

Gas-Phase H/D Exchange of Sodiated Glycine Oligomers with ND₃: Exchange Kinetics Do Not Reflect Parent Ion Structures

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Abstract: H/D exchange is a method commonly used to probe molecular structure. The majority of studies in the gas phase have involved protonated molecular ions. The present study gives attention to molecular ions formed by coordination with a sodium ion. In particular, ND₃ is reacted with sodiated glycine oligomers, Gly_n, where $n = 1-5$, and the results are interpreted using density functional calculations. Experimentally, Gly₁Na⁺, Gly₄Na⁺, and Gly₅Na⁺ all undergo three fast exchanges with ND₃, while Gly₂Na⁺ and Gly₃Na⁺ undergo one fast and two slow exchanges with ND₃. The methyl esters Gly₃OMeNa⁺ and Gly₅OMeNa⁺ do not exchange with ND₃. In agreement with earlier experimental studies, theoretical calculations show that the lowest-energy conformers of the sodiated glycine oligomers are charge-solvated structures. Calculations further indicate that, in the process of H/D exchange with ND₃, sodiated monoglycine and tetraglycine adopt zwitterionic structures, sodiated diglycine adopts a salt-bridge form, and sodiated triglycine takes on an ion-stabilized ion pair form. Sodiased monoglycine and diglycine exchange via an onium-ion mechanism. The proposed exchange mechanisms require a carboxylic acid hydrogen to complete the exchange, which is in agreement with the experimental results showing that no exchange occurs with methyl ester glycine oligomers. These studies clearly demonstrate that, in the process of H/D exchange, noncovalent complexation of the exchange reagent provides the energy required to access intermediates structurally distinct from the parent ions. H/D exchange is facile for these intermediates. Contrary to the assumption often expressed in earlier studies, H/D exchange kinetics may not directly reflect ion structures.

Introduction

The introduction of an isotopic label by H/D exchange can provide structural information for biomolecules in both solution¹⁻⁵ and the gas phase.⁶⁻¹³ In a typical experiment, a reagent molecule exchanges hydrogen for deuterium at exposed labile sites on the target species. The rate of exchange, and number of

exchanges that occur, provides information about the structure of the target molecule.

For gas-phase H/D exchange, an ionized molecule, typically protonated, is introduced into a mass spectrometer in the presence of an exchange reagent. The extent of exchange is monitored at different time intervals following the introduction of the target molecule into the instrument. H/D studies of biomolecules in the gas phase have been used to identify different conformers of proteins and to characterize these conformers as compact or extended.^{11,12,14} In a more recent application involving studies of the structure of noncovalent complexes, Geller and Lifshitz have used H/D exchange to determine that only one conformer of serine dipeptide exists in the gas phase and have assigned this conformation as the nonzwitterionic form.¹⁰ H/D exchange is a complicated process. For example, different reagents can result in different levels of H/D exchange for the same molecule,⁷ with the extent of exchange typically increasing with the basicity of the exchange reagent.¹⁵ By focusing on small model systems of protonated glycine oligomers, Campbell et al. were able to characterize several different H/D exchange mechanisms for these species.⁷

Protonated compounds are most often studied in gas-phase H/D exchange experiments, but it is also common to observe

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molecules with attached alkali metal ions. H/D exchange of peptides complexed with alkali metals has been studied previously, but the role of the exchange reagent was not taken into account.¹⁶ Several studies have been done on small lithiated and sodiated peptides, specifically glycine, oligoglycines, and their derivatives, to determine the lowest-energy conformation of such species in the gas phase.^{17–19} There is general agreement that the lowest-energy structures of sodiated oligoglycines are charge-solvated forms, in which the peptide carries no charge and serves to solvate the alkali metal charge carrier. The behavior of peptides complexed with alkali metals can be quite different from that of their protonated counterparts. For example, while singly sodiated bradykinin exchanges all 17 labile hydrogens with D₂O, singly protonated bradykinin is unreactive under similar conditions.⁸ Williams et al. investigated several protonated and sodiated peptides.²⁰ They found that sodiated peptides typically exchange with D₂O more rapidly than protonated peptides. Full methyl esterification of carboxylic acid functional groups, or replacement of all acidic hydrogens with sodium ions, inhibited the exchange of labile hydrogens with D₂O. The paper by Williams et al. also provides an excellent review of earlier H/D exchange studies involving peptides and proteins in the gas phase.²⁰

