala-Gly	Gly-β-Ala	Ala-Gly	Gly-Ala	acetylglycine	acetylglycine amide
pyrrole (201.6)	0.05					0.08	0.01
aniline (202.5)	0.01					1.0	0.01
dmf (203.6)	0.01	0.01	0.03	0.04	0.07	0.69	0.06
cyclapropylamine (206.6)	0.01	0.01	0.02	0.03	0.02	1.00	0.12
allylamine (207.9)	0.03	0.002	0.12	0.05	0.20		0.39
n-propylamine (210.1)	0.52	0.05	0.26	0.42	0.52	0.57	0.78
isobutylamine (211.0)	1.0	0.06	0.40	1.0	1.0		
tert-butylamine (213.0)	0.69	0.07	0.31	1.0	1.0		
cyclohexylamine (213.7)		0.04	1.0	1.0	1.0		
ethylmethylamine (215.1)	0.93	0.06	0.84		0.85		
diethylamine (217.7)	0.87	0.58	0.99	1.0	1.0		
di-*n*-propylamine (219.7)	1.0	1.0	1.0	1.0	1.0		

[a] All values are for reaction efficiencies based on: efficiency = k_{exp}/k_{ADO}, where k_{exp} is the experimental rate and k_{ADO} is the rate predicted by ADO theory. See text for details. GB values are obtained from ref 37.

Table 2. Assigned Gas-Phase Basicity Values of Peptides and Peptide Derivatives Used in the Experiments

amino acid/peptide	assigned gas-phase basicities (kcal/mol, 300 K)
β-Ala	209.0 ± 1.1
β-Ala-Gly	216.4 ± 1.3
Gly-β-Ala	209.0 ± 1.0
Ala-Gly	209.0 ± 1.0
Gly-Ala	209.0 ± 1.0
N-acetylglycine	202.0 ± 0.5
N-acetylglycine amide	207.2 ± 0.7

on the surface of the vacuum chamber. Its effect on the analysis has also been discussed earlier.[26]

Theoretical Procedures. Molecular orbital calculations were performed on a Indigo Elan (Silicon Graphics Inc.) computer using the AM1 theoretical model contained in the Spartan molecular orbital package (Wavefunction Corp.). The graphics interface feature of this program has been extremely useful in identifying reasonable structures for global and local minima. AM1 has been evaluated for its applicability to protonated species.[17,22] All structures are fully optimized. For each structure reported, several other conformations were analyzed to ensure that global minimum of a specific proton binding site was found.

Results

Gas-Phase Basicity. The compounds investigated included β-alanine, β-Ala-Gly, Gly-β-Ala, Ala-Gly, and Gly-Ala to allow direct comparisons between α- and β-amino acids. The GB's of *N*-acetylglycine and *N*-acetylglycine amide were determined to characterize the protonation involving exclusively the carbonyl groups.

The use of bracketing methods to obtain gas-phase basicities is described in several recent publications.[17,22] The GB values were assigned by performing proton transfer reactions with bases of known GB (reference bases). Rate constants were measured and converted to efficiencies (Table 1) using the expression:

$$efficiency = k_{exp}/k_{ADO}$$

where k_{exp} and k_{ADO} are the experimental and theoretical (obtained by average dipole orientation theory[34,35]) rate constants, respectively. The efficiencies of proton transfer to each reference base are compared from the least to the most basic (Table 1). A large change in reaction efficiencies signifies the transition between endergonic and exergonic reactions. By convention we have selected 50% as the lowest efficiency for exergonic (or exoergic) reactions. The rational for this cutoff is based on the results by Bohme et al.[36] and has also been discussed in earlier publications.[17,22]

For the compounds β-alanine, β-Ala-Gly, Ala-Gly, Gly-Ala, and *N*-acetylglycine the boundaries between exergonic and endergonic reactions are easily defined because the changes in efficiencies are both large and abrupt (Table 1). For example, β-alanine reacts with less than 5% efficiency with compounds whose GB is less than *n*-propylamine. With *n*-propylamine the reaction efficiency jumps to 52%.

