Chapter 7. Gas Phase H/D Exchange of Sodiated Glycine Oligomers with ND_3: Exchange Kinetics Do Not Reflect Parent Ion Structure

Reproduced with permission from Cox, H. A.; Julian, R.R.; Lee, S.-W.; Beauchamp, J.L. *J. Am. Chem. Soc.* 2004, **126**(20); 6485 – 6490.

7.1. 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, ND_3 is reacted with sodiated glycine oligomers, Gly_n, where n = 1 to 5, and the results are interpreted using density functional calculations. Experimentally, Gly_1Na^+, Gly_4Na^+, and Gly_5Na^+ all undergo three fast exchanges with ND_3, while Gly_2Na^+ and Gly_3Na^+ undergo one fast and two slow exchanges with ND_3. The methyl esters Gly_3OMeNa^+ and Gly_5OMeNa^+ do not exchange with ND_3. 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 ND_3, 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. Sodiated monoglycine and diglycine exchange via an onium-ion mechanism. The proposed exchange mechanisms require a carboxylic acid hydrogen in order 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, non-covalent 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.

7.2. Introduction

The introduction of an isotopic label by H/D exchange can provide structural information for biomolecules in both solution[1-5] 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 non-covalent 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 non-zwitterionic 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 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 D_2O, 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 D_2O 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 D_2O. 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, Gly_1 to Gly_5. These model compounds are used to elucidate the fundamental mechanism of H/D exchange involving carboxylic, amide, and amine hydrogens with the exchange reagent

ND_3. The lowest energy structures of the sodiated glycine oligomers are found to be charge solvated structures. However, in the process of H/D exchange, non-covalent complexation of the exchange reagent ND_3 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.

7.3. Methods

7.3.1. 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, ND_3 (~7×10^{-8} torr), was maintained in the cell. MALDI solutions were prepared by mixing 1M 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 (~ 1.5 μL) 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 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 1 μL/min flow rate using a syringe pump (Harvard Apparatus, Model 22, South Natick, MA). No acid was added to the solution.

7.3.2. 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]

7.4. Results and Discussion

The results of the H/D exchange experiments are summarized in Table 7.1.

Table 7.1. 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)
$[Gly_3OMe+Na]^+$	0 (not observed)
$[Gly_5OMe+Na]^+$	0 (not observed)

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 ND_3 and sodiated pentaglycine, as well as between ND_3 and the sodiated methyl ester of pentaglycine, is shown at several reaction times in Figure 7.1. While there are seven exchangeable hydrogens in sodiated pentaglycine, only three hydrogens have been exchanged in 100 seconds, and no evidence of further exchange is observed in 800 seconds of reaction time. No deuterium exchange is observed in 800 seconds 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 7.2. For sodiated pentaglycine, the rate constants for

H/D exchange with ND_3 were determined to be 3.0×10^{-10}, 2.5×10^{-10}, and 1.5×10^{-10} cm^3 $molecule^{-1}$ sec^{-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 ND_3.