H/D exchange results are presented herein for sodiated glycine oligomers, Gly₁ to Gly₅. These model compounds are used to elucidate the fundamental mechanism of H/D exchange involving carboxylic, amide, and amine hydrogens with the exchange reagent ND₃. The lowest-energy structures of the sodiated glycine oligomers are found to be charge-solvated structures. However, in the process of H/D exchange, noncovalent complexation of the exchange reagent ND₃ to a sodiated glycine oligomer provides the energy required to access intermediates structurally distinct from the parent ions. H/D exchange is facile for these intermediates. Because of this, H/D exchange dynamics do not directly reflect the structure of sodiated glycine oligomers.

Methods

Experimental Methods. Experiments were performed in an external ion source 7-T FT-ICR mass spectrometer that has been described in detail elsewhere.²¹ Sodiated peptide ions were generated by MALDI except for sodiated monoglycine and diglycine, which were generated by electrospray. For MALDI experiments, samples were deposited on a stainless steel probe tip directly inserted into the octopole ion guide. A pulsed nitrogen laser (LSI Laser Science, Inc., 337 nm) was focused onto the probe tip to desorb ions that were transported by an octopole ion guide through three stages of differential pumping to the ICR cell. A static pressure of the H/D exchange gas, ND₃ ($\sim 7 \times 10^{-8}$ Torr), was maintained in the cell. MALDI solutions were prepared by mixing 1 M 2,5-dihydroxybenzoic acid in ethanol, 0.03 M peptide in water/ acetonitrile (3:7 v/v), and 1 M D-fructose in water with the mixing ratio of 6:3:2. An aliquot ($\sim 1.5 \mu\text{L}$) of the sample-matrix solution was deposited onto the probe and allowed to air-dry at room temperature. Electrospray solutions were prepared by dissolving peptides in methanol/water (1:1) solution at a concentration of 10 pmol/ μL and then mixed

Table 1. Summary of H/D Exchange Results for Sodiated Glycine Oligomers

species	observed number of exchanges
[Gly+Na] ⁺	3 (3 fast)
[Gly ₂ +Na] ⁺	3 (1 fast, 2 slow)
[Gly ₃ +Na] ⁺	3 (1 fast, 2 slow)
[Gly ₄ +Na] ⁺	3 (3 fast)
[Gly ₅ +Na] ⁺	3 (3 fast)
[Gly ₃ OMe+Na] ⁺	0 (not observed)
[Gly ₅ OMe+Na] ⁺	0 (not observed)

with an equal volume of 50 pmol/ μL NaCl solution in methanol/water (1:1). An Analytica of Branford (Branford, CT) electrospray source was used. The solutions were continuously sprayed at a 1 $\mu\text{L}/\text{min}$ flow rate using a syringe pump (Harvard Apparatus, model 22, South Natick, MA). No acid was added to the solution.

Computational Methods. Candidate structures were initially evaluated at the PM5 level using CAChe 5.04 (Fujitsu, Beaverton, OR). In some cases, the PM3 level was used for a more accurate description of hydrogen bonding, with the sodium replaced by lithium. Following minimization at the lower level of theory, structures were optimized using density functional theory (DFT). The DFT calculations were carried out using Jaguar 4.1 (Schrödinger, Inc., Portland, OR). Full geometry optimization was performed at the B3LYP/6-31G** level.²² These structures were used as starting points for further optimization at the B3LYP/6-31++G** level, which has been shown to be an appropriate basis set for hydrogen-bonded complexes.^{23,24}

Results and Discussion

The results of the H/D exchange experiments are summarized in Table 1.