Protonated Gly-β-Ala and *N*-acetylglycine amide yield more gradual changes in efficiencies with reference bases of increasing GB. The GB of Gly-β-Ala is assigned between allyl amine and *n*-propylamine and that of *N*-acetylglycine amide between cyclopropylamine and allylamine. The low efficiencies observed in the reaction of protonated Gly-β-Ala with *n*-propylamine, isobutylamine, and *tert*-butylamine are likely the result of steric interactions that decrease the rate of proton transfer. Similar behavior was observed in the GB determination of valine oligomers where the large alkyl side chains of the peptides proved to be effective in decreasing the rates of proton transfer.[22]

The assigned GB values for all the compounds are presented in Table 2. The gas-phase basicity of β-alanine (209.0 kcal/mol) lies between allylamine and *n*-propylamine, making it 3.0 kcal/mol greater than alanine (206.0 kcal/mol).[37] The higher GB of β-alanine relative to alanine contrasts with the behavior of simple amines where the NH_2 groups on primary carbons are often less basic than the those on secondary carbons, e.g. *n*-alkylamines of propyl and butylamines are 1 kcal/mol less basic than their respective *sec*-alkylamines.[37] The greater basicity of β-alanine compared to alanine is, however, partly explained by the greater separation of the electron-withdrawing carboxylic acid group from the amine.

The GB of the dipeptide β-Ala-Gly (216.4 kcal/mol) is greater than that of β-alanine by 7.4 kcal/mol. This increase is significantly larger than those observed between glycine, alanine, and valine and their respective dipeptides (5.5−5.8 kcal/mol).[22] The dipeptide Gly-β-Ala (209.0 kcal/mol) is, however, 7.4 kcal/mol less basic than β-Ala-Gly, illustrating a clear preference for the β-alanine residue to be on the N-terminus rather than the C-terminus. The GB of Gly-β-Ala is similar to that of Ala-Gly (209.0 kcal/mol) and Gly-Ala (209.0 kcal/mol). The latter two are both expected to protonate on the terminal amine. The similarities in GB's makes it likely that Gly-β-Ala is similarly protonated on the terminal amine, even though glycine is the less basic residue (5.9 kcal/mol less basic than β-alanine).

The dipeptide analog *N*-acetylglycine has a GB (202.0 kcal/

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Table 3. Sequential H/D Exchange Rates Determined for the Reaction of CH_3OD with the Protonated Peptides[a]

compound	labile hydrogens	k_1	k_2	k_3	k_4	k_5
β-alanine	4	1.7	0.029	nrx	nrx	
β-Ala-Gly	5	0.58	0.010	nrx	nrx	nrx
Gly-β-Ala	5	33.6	35.9	9.7	3.2	1.5
glycine	4	10.6	3.7	2.6	1.2	
alanine	4	8.4	0.8	nrx	nrx	
N-acetylglycine	3	20.9	17.1	nrx		
N-acetylglycine amide	4	22.6	nrx	nrx	nrx	

[a] All values for k are $k \times 10^{-11}$ cm^3 molecule^{-1} s^{-1}.

mol) slightly less than glycine (203.1 kcal/mol). This value is also similar to the GB of other amides, for example $(CH_3)_2$-NCHO (203.6 kcal/mol) and CH_3CONH_2 (198.4 kcal/mol).[37] The closeness of the GB values suggests that the carboxylic acid in *N*-acetylglycine does not strongly contribute to the stabilization of the charge on the carbonyl amide and that the bridging interactions are very weak in protonated *N*-acetylglycine. The GB of *N*-acetylglycine is 6.5 kcal/mol less than diglycine (208.5 kcal/mol), providing further evidence that the terminal amine is the primary site of protonation in dipeptides which have no basic side chains.

The GB of the *N*-acetylglycine amide (207.2 kcal/mol) is 5.2 kcal/mol greater than the acid due, most likely, to the strong intramolecular interaction between the two carbonyl groups. This large increase clearly illustrates that the carbonyl bridged species (**2**) is the most favorable protonated tautomer of *N*-acetylglycine amide. The similarity between the GB of this compound and the GB of triglycine (209.6 kcal/mol),[17] which also has two amide groups, indicates that both modes of protonation (**1** and **2**) probably occur in the gas phase for triglycine.