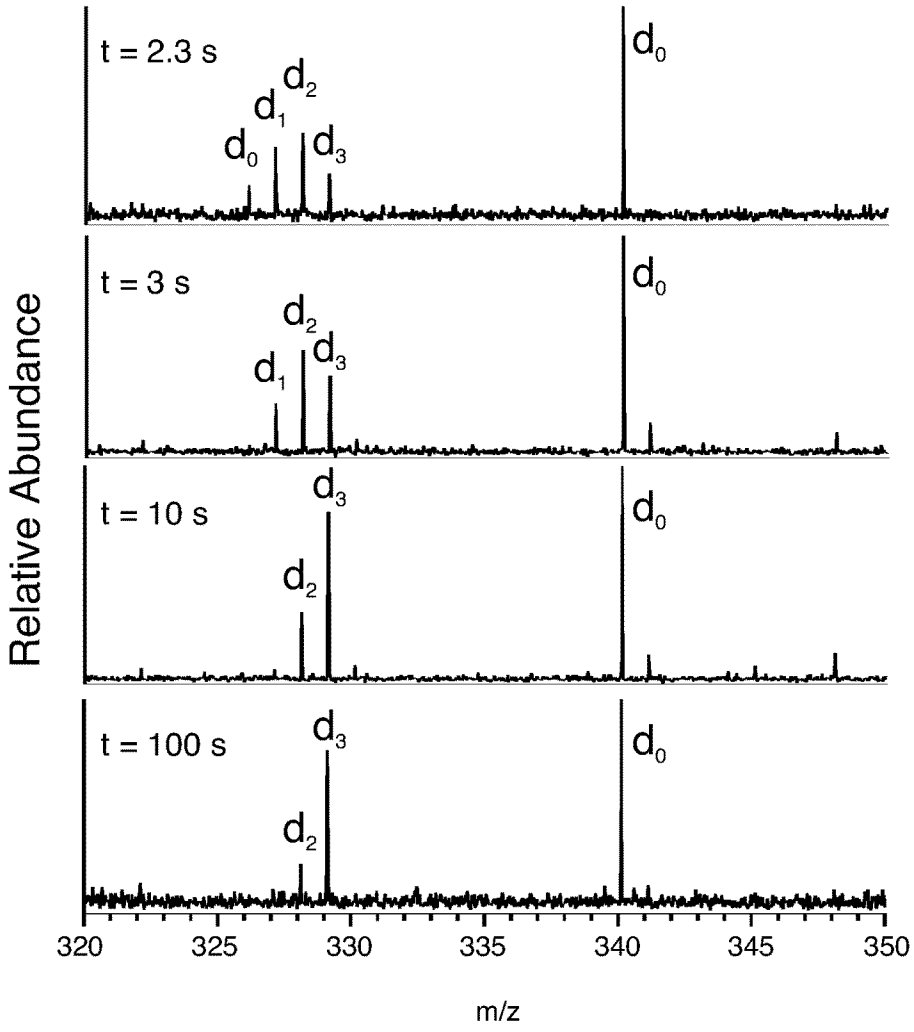

Figure 7.1. H/D exchange of $[Gly_5+Na]^+$ (left) and $[Gly_5OMe+Na]^+$ (right) with 1.9×10^{-8} torr ND_3 over 100 seconds. $[Gly_5OMe+Na]^+$ exchanges no hydrogens in 100 seconds, while [Gly5+Na]+ exchanges three hydrogens. d_n refers to the number of exchanged hydrogens.

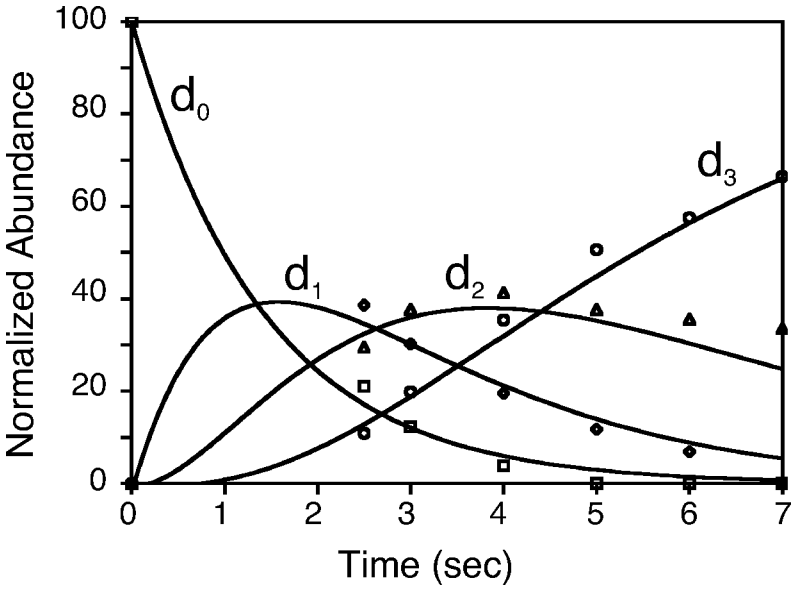

Figure 7.2. H/D exchange kinetics for Gly_5Na^+ reacting with ND_3 at 1.9×10^{-8} torr. d_n refers to the number of exchanged hydrogens.

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

7.4.1. Sodiated glycine

Sodiated glycine has previously been studied, both theoretically and experimentally. Bouchonnet and Hoppillard 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 Gly_1Na^+ potential energy surface. Our calculations are also in agreement with these findings; the charge solvated structure designated as G1CS in Figure 7.3 is 10.0 kJ mol^{-1} more stable than the zwitterionic structure G1ZW.

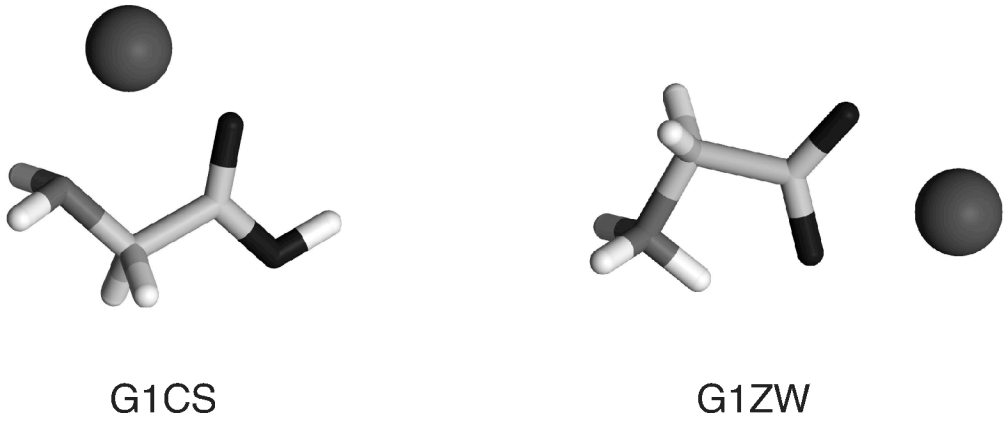

Figure 7.3. Two structures of Gly_1Na^+. The charge solvated structure G1CS is 10.0 kJ mol^{-1} more stable than the zwitterionic structure G1ZW.