In selected cases, the rate constants for H/D exchange were determined by fitting ion abundance to a series of first-order differential equations, assuming a single exchange in each encounter. The extent of H/D exchange between ND₃ and sodiated pentaglycine, as well as between ND₃ and the sodiated methyl ester of pentaglycine, is shown at several reaction times in Figure 1. While there are seven exchangeable hydrogens in sodiated pentaglycine, only three hydrogens have been exchanged in 100 s, and no evidence of further exchange is observed in 800 s of reaction time. No deuterium exchange is observed in 800 s for the sodiated methyl ester of pentaglycine, implying that the presence of a C-terminal hydrogen is necessary for H/D exchange. It is clear that, while the sodiated glycine pentamer exchanges three hydrogens, no exchange is observed with the methyl ester. The kinetic analysis of these data for the sodiated glycine pentamer is shown in Figure 2. For sodiated pentaglycine, the rate constants for H/D exchange with ND₃ were determined to be 3.0×10^{-10} , 2.5×10^{-10} , and $1.5 \times 10^{-10} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ for the three observed hydrogen exchanges. The ratio of these exchange rates is close to 3:2:1, suggesting three equivalent hydrogens exchange with ND₃.

In the discussion below, we characterize fast exchanges as those that occur at a rate of $1 \times 10^{-10} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ or greater, slow exchanges as those with rates between 1×10^{-10} and $1 \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$, and exchanges not observed in our instrument as those with rate constants of less than $1 \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$.

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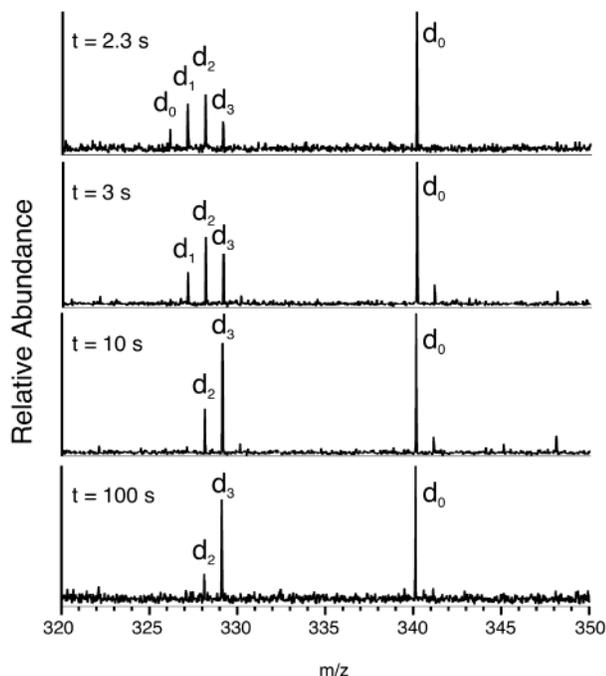


Figure 1. H/D exchange of [Gly₅+Na]⁺ (left) and [Gly₅OMe+Na]⁺ (right) with 1.9×10^{-8} Torr ND₃ over 100 s. [Gly₅OMe+Na]⁺ exchanges no hydrogens in 100 s, while [Gly₅+Na]⁺ exchanges three hydrogens. d_n refers to the number of exchanged hydrogens.

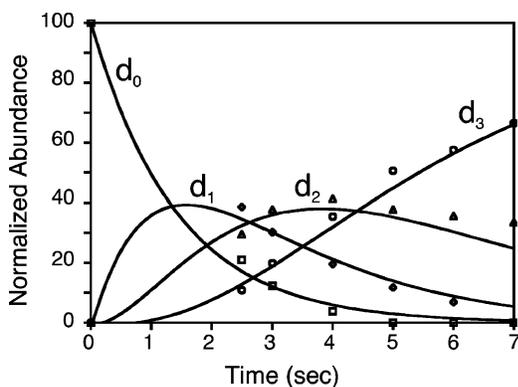


Figure 2. H/D exchange kinetics for Gly₅Na⁺ reacting with ND₃ at 1.9×10^{-8} Torr. d_n refers to the number of exchanged hydrogens.