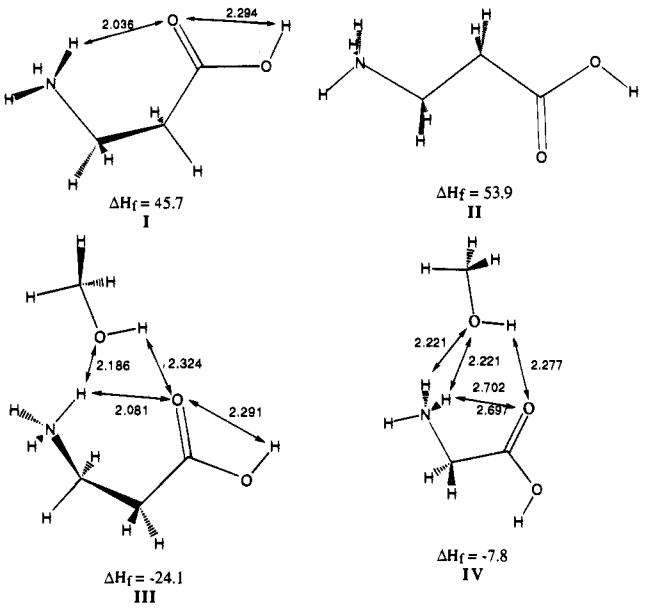
H/D Exchange. Rate constants associated with the incorporation of specific numbers of deuteriums can be obtained from the time-dependence behavior of the corresponding deuterated product.[26,33] These rate constants, obtained by using CH_3OD as the deuterating reagent, are listed in Table 3. The values associated with k_n are the rate constants of the reactions that incorporate the nth deuterium. The deuterating reagent CH_3-OD was used as it has been found in this laboratory to show H/D exchange selectivity between various labile hydrogens.[26,33]

The rate constants for protonated β-alanine exchanging with deuterated methanol are significantly smaller than for any α-amino acids. Incorporation of the first deuterium is slow, with a rate constant of 1.7×10^{-11} cm^3 molecule^{-1} s^{-1}. The first exchange probably occurs on the carboxylic acid, as it does with the α-amino acids. This hypothesis is supported by the nonstatistical nature of the rate constants k_1, k_2, and k_3. Fast exchange on the protonated amide would result in a roughly 3:2:1 ratio. The value of k_1 is nearly one order of magnitude less than the corresponding values for α-amino acids. Glycine and alanine, for example, have k_1 values of 10.6 and 8.4×10^{-11} cm^3 molecule^{-1} s^{-1}, respectively. The large difference in the rates between the α- and β-amino acids is not simply explained by differences in basicity, as compounds more basic than β-alanine undergo exchange more rapidly. The structure of the protonated species and its interaction with methanol, as we will show, play a strong role and accounts for the differences in reactivities (*vide supra*).

Earlier investigations have shown that the dipeptides of glycine and alanine are significantly more reactive than the corresponding amino acids.[33] In contrast, protonated β-Ala-Gly is much less reactive than β-Ala. The position of the β-Ala residue is a major factor in the reactivity of the dipeptides. When the β-Ala residue is the N-terminus, the protonated dipeptide is less reactive to H/D exchange than when it is the C-terminus. The rate constant associated with the exchange of the first deuterium, k_1, of β-Ala-Gly (0.58×10^{-11} cm^3 molecule^{-1} s^{-1}) is nearly two orders of magnitude less than Gly-β-Ala (33.6×10^{-11} cm^3 molecule^{-1} s^{-1}). Under these experimental conditions only two exchanges are observed with either β-alanine or β-Ala-Gly while the protonated dipeptides of alanine and glycine exchange all five labile hydrogens.[33]

The H/D exchange behaviors of *N*-acetylglycine (**5**) and *N*-acetylglycine amide (**6**) do not resemble either diglycine or dialanine. Both protonated compounds **5** and **6** exchange only a fraction of the total number of labile hydrogens; acetylglycine, with three labile hydrogens, exchanges two while the amide, with four, exchanges only one. In comparison, protonated diglycine and dialanine exchange all five labile hydrogens under similar reaction conditions. The rate constants of the first two exchanges, k_1 and k_2, of protonated **5** are both large and nearly equal (20.9 and 17.1×10^{-11} cm^3 molecule^{-1} s^{-1}, respectively). The single exchangeable hydrogen of protonated **6** reacts as rapidly (22.6×10^{-11} cm^3 molecule^{-1} s^{-1}).