When the deuterated exchange reagent approaches sodiated glycine, the most favorable configuration of the complex is shown in Figure 7.4 (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, 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 G1ZW. 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 7.4 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. Since 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.

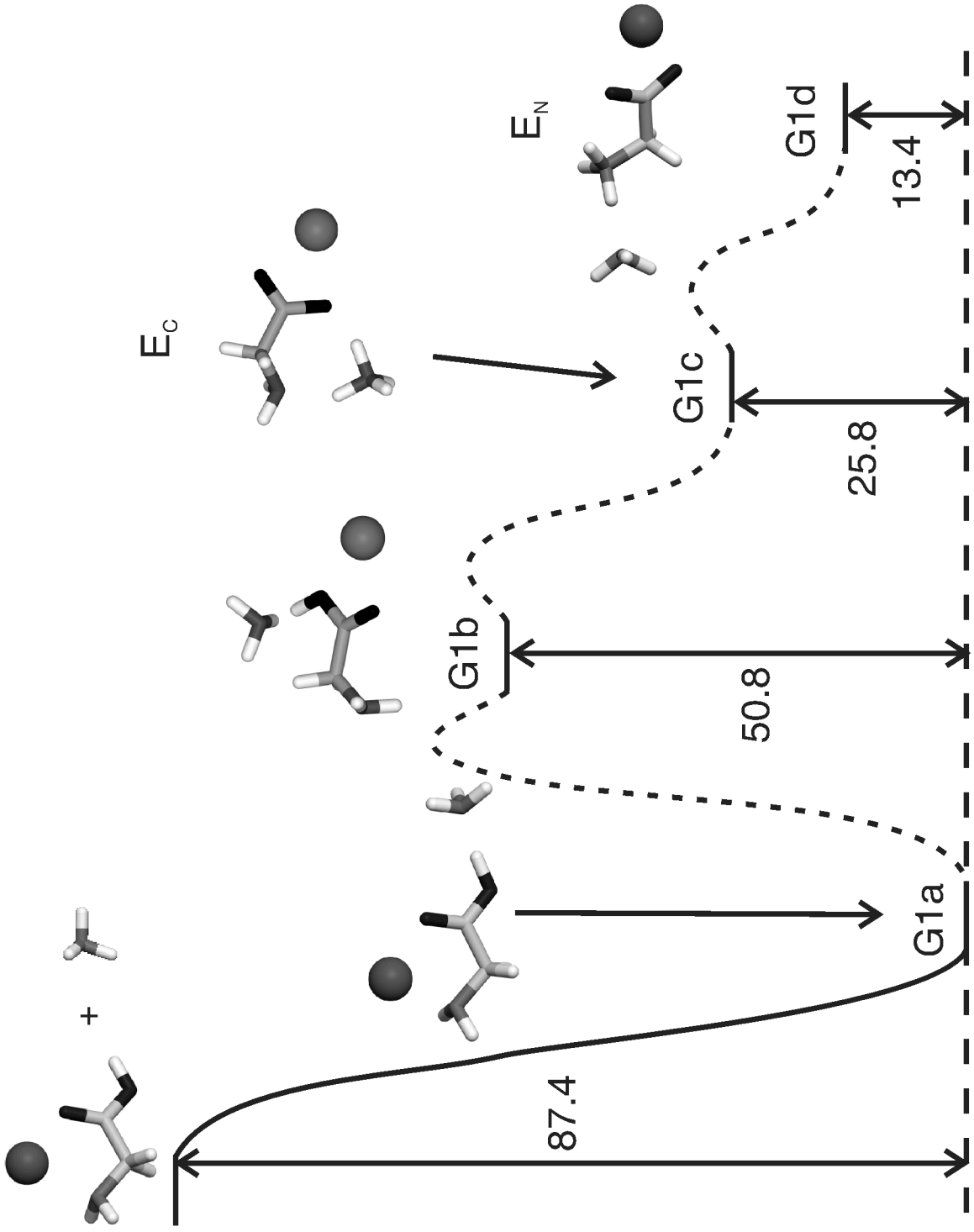

Figure 7.4. Illustration of the energetics of the exchange process between Gly_1Na^+ and ammonia. All energies are given in kJ mol^{-1}.

7.4.2. Sodiated Diglycine

The lowest-energy structure of sodiated diglycine is a charge solvated structure (G2CS in Figure 7.5). 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 7.5). 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.