Sodiated Glycine. Sodiated glycine has previously been studied, both theoretically and experimentally. Bouchonnet and Hoppiliard optimized sodiated glycine at the 3-21G level and found that the most stable structure was one in which the sodium cation is bound both to the nitrogen atom and to the oxygen atom of the carbonyl functional group.²⁵ Higher level calculations by Jensen (optimized at the 6-31G* level),¹⁷ Moison and Armentrout (optimized at the MP2/6-31G* level),¹⁸ and Wyttenbach et al. (optimized at the B3LYP/6-311++G** level)¹⁹ all agree that this structure is a global minimum on the Gly₁Na⁺ potential energy surface. Our calculations are also in agreement with these findings; the charge-solvated structure designated as G1CS in Figure 3 is 10.0 kJ mol⁻¹ more stable than the zwitterionic structure G1ZW.

When the deuterated exchange reagent approaches sodiated glycine, the most favorable configuration of the complex is shown in Figure 4 (G1a). No local minimum was identified in which the proton was transferred from the C-terminus of sodiated glycine to ammonia in the configuration G1a. However,

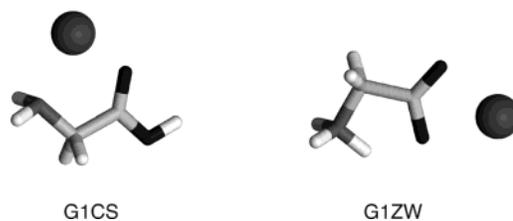


Figure 3. Two structures of Gly₁Na⁺. The charge-solvated structure G1CS is 10.0 kJ mol⁻¹ more stable than the zwitterionic structure G1ZW.

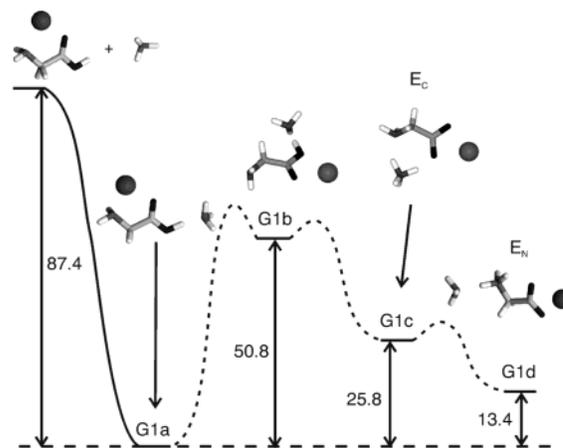


Figure 4. Illustration of the energetics of the exchange process between Gly₁Na⁺ and ammonia. All energies are given in kJ mol⁻¹.

the complex can undergo an onium-ion-type mechanism by which the carboxylic proton is transferred to the N-terminus.

In the onium-ion mechanism, the complex G1a rearranges to form G1b, which is significantly higher in energy than G1a (50.8 kJ mol⁻¹). This complex can then go through the onium-ion intermediate G1c, which is a local minimum, and then rearrange to G1d, which is a local minimum 13.4 kJ mol⁻¹ above G1a. Not surprisingly, this is close to the 10.0 kJ mol⁻¹ difference in energy between G1CS and G1ZZW. In G1d, all three labile hydrogens become equivalent, as experimentally observed. It appears that G1c does not dissociate to give exchanged products without forming G1d.

The structure in which ammonia abstracts the C-terminal proton is shown in Figure 4 as E_C, and the point at which ammonia can exchange with the N-terminal protons is marked as E_N. E_N is energetically downhill from E_C in this case. Because we observe exchanges of three apparently equivalent hydrogens for the sodiated monoglycine exchange with ammonia, we conclude that there is little barrier to reaching E_N from E_C. Once the system is in the configuration E_C, there is little probability of reversing the process and dissociating prior to forming E_N.

Sodiated Diglycine. The lowest-energy structure of sodiated diglycine is a charge-solvated structure (G2CS in Figure 5). Instead of forming a zwitterionic structure like sodiated glycine, sodiated diglycine can take on a ring-type structure, in which the carboxylic acid hydrogen is 1.72 Å away from the N-terminus nitrogen (G2R in Figure 5). For sodiated diglycine, the charge-solvated structure is lower in energy than G2R by 71.2 kJ mol⁻¹. These results are in accordance with the results of Wyttenbach et al.¹⁹ and indicate that the charge-solvated structure is the most likely gas-phase structure for the sodiated diglycine.