Chart 1



Discussion

N—H$^+$···O=C Interactions. The GB and H/D exchange reactivity of β-Ala and dipeptides containing this amino acid residue point to features that are different from the common α-amino acids. Both β-Ala and β-Ala-Gly are considerably more basic their α-amino acid isomers. Molecular orbital calculations performed on protonated β-alanine using AM1 predict that a closed ring structure is the most stable protonated conformer. The ability of the carbonyl functional group to solvate the protonated amine is key to further stabilization. Shown in Chart 1 are the optimized structures of the open (**I**) and closed (**II**) forms of protonated β-alanine (AM1 structures are designated by Roman numerals). The distance between the closest hydrogen and the carbonyl group of the closed form is only 2.036 Å, indicative of a strong hydrogen-bonding interaction. This species is more stable than the open form by 8.2 kcal/mol. In the α-amino acids the N—H···O=C interaction between the protonated amine and the carboxylic acid is shown by experiments[28] and MO calculations (semiempirical[17] and *ab initio*[30,31]) to be weak or absent.

The increased hydrogen-bonding interaction between the protonated terminal amine and the carbonyl group in β-alanine

Table 4. Assignment of Proton Affinities of Individual Basic Sites on Amino Acids and Peptides Using Reference Compounds with Single Basic Sites

	N-site	PA (kcal/mol)[a]	O-site	PA (kcal/mol)[a]	$\Delta H_D°$ (kcal/mol)[b]
alanine	alanine	214.8	acetic acid	190.2	23.6
β-alanine	ethylamine	217.0	acetic acid	190.2	23.0
	n-propylamine	217.9	acetic acid	190.2	22.8
β-Ala-Gly	ethylamine	217.0	CH_3CONH_2	206.2	27.2
N-acetylglycine	CH_3CONH_2	206.2	acetic acid	190.2	25.6
N-acetylglycine amide	CH_3CONH_2	206.2	CH_3CONH_2	206.2	30.4

[a] Values from ref 37. [b] Values are calculated using eq 1. See text for details.

stems from a combination of two factors: the relative intrinsic proton affinity (PA) of the base sites and the ability of the sites to freely interact. We attempt to decouple the effects of these two factors by using an empirical relationship suggested by Meot-Ner between the strength of the hydrogen bonding interaction of two basic sites and their difference in PA.[38] The strength of the interaction is given by the expression:

$$\Delta H_D° = \Delta H_D°(0) - b\Delta PA \quad (1)$$

The values $\Delta H_D°(0)$ and b differ for the interacting sites. For the interaction of a protonated nitrogen with an oxygen, N—H$^+$···O, the values are 30.0 ± 1.5 kcal/mol and b = 0.26 ± 0.03, respectively. For O—H$^+$···O, the corresponding values are 30.4 ± 0.4 kcal/mol and b = 0.30 ± 0.01, respectively. The study performed by Meot-Ner used hydrogen-bound dimers that are free to find the most favorable interaction. In peptides, the two base sites are constrained by the molecular tether that connects them. By comparing the hypothetical $\Delta H_D°$ with the determined GB values of the various compounds, we may isolate differences resulting from different structural constraints.

The different structural constraints present in protonated alanine and β-alanine are evaluated by comparing the differences in $\Delta H_D°(\Delta\Delta H_D°)$ with those in GB. The binding sites in each compound are identified and the respective proton affinities approximated by employing monofunctional compounds to represent individual base sites in the amino acid. For purposes of obtaining PA values, the amine site of alanine is represented by alanine (since we know from glycine that interaction between the two most basic sites is negligible) and the acid site by acetic acid (Table 4). β-Alanine is represented by a short-chain primary amine (ethylamine or *n*-propylamine) and acetic acid. The calculated value ($\Delta H_D°$) corresponds to the strength of the interaction of the protonated amine with the carboxylic acid *in the absence of structural constraints*. For both alanine and β-alanine the above equation yields similar numbers, 23.6 and 23.0 kcal/mol, respectively. Use of either ethylamine or *n*-propylamine as models for the amino group of β-alanine does not significantly alter the calculated value. If the intrinsic proton affinity of the sites is the primary source for the magnitude of their GB, then both alanine and β-alanine should have identical values. Instead, we find that β-alanine is 2.6 kcal/mol more basic than alanine. The discrepancy, although small, is significant and likely results from the structural differences between the two protonated compounds: that is, in β-alanine the N—H···O interaction is allowed to assume a more linear geometry than in the α-amino acid.