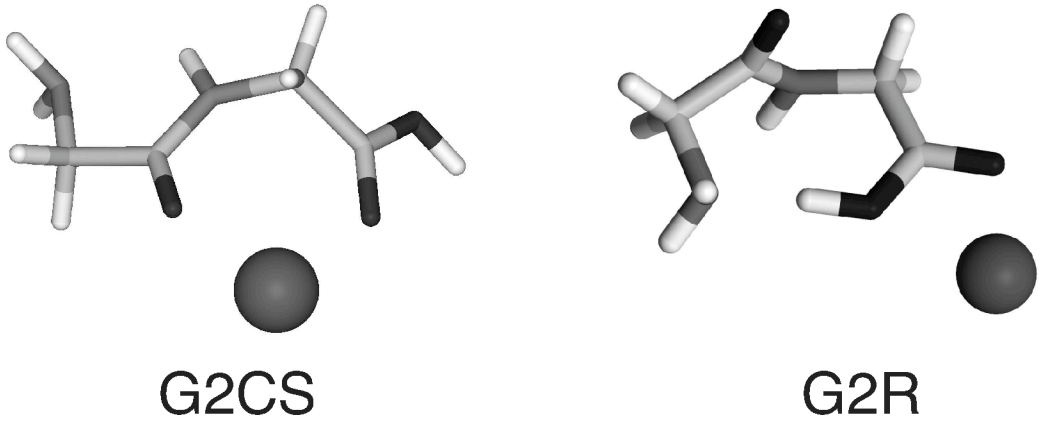

Figure 7.5. Charge solvated sodiated diglycine (G2CS) and a ring-type structure for sodiated diglycine (G2R). The charge solvated structure is lower in energy by 71.2 kJ mol^{-1}.

Binding the exchange reagent ND_3, however, provides 83.1 kJ mol^{-1} to the molecular complex (Figure 7.6). 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 mechamism.

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 it can rearrange to exchange with the N-terminus of the diglycine adduct, shown in the latter half of Figure 7.6. 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.

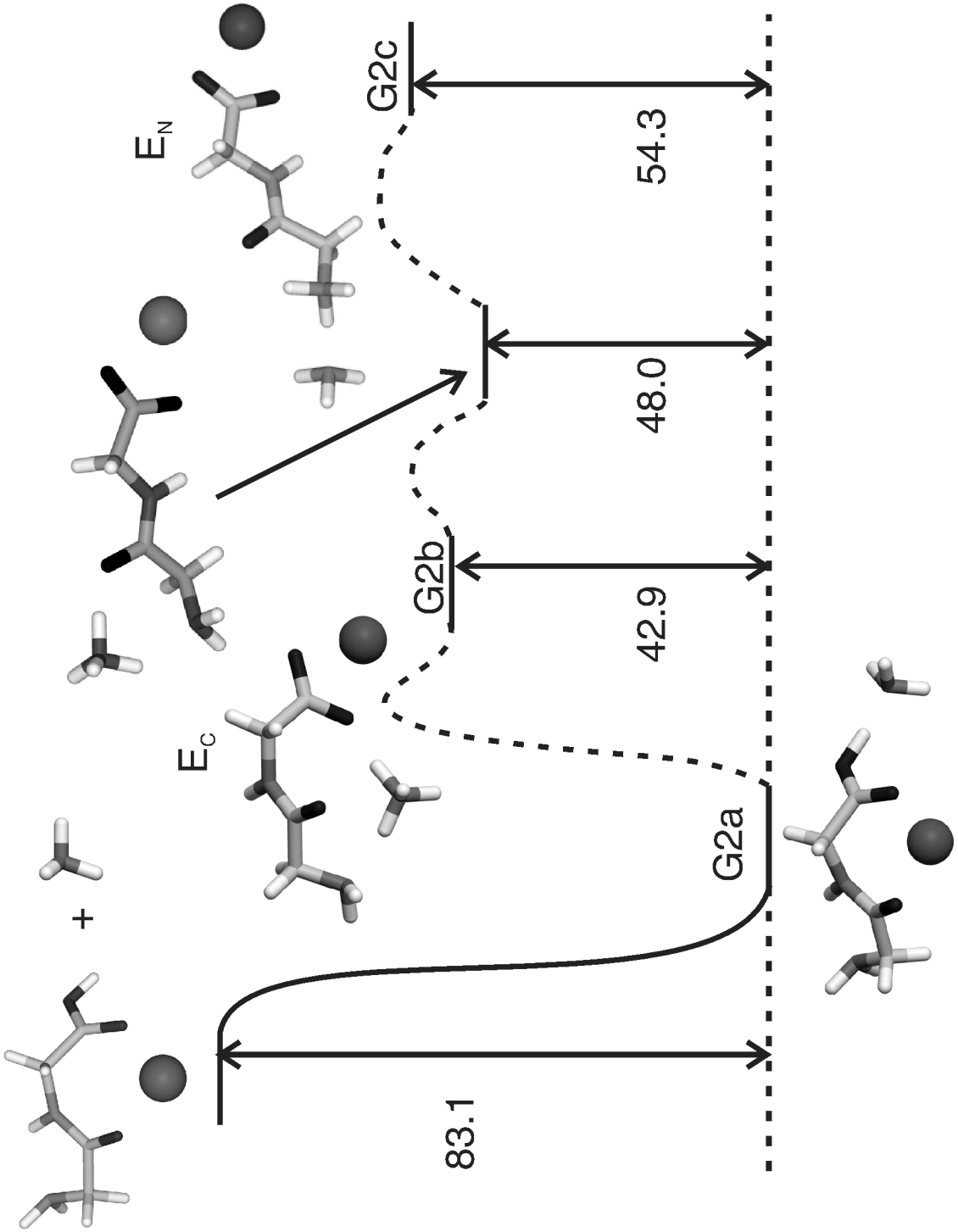

Figure 7.6. Illustration of the energetics of the exchange process between Gly_2Na^+ and ammonia. All energies are given in kJ mol^{-1}. The barriers separating the energy minimized structures shown were not evaluated.