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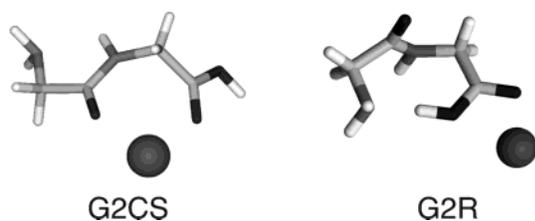


Figure 5. Charge-solvated sodiated diglycine (G2CS) and a ring-type structure for sodiated diglycine (G2R). The charge-solvated structure is lower in energy by 71.2 kJ mol^{-1} .

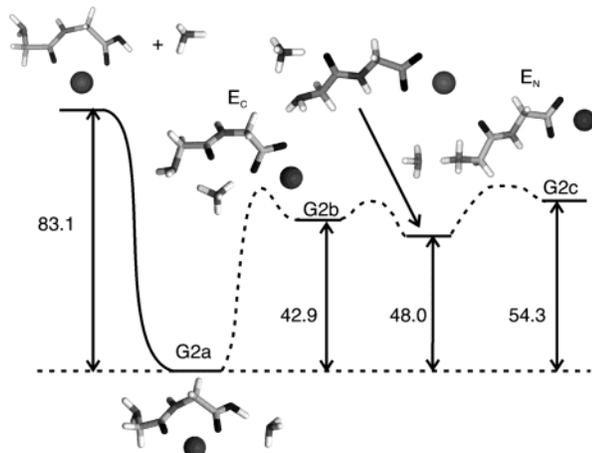


Figure 6. Illustration of the energetics of the exchange process between Gly_2Na^+ and ammonia. All energies are given in kJ mol^{-1} . The barriers separating the energy-minimized structures shown were not evaluated.

Binding the exchange reagent ND_3 , however, provides 83.1 kJ mol^{-1} to the molecular complex (Figure 6). This excess energy allows the adduct to rearrange from structure G2a to structure G2b. ND_3 abstracts a proton from the C-terminus, and the complex rearranges, so that NHD_3^+ forms hydrogen bonds with the N and C termini and the sodium ion is complexed to the C-terminus. The G2b structure corresponds to a salt-bridge structure comprising Na^+ and NHD_3^+ separated by the carboxylate group. In this case, we can infer that the carboxylate hydrogen is exchanged by an onium-ion mechanism.

When the complex has the structure G2b (also denoted E_C), the NHD_3^+ can either donate a deuterium to the C-terminus of the sodiated diglycine or rearrange to exchange with the N-terminus of the diglycine adduct, shown in the latter half of Figure 6. G2c (also denoted E_N) is the structure in which exchange with the N-terminus hydrogens can occur. The observation of one fast and two slow exchanges indicates that the three hydrogens do not equilibrate prior to dissociation of the complex. Our theoretical calculations indicate that the exchange process with the N-terminus requires more rearrangement of the complex, and is higher in energy, than the exchange with the C-terminus of the peptide. This provides a reasonable explanation for the one fast exchange and two slow exchanges observed, where the fast exchange would occur at the C-terminus and the two slow exchanges would correspond to exchange of the N-terminus hydrogens.

Unlike protonated diglycine, which exchanges all five of its labile hydrogens with ND_3 ,⁷ sodiated diglycine exchanges only three of its four exchangeable hydrogens. This suggests that the amide hydrogen is unreactive with respect to exchange. The gas-phase acidity of the amide hydrogen for protonated diglycine



Figure 7. Charge-solvated sodiated triglycine (G3CS) and ring form sodiated triglycine (G3R). The distance between the carboxylic acid hydrogen and the N-terminal nitrogen is 1.77 \AA .