The method applied to β-Ala-Gly, using ethyl amine and acetamide to represent the two base sites, produces a $\Delta H_D°$ that is 4.2 kcal/mol greater than that for β-alanine (Table 4). If structural effects are the same in both β-Ala and β-Ala-Gly then the difference in $\Delta H_D°$ should be equal to the difference in GB (ΔGB). Instead ΔGB, GB(β-Ala-Gly) − GB(β-Ala), is 7.4 kcal/mol, or 3.2 kcal/mol more than $\Delta\Delta H_D°$. The greater stabilization found in protonated β-Ala-Gly is likely the result of further interactions with the second carbonyl group. Recent MO calculations suggest that both the carbonyl amide and the carboxylic acid terminus may coordinate with the protonated terminal amine.[17,22,30]

The relatively poor reactivities of β-Ala and β-Ala-Gly toward H/D exchange are likely rooted in structural factors. We have shown that no simple relationship exists between GB and H/D exchange reactivity.[33] For example among the amino acids, lysine and histidine are considerably more basic and more reactive to H/D exchange than either glycine or alanine. Instead, it appears that relative proton affinities (PA) of individual base sites are more strongly correlated to H/D exchange reactivity.[33] MO calculations of methanol coordinated to protonated β-alanine and glycine also support the importance of structural constraints in H/D exchange. The optimized structures of methanol interacting with the most stable conformers of protonated β-alanine (**III**) and glycine (**IV**) are shown. Experimental evidence to support the types of interactions portrayed in **III** and **IV** has been provided and discussed.[33] Calculations predict extensive hydrogen bonding between the protonated amine and the methanol in both systems as shown by the distances of less than 2.5 Å between the hydrogen and the nearest nonbonded heteroatom. The major difference between the structures of protonated β-alanine (**III**) and protonated glycine (**IV**) is the distance between the hydrogen on the terminal amine and the carbonyl oxygen. For **III** this distance is 2.081 Å while for **IV** it is 2.697 Å. This means that protonated β-alanine forms a stable closed structure that prohibits insertion of the methanol thereby decreasing the rate of H/D exchange. This effect is further enhanced in β-Ala-Gly where the carbonyl amide makes the N—H$^+$···O=C interaction even stronger.

C=O—H$^+$···O=C Interactions. Both *N*-acetylglycine (**5**) and *N*-acetylglycine amide (**6**) provide structural analogs to the peptide backbone found in diglycine and triglycine, with the exclusion of the terminal amine. The GB of *N*-acetylglycine (202.0 kcal/mol) is similar to that of glycine (203.1 kcal/mol) but considerably less than that of diglycine (208.5 kcal/mol). Protonation on glycine involves the terminal amine with little interaction to the carbonyl group of the acid. Similarly, protonation on *N*-acetylglycine involves the carbonyl amide with little interaction to the carbonyl of the acid. The similarities in the GB of the two compounds indicate that the terminal amine and the carbonyl amide groups in peptides have similar intrinsic basicities. Furthermore, it is the interaction of the protonated terminal amine with the carbonyl amide (bridging interactions) in protonated diglycine that makes the latter more strongly basic.[17]

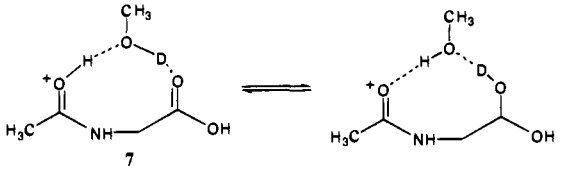
The GB of *N*-acetylglycine amide (209.0 kcal/mol) is similar to the GB of triglycine (209.6). As both species contain two carbonyl amide groups, the results suggest that protonation of triglycine is more akin to **2** than **1**. MO calculations, however, suggest that the interactions depicted by **1** and **2** have similar stabilities, within experimental error.[17] In any case, **2** is clearly a stable interaction and not, as predicted by low-level *ab initio*

calculations (STO-3G basis sets),[30] a transition state. The similarities in GB further support our earlier hypothesis that proton migration between the terminal amine and the carbonyl groups is thermodynamically feasible in peptides without strongly basic side chains as many of the base sites have similar intrinsic basicities.[17]

N-Acetylglycine and *N*-acetylglycine amide are ideal compounds to test the relationship proposed by Meot-Ner.[38] Structural constraints imposed on the intramolecular interactions are expected to be the same in the two compounds so that $\Delta H_D°$ should equal ΔGB. The values predicted by equation **1** (Table 4), using acetamide as the model for the amide sites and acetic acid for the carboxylic acid, are $\Delta H_D° = 25.6$ kcal/mol for *N*-acetylglycine and 30.4 kcal/mol for *N*-acetylglycine amide. The difference, $\Delta\Delta H_D°$, of 4.8 kcal/mol agrees remarkably well with ΔGB = 5.2 kcal/mol. Clearly, the increase in GB between *N*-acetylglycine and its amide derivative is due solely to the increase in hydrogen-bonding interactions brought about by the conversion of the carboxylic group to an amide. These experiments provide a quantitative value for the interaction of two neighboring carbonyl amide groups in peptides corresponding to 5.2 kcal/mol.