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 is 1076.1 kJ mol^{-1} at the 6-31++G** level. The gas phase acidity of the amide hydrogen for sodiated diglycine is higher, 1117.0 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.

7.4.3. Sodiated Triglycine

The charge solvated form of Gly_3Na^+ is more stable than the ring form by 22.2 kJ mol^{-1} (Figure 7.7). Binding one ammonia molecule to this species stabilizes the complex by 101.8 kJ mol^{-1}.

To more fully understand the H/D exchange process, the energy of this non-covalent 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 7.8 and given in Table 7.2.

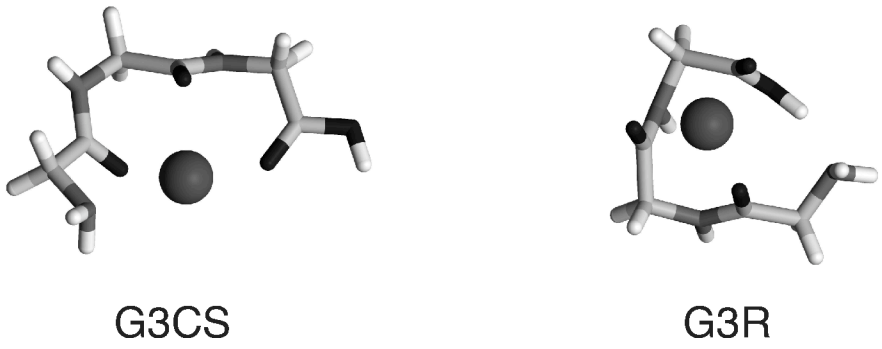

Figure 7.7. Charge solvated sodiated triglycine (G3CS) and ring form sodiated triglycine (G3R). The distance between the carboxylic acid hydrogen and the N-terminal nitrogen is 1.77 Å.

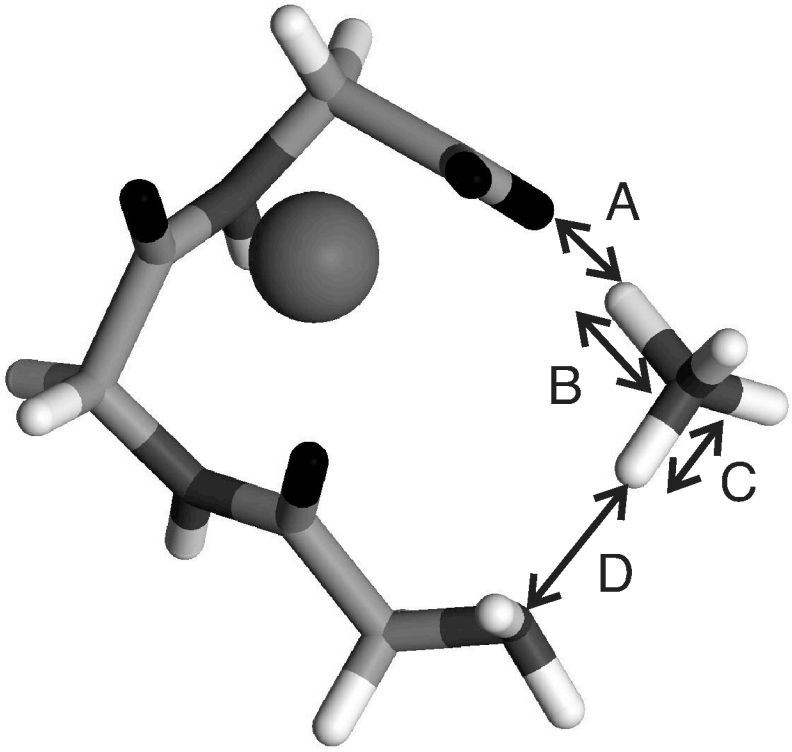

Figure 7.8. A structure of the sodiated triglycine-ammonia complex illustrating the distances listed in Table 7.2.

Table 7.2. Calculated interatomic distances (Å) for sodiated triglycine structures shown in Figure 7.8.

	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.

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 7.9. 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 7.2. Because the surface was sampled directly, we do not include possible barriers in Figure 7.9.

When a proton from the C-terminus is transferred to the ammonia, creating an ammonium ion, and a proton is stepped 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 ND_3 as compared to that between the C-terminus and ND_3. 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.