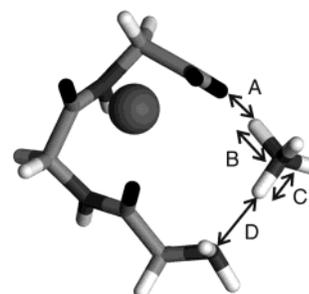


Figure 8. Structure of the sodiated triglycine–ammonia complex illustrating the distances listed in Table 2.

Table 2. Calculated Interatomic Distances (\AA) for Sodiated Triglycine Structures Shown in Figure 9

	A (O...H)	B (H...N)	C (N...H)	D (H...N)
G3b	1.10 ^a	1.51	1.03	2.25
G3c	1.40 ^a	1.15	1.04	2.00
G3d	1.67	1.02 ^b	1.20 ^a	1.59
G3e	1.89	1.02 ^b	1.40 ^a	1.26
G3f	1.89	1.02 ^b	1.60 ^a	1.13

^a This value was fixed before the minimization was carried out. ^b The proton shared between the carboxylate terminus and the ammonia molecule was fixed on the ammonia molecule for these calculations.

is $1076.1 \text{ kJ mol}^{-1}$ at the 6-31++G** level. The gas-phase acidity of the amide hydrogen for sodiated diglycine is higher, $1117.0 \text{ kJ mol}^{-1}$, at the same level of theory. While protonated diglycine can slowly exchange an amide hydrogen, the higher gas-phase acidity of the sodiated species appears to mitigate this exchange process.

Sodiated Triglycine. The charge-solvated form of Gly_3Na^+ is more stable than the ring form by 22.2 kJ mol^{-1} (Figure 7). Binding one ammonia molecule to this species stabilizes the complex by $101.8 \text{ kJ mol}^{-1}$.

To more fully understand the H/D exchange process, the energy of this noncovalent complex was examined as a function of the distance between an oxygen on the C-terminus of the peptide and a proton abstracted from the C-terminus. The interatomic distances between the C-terminus oxygen, the N-terminus nitrogen, the nitrogen in ammonia, and the transferable protons are defined in Figure 8 and given in Table 2.

As the proton moves away from the C-terminus and toward the ammonia, the potential energy surface is very flat, changing only by 5.0 kJ mol^{-1} , as shown in Figure 9. Because this surface is flat, we were unable to converge on a local minimum for this structure. Instead, we chose to examine the potential energy surface by sampling the potential energy surface as a function of interatomic distances, as shown in Table 2. Because the surface was sampled directly, we do not include possible barriers in Figure 9.

When a proton from the C-terminus is transferred to the ammonia, creating an ammonium ion, and a proton is stepped