The contrasting H/D exchange behavior of the two protonated compounds (**5** and **6**) reveals which hydrogens undergo exchange. The acid with three labile hydrogens undergoes two rapid exchanges, while the amide derivative with four undergoes only a single exchange. For protonated *N*-acetylglycine amide, H/D exchange on the nitrogen of the amide groups should yield a minimum of three exchanges. The single exchange means that only the proton coordinated to the carbonyl groups undergoes H/D exchange. With the same reasoning applied to the acid, we conclude that exchange occurs with the carbonyl coordinated proton and the hydrogen of the carboxylic acid.

Complexes of methanol and protonated **5** and **6** are proposed (structure **7**) that are analogous to complexes of methanol with β-alanine and glycine. The bidentate interaction of methanol allows optimal hydrogen bonding that in turn facilitates H/D exchange. Similar bidentate complexes have been proposed in the interactions of protonated amines and polyethers.[39] H/D exchange of the carbonyl coordinated hydrogen in **7** may proceed by a simultaneous decrease of $CH_3O\cdots H$ distance and increase of $D\cdots OCH_3$ distance followed by the loss of CH_3OH (see below). This intermediate tautomerizes after the complex



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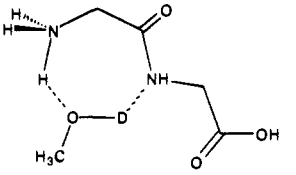
dissociates, transferring either the deuterium or the hydrogen to the more basic carbonyl group. The similarities between k_1 and k_2 (the rate constants for the first and second exchange with deuterium, 20.9 and 17.1 × 10^{-11} cm^3 $molecule^{-1}$ s^{-1}) supports this type of a mechanism as the proton and the acidic hydrogen become nearly equivalent. The whole reaction is likely driven by the energy of dimerization (*ca.* 25 kcal/mol[39]). A similar mechanism may be drawn for the H/D exchange reaction of protonated *N*-acetylglycine amide. In this reaction, however, only the hydrogen coordinated to the carbonyl group can transfer between the two amide groups. The nitrogen bound hydrogens in **6** do not migrate as the carboxylic acid hydrogen in **5** even when the molecule is excited by the energy of dimerization.

The lack of observable exchange in the amide hydrogens of protonated **5** and **6** contrasts with that of protonated diglycine and dialanine where all labile hydrogens, including the amide hydrogen, undergo H/D exchange.[33] The determined rate constant for the exchange of the fifth (and last) labile hydrogen in protonated diglycine, 1.5 × 10^{-11} cm^3 $molecule^{-1}$ s^{-1}, is one order of magnitude greater than that for the smallest detectable rate, less than 1 × 10^{-12} cm^3 $molecule^{-1}$ s^{-1}. Several factors may account for the differing behavior. The coordination of methanol may differ between the groups of compounds producing different dimeric structures and heats of dimerization. For example, coordination of methanol with protonated dipeptides may also occur between the terminal amine and the amide group as shown in structure **8**. Alternatively, proton migration between the terminal amine and the amide of diglycine make take place allowing the exchange of the N−H groups to occur indirectly. The latter is supported by MO calculations (AM1) that predict such a transfer must overcome a barrier of only 32 kcal/mol, an amount accessible from the heat of dimerization.[40] However, more experimental evidences are required to resolve the two possibilities.



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Conclusion

Short-range interactions in protonated peptides are governed primarily by the formation of strong hydrogen bonds. These interactions are affected by the relative intrinsic proton affinity of the sites and the structural constraints which may prohibit two sites from interacting favorably. Peptides that contain the β-Ala residue as the terminal amine are more basic and less reactive to H/D exchange than their α-amino acid analogs. Structural constraints in the α-amino acid analog prohibit the favorable interaction of the protonated terminal amine with the neighboring carbonyl group. These constraints are relaxed with β-amino acids. When the terminal amine is removed, protonation on the carbonyl group takes place, and is also stabilized by the intramolecular hydrogen bonding interaction with neighboring carbonyl groups. This interaction is stronger when the neighboring group is an amide rather than an acid due to the greater intrinsic proton affinity of the carbonyl group.

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