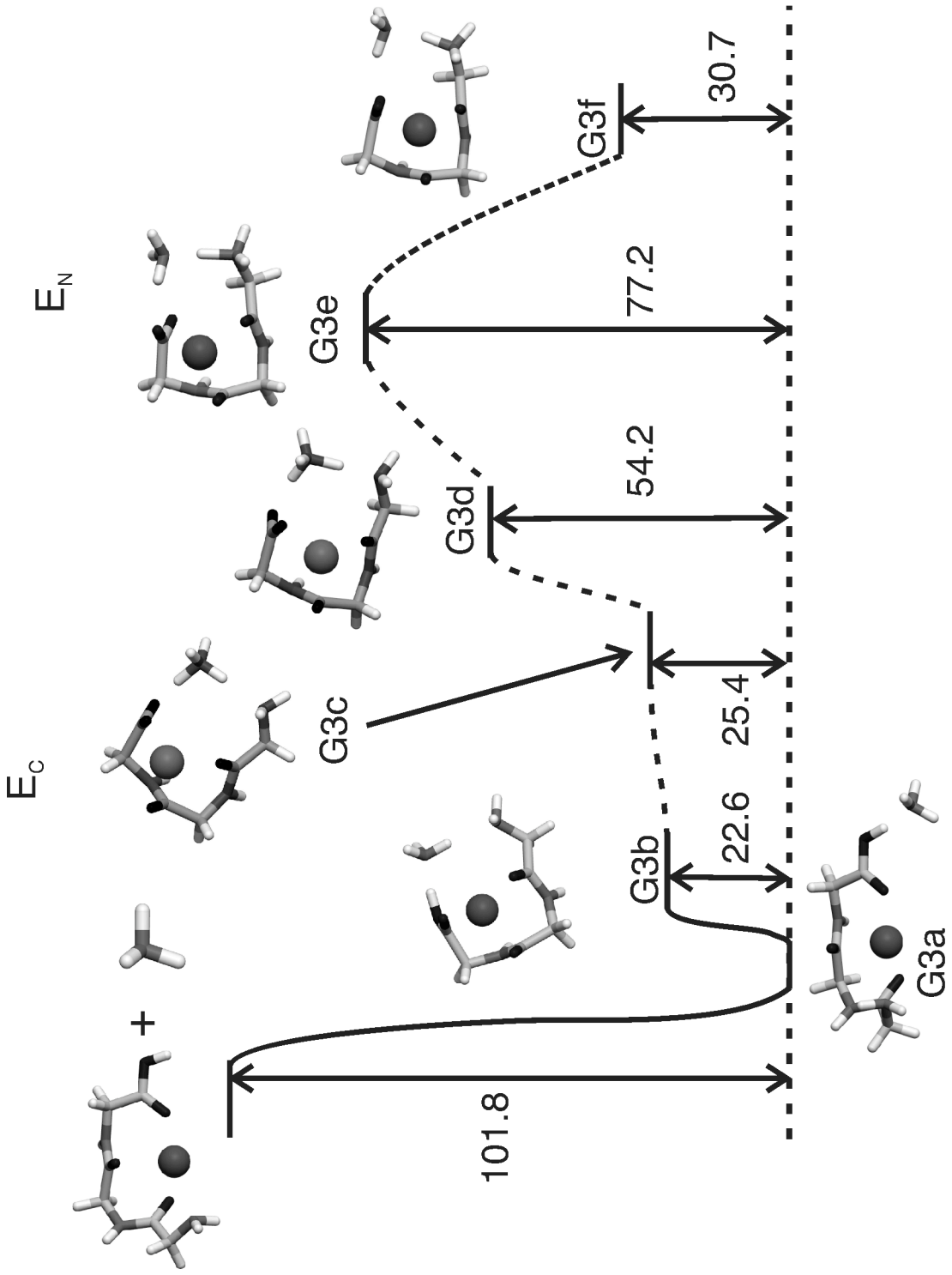

Figure 7.9. Illustration of the energetics of the exchange process between sodiated triglycine and ammonia. All energies are in kJ mol^{-1}.

7.4.4. Sodiated Tetraglycine and Pentaglycine

Unlike diglycine and triglycine, sodiated tetraglycine undergoes three fast exchanges with ND_3. Again, the charge solvation form of sodiated tetraglycine is more stable than the ring form, this time by 33.5 kJ mol^{-1} (Figure 7.10).

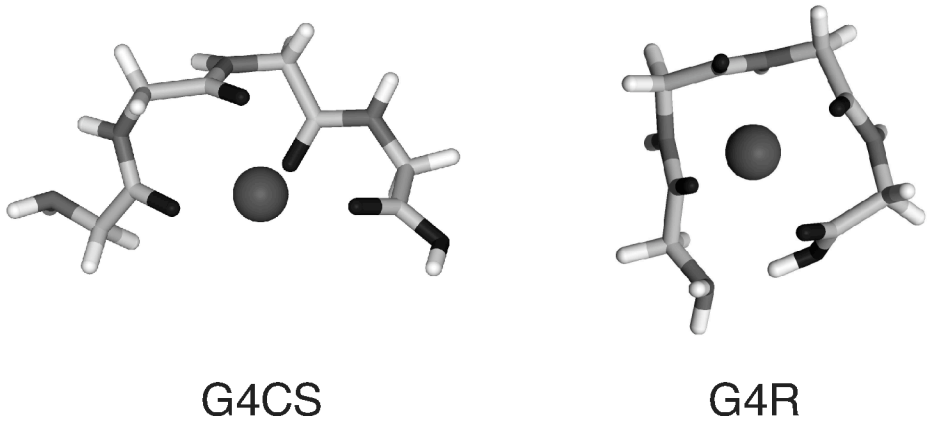

Figure 7.10. The charge solvated form of sodiated tetraglycine (G4CS) and the ring structure (G4R).