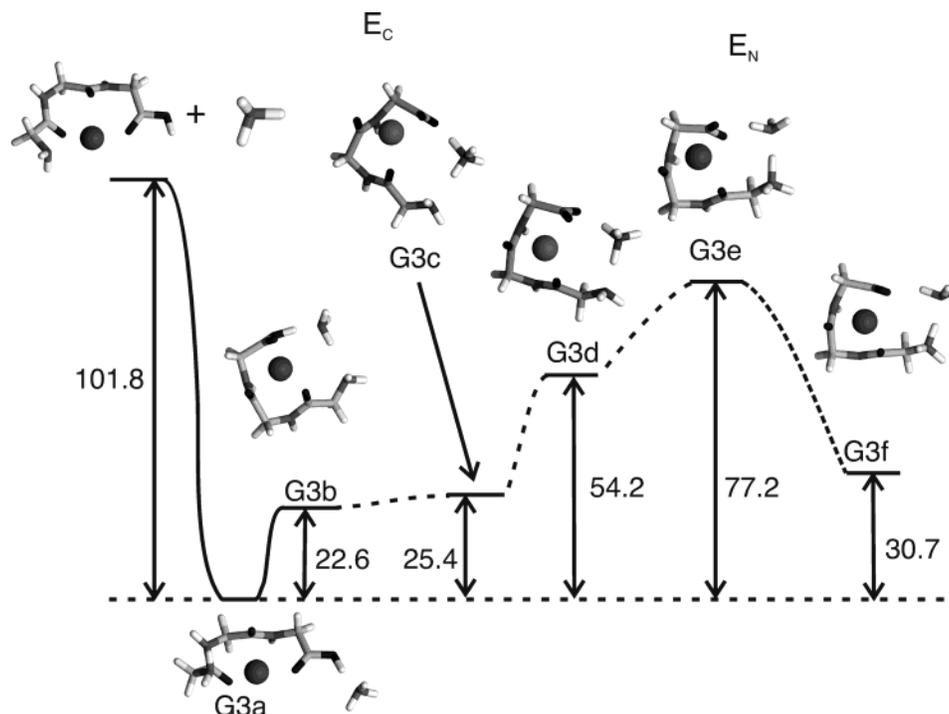


Figure 9. Illustration of the energetics of the exchange process between sodiated triglycine and ammonia. All energies are in kJ mol^{-1} .

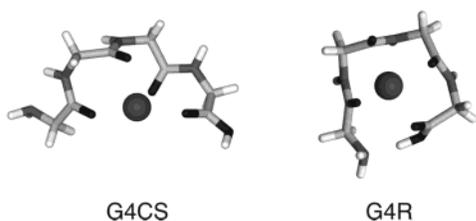


Figure 10. The charge-solvated form of sodiated tetraglycine (G4CS) and the ring structure (G4R).

between the ammonium and the N-terminus of the peptide, there is a much higher potential energy barrier (up to 77.2 kJ mol^{-1}). Although this is still energetically accessible, the higher barrier may be responsible for the slower exchange observed between the N-terminus and ND₃ as compared to that between the C-terminus and ND₃. The energy minimum of this transition is shown as G3c. In reaching this minimum, the complex goes through an ion-stabilized ion pair structure, where the ammonium ion and negatively charged C-terminus are stabilized by the proximity of a sodium cation. Note that, if this mechanism is correct, ammonia must abstract a proton from the carboxylic acid before exchanging with the N-terminal hydrogens. In the O-methyl ester of triglycine, the carboxylic acid site is blocked, so no exchanges are observed.

Sodiased Tetraglycine and Pentaglycine. Unlike diglycine and triglycine, sodiated tetraglycine undergoes three fast exchanges with ND₃. Again, the charge solvation form of sodiated tetraglycine is more stable than the ring form, this time by 33.5 kJ mol^{-1} (Figure 10).

When ND₃ interacts with sodiated tetraglycine, the minimum energy state of the complex has ammonia bound to the C-terminus of the peptide through the acidic hydrogen (Figure 11, structure G4a). The binding of ND₃ releases 56.9 kJ mol^{-1} of energy, and the complex can rearrange to the structure labeled G4b in Figure 11, most likely via a relay mechanism. Here, the

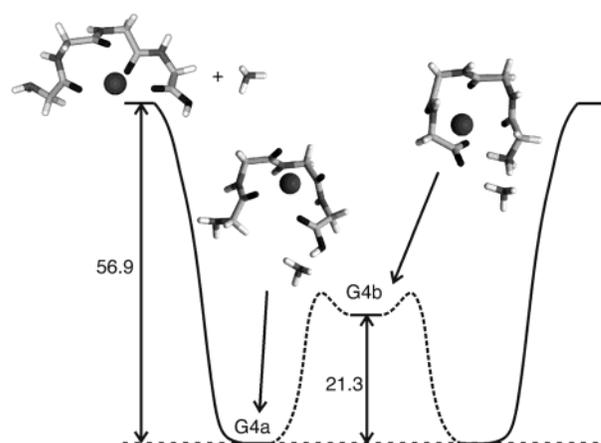


Figure 11. Illustration of the energetics of the exchange process between Gly₄Na⁺ and ammonia. All energies are given in kJ mol^{-1} . Energetics of intermediate species between G4a and G4b were not evaluated.

peptide is in a zwitterionic form, and the three labile hydrogens become equivalent. These results can be extended to the case of the sodiated glycine pentamer, as the structures examined for the sodiated glycine pentamer are very similar in form to those found for the sodiated glycine tetramer. Note that, once again, the acidic hydrogen of the C-terminus is required for the exchange process to occur, which explains why ND₃ does not exchange with the O-methyl ester of sodiated pentaglycine.