When ND_3 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 7.11, structure G4a). The binding of ND_3 releases 56.9 kJ mol^{-1} of energy, and the complex can rearrange to the structure labeled G4b in Figure 7.11, most likely via a relay mechanism. Here, the 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 ND_3 does not exchange with the O-methyl ester of sodiated pentaglycine.

7.4.5. Comparison of ND_3 and D_2O as Exchange Reagents for Sodiated Peptides

We have examined the behavior of ND_3 as an exchange reagent for sodiated peptides. D_2O 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 D_2O exchange data with sodiated peptides to draw limited comparisons. For the singly charged sodiated peptide VEPIPY, 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 ND_3 and D_2O exhibit similar behavior, although the mechanisms of exchange are likely to be different. For example, ND_3 can participate in an onium ion exchange mechanism, while the comparatively less basic D_2O is more likely to participate in a relay exchange mechanism.

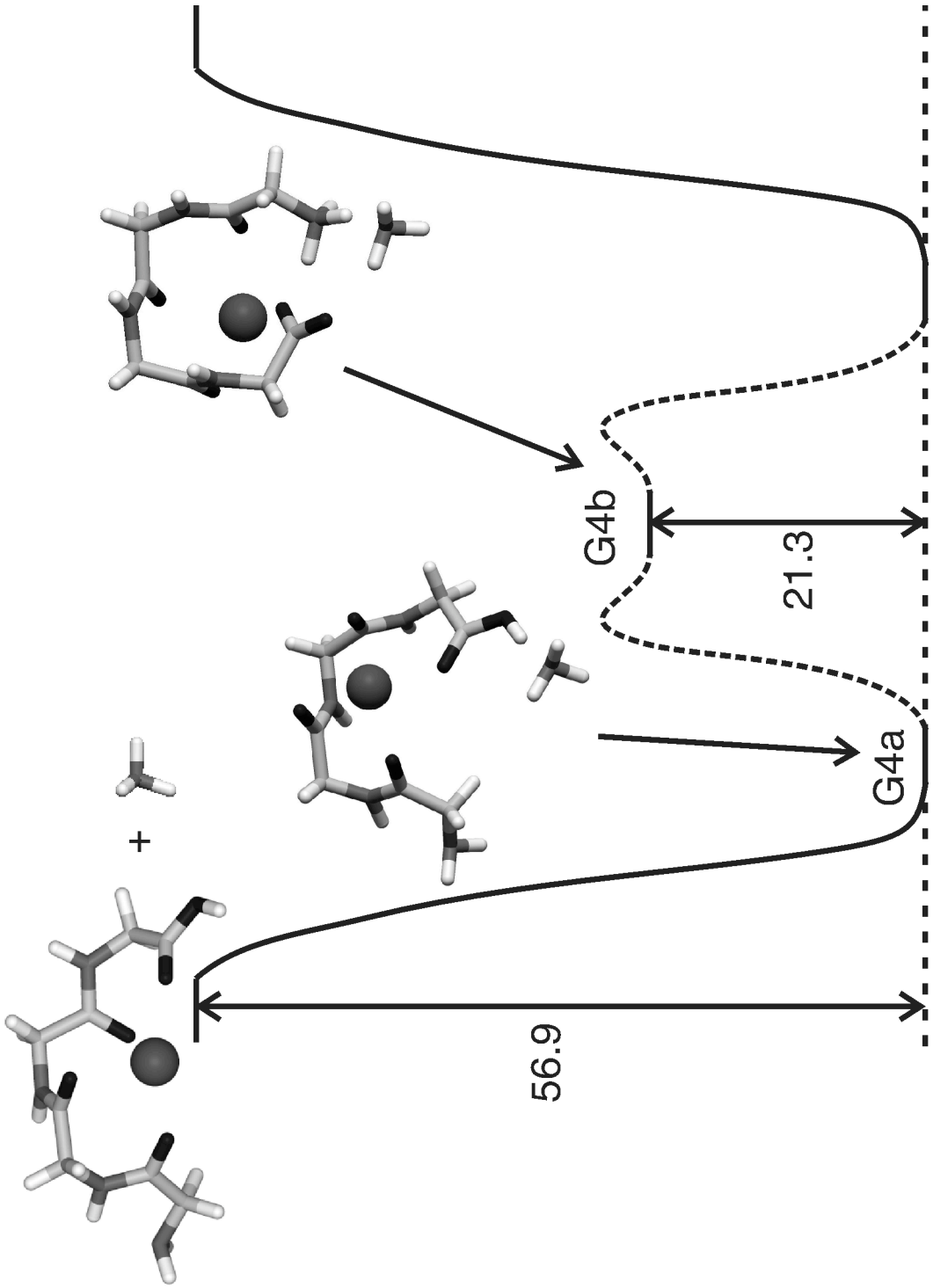

Figure 7.11. Illustration of the energetics of the exchange process between Gly_4Na^+ and ammonia. All energies are given in kJ mol^{-1}. Energetics of intermediate species between G4a and G4b were not evaluated.