Comparison of ND₃ and D₂O as Exchange Reagents for Sodiased Peptides. We have examined the behavior of ND₃ as an exchange reagent for sodiated peptides. D₂O is more commonly used as an exchange reagent, however, and it is interesting to compare our results to those of other studies. Williams et al. provide enough D₂O exchange data with sodiated peptides to draw limited comparisons. For the singly charged sodiated peptide VEIPY, they observe five hydrogens exchanged, which could correspond to the two carboxyl hydrogens,

two N-terminal hydrogens, and the hydrogen on the tyrosine side chain. In this case, the three amide hydrogens do not exchange. This is consistent with the results they observe with singly charged sodiated FLEEL, in which the three carboxylic hydrogens and two N-terminus hydrogens underwent rapid exchange. The four amide hydrogens appear to exchange slowly, if at all.²⁰ In this case, the exchange reagents ND₃ and D₂O exhibit similar behavior, although the mechanisms of exchange are likely to be different. For example, ND₃ can participate in an onium-ion exchange mechanism, while the comparatively less basic D₂O is more likely to participate in a relay exchange mechanism.

In another study, Solouki et al. looked at a single tripeptide, RGD, and observed the extent of exchange when RGD was complexed to various alkali cations, using ND₃ as the exchange reagent. In contrast to our observations, singly charged sodiated RGD appears to exchange one hydrogen slowly (and possibly a second, more slowly) with ND₃. Including the strongly basic arginine residue can drastically affect H/D exchange patterns. Singly protonated RGD exchanged hydrogens more rapidly and completely with ND₃ than did the singly sodiated peptide.

Another interesting study of H/D exchange of singly protonated bradykinin (RPPGFSPFR) and singly sodiated bradykinin, conducted by Freitas and Marshall, found that the sodiated species rapidly exchanged all labile hydrogens with D₂O, while the protonated species was unreactive under similar conditions.⁸ This behavior is quite remarkable and contrasts with the results on smaller peptides obtained by Williams et al., using D₂O as the exchange reagent.²⁰

A recent study by Wyttenbach et al. of H/D exchange of AARAA suggests that, although the lowest-energy conformation of the peptide is a charge-solvated structure, the peptide accesses a salt-bridge-type structure when undergoing exchange with D₂O.²⁷ This provides an additional example where H/D exchange proceeds through structures distinct from the lowest-energy form of the parent ion.

It is difficult to draw any general conclusions about the behavior of ND₃ and D₂O as exchange reagents. It seems that each case must be examined independently and that the use of H/D exchange as a general structural probe in the gas phase must be supplemented with detailed examinations of possible exchange mechanisms.

Conclusions

When using H/D exchange as a probe of molecular structure, the effects of solvation by the exchange reagent must be taken into account, particularly in the gas phase. In the present study, we have shown that interaction of the exchange reagent ND₃ with sodiated oligoglycines can lead to the formation of a chemically activated adduct with sufficient internal excitation to access exchange intermediates which are structurally distinct from the target molecules. This has also been observed in the H/D exchange reaction of ND₃ with arginine monomers and dimers.²⁶ Collision cross sections of the Gly_nNa⁺ complexes (for $n = 1-6$) indicate that sodiated oligoglycines form solvated ion rather than salt-bridge structures in the gas phase.¹⁹ Our calculations concur with these results. However, in the process of H/D exchange with ND₃, sodiated monoglycine and tetraglycine adopt zwitterionic structures, sodiated diglycine adopts a salt-bridge form, and sodiated triglycine takes on an ion-stabilized ion pair form. H/D exchange is facile for these intermediates. The proposed exchange mechanisms require a carboxylic acid hydrogen to complete the exchange, which is in agreement with the experimental results showing that no exchange occurs with methyl ester glycine oligomers. Contrary to the assumption often expressed in earlier studies, H/D exchange kinetics may not directly reflect ion structures.

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