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 ND_3 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 ND_3. Including the strongly basic arginine residue can drastically affect H/D exchange patterns. Singly protonated RGD exchanged hydrogens more rapidly and completely with ND_3 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 D_2O, while the protonated speices 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 D_2O 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 D_2O.[26] 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 ND_3 and D_2O 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.

7.5. 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 ND_3 with sodiated oligoglycines can lead to the formation of a chemically activated adduct with sufficient internal excitation to access exchange intermediates that are structurally distinct from the target molecules. This has also been observed in the H/D exchange reaction of ND_3 with arginine monomers and dimers.[27] Collision cross sections of the Gly_nNa^+ complexes (for n = 1 to 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 ND_3, 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 in order 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.

7.6. Acknowledgement

The authors gratefully acknowledge support from the Beckman Institute and computational resources provided by the Materials Simulation Center. This work was supported in part by NSF Grant CHE-9727566.

7.7. References

(1) Watson, A. A.; Fairlie, D. P.; Craik, D. J. *Biochem.* **1998**, *37*, 12700.

(2) Zhang, Z.; Li, W.; Logan, T. M.; Li, M.; Marshall, A. G. *Protein Sci.* **1997**, *6*, 2203.

(3) Konermann, L.; Simmons, D. A. *Mass Spectrom. Rev.* **2003**, *22*, 1.

(4) Miranker, A.; Robinson, C. V.; Radford, S. E.; Dobson, C. J. *FASEB J.* **1996**, *10*, 93.

(5) Smith, D. L.; Deng, Y.; Zhang, Z. *J. Mass Spectrom.* **1997**, *32*, 135.

(6) He, F.; Marshall, A. G.; Freitas, M. A. *J. Phys. Chem. B* **2001**, *105*, 2244.

(7) Campbell, S.; Rodgers, M. T.; Marzluff, E. M.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1995**, *117*, 12840.

(8) Freitas, M. A.; Marshall, A. G. *Int. J. Mass Spectrom.* **1999**, *182/183*, 221.

(9) Mao, D.; Douglas, D. J. *J. Am. Soc. Mass Spectrom.* **2003**, *14*, 85.

(10) Geller, O.; Lifshitz, C. *Int. J. Mass Spectrom.* **2003**, *227*, 77.

(11) Valentine, S. J.; Clemmer, D. E. *J. Am. Chem. Soc.* **1997**, *119*, 3558.

(12) Wood, T. D.; Chorush, R. A.; Wampler III, F. M.; Little, D. P.; O'Connor, P. B.; McLafferty, F. W. *PNAS USA* **1995**, *92*, 2451.

(13) McLafferty, F. W.; Guan, Z.; Haupts, U.; Wood, T. D.; Kelleher, N. D. *J. Am. Chem. Soc.* **1998**, *120*, 4732.

(14) Mao, D.; Babu, K. R.; Chen, Y.-L.; Douglas, D. J. *Anal. Chem.* **2003**, *75*, 1325.

(15) Ausloos, P.; Lias, S. G. *J. Am. Chem. Soc.* **1981**, *103*, 3641.

(16) Solouki, T.; Fort, J., R. C.; Alomary, A.; Fattahi, A. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 1272.

(17) Jensen, F. *J. Am. Chem. Soc.* **1992**, *114*, 9533.

(18) Moision, R. M.; Armentrout, P. B. *J. Phys. Chem. A* **2002**, *106*, 10350.

(19) Wyttenbach, T.; Bushnell, J. E.; Bowers, M. T. *J. Am. Chem. Soc.* **1998**, *120*, 5098.

(20) Jurchen, J. C.; Cooper, R. E.; Williams, E. R. *J. Am. Soc. Mass Spectrom.* **2003**, *14*, 1477.

(21) Rodgers, M. T.; Campbell, S.; Marzluff, E. M.; Beauchamp, J. L. *Int. J. Mass Spectrom.* **1995**, *148*, 1.

(22) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.

(23) Del Bene, J. E.; Person, W. B.; Szczepaniak, K. *J. Phys. Chem.* **1995**, *99*, 10705.

(24) Balta, B.; Basma, M.; Aviyente, V.; Zhu, C.; Lifshitz, C. *Int. J. Mass Spectrom.* **2000**, *201*, 69.

(25) Bouchonnet, S.; Hoppilliard, Y. *Org. Mass Spectrom.* **1992**, *27*, 71.

(26) Wyttenbach, T.; Paizs, B.; Barran, P.; Breci, L.; Liu, D.; Suhai, S.; Wysocki, V. H.; Bowers, M. T. *J. Am. Chem. Soc.* **2003**, *125*, 13768.

(27) Geller, O.; Lifshitz, C. *J. Phys. Chem. A* **2003**, *107*, 5654.