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**Probing the Mechanisms and Dynamics of Gas Phase
Hydrogen – Deuterium Exchange Reactions of Sodiated Polyglycines**

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Abstract

The rate constants for H/D exchange reactions of sodiated polyglycines (G_nNa^+ , $n=2-8$) and polyalanines (A_nNa^+ , $n=2, 3$ and 5) with ND_3 have been measured in the cell of an FT-ICR mass spectrometer. All peptides except G_2Na^+ are found to undergo three exchange reactions, all of which are consecutive with no sign of multiple exchanges within a single collision event. This information has been used to construct full mechanistic scenarios with the help of detailed quantum chemical calculations of the possible reaction paths for H/D exchange. The first exchange is always located at the C terminus however with different mechanisms depending upon whether the peptide termini can (larger peptides) or cannot (smaller peptides) interact directly without strong energy penalty. The most favourable mechanisms for the second and third exchanges of the N terminus protons, are found to be different from those for the first for all peptide sizes. The peptide distortions that are necessary in order for some of these reactions to occur are made possible by the energy reservoir provided by the favorable interaction of the peptide ion with ND_3 . Their occurrence and variety preclude any general relationship between H/D exchange kinetics and the most stable ion structures. There is however a break at G_7Na^+ in the kinetics trend, with a first exchange rate which is much smaller than for all other peptide sizes. This break can be directly related to a different structural type in which the C terminus is neither free nor close to the N terminus.

Introduction

H/D exchange reactions and their kinetics in the gas phase have long been used to obtain structural information on mass-selected ions^{1,2}. One approach is to use kinetic behaviour to infer the existence of a single isomer or of a mixture, assuming that isomers react at different rates. Analysis of kinetic data may even provide access to relative populations of isomers³. Another tool is to identify the maximum number of hydrogens which can be replaced by deuteria to distinguish between isomers or conformers. This has been successful for distinguishing between isomers of small organic ions such as keto and enol isomers⁴⁻⁷, however it requires complementary information to relate isomers and exchange rates. The distinction between conformers of complex molecules such as peptides is even more challenging. Even though it is intuitively appealing to consider that, in analogy with condensed phase experiments, the more compact the structure, the fewer the accessible exchangeable hydrogens and therefore the smaller the number of exchanges to be observed, there are several recent reports which provide counter examples⁸⁻¹². It can be argued that the electrostatic stabilization which arises from the association of the charged analyte and the H/D exchange reagent may be enough to trigger conformational changes in the analyte, precluding any direct relationship between the rate and number of H/D exchanges and a specific structure¹³⁻¹⁵. It remains, however, that reactions occurring at different rates within a family of related species may be interpreted as showing that starting structures are different. In any case, exchange mechanisms must be considered in detail in order to improve the relevance of exchange kinetics data.

Several mechanisms for H/D exchange of protonated peptides have been proposed¹⁶, involving different types and sequences of N-H and/or O-H bond breaking and making steps. Some of them have gained confirmation in recent detailed studies¹⁷. The situation is different for sodiated peptides because a sodium ion exerts a much weaker bond activation effect than does a proton. Most of the mechanisms invoked in the relatively few studies published so far involve proton transfer from a strongly acidic site (either the C terminus or Asp or Glu side chains) to a basic site, either the N terminus or the side chains of Arg, Lys or potentially less basic side chains¹⁸⁻²¹. The required involvement of an acidic site is reinforced by the observation that when the C-terminus of sodiated tri- or pentaglycine is modified to be a methyl ester, no

exchange is observed¹⁴. In the present work, H/D exchange kinetics are used to compare the sodiated glycine oligomers, in an attempt to relate different kinetics to different starting structures. To this end, the results of H/D exchange experiments of mass-selected G_nNa^+ ($n=2-8$) ions with ND_3 are described below, and the mechanisms based on detailed quantum mechanical investigations of the potential energy surfaces associated with H/D exchange are discussed. We have used ND_3 as the exchange reagent, rather than, for example, D_2O or CH_3OD in order to generate faster exchanges and thus to establish more easily the kinetics and maximum number of hydrogens which can be exchanged.

Experimental and computational section

Gas phase H/D exchange experiments were carried out on a Bruker BioAPEX III Fourier-transform ion cyclotron resonance mass spectrometer with a 7 T magnet which has been described elsewhere²².

The pressure of ND_3 in the FTICR cell was determined via calibration of the ionization gauge reading using well-known reaction rate constants for ion–molecule reactions involving ammonia, as well as other species. The walls of the vacuum system were conditioned by repetitive additions and evacuations of ND_3 in the FT-ICR cell and inlet lines. Each of the sodiated polyglycines (G_nNa^+) was generated by electrospray ionization and then accumulated in an rf-only quadrupolar field before being transferred as a pulse to the FT-ICR cell via a series of high voltage ion optical elements. The reactant ions are decelerated to ≤ 1 eV by a cylindrical decelerator lens assembly in the fringing field of the superconducting magnet and then trapped in the FTICR cell, following which any excess kinetic energy was removed by a pulse of Ar gas into the cell to effect efficient collisional cooling. The G_nNa^+ species of interest was then isolated in the FTICR cell by a series of rf ejection events and allowed to react with the ND_3 at pressures between 5×10^{-9} and 5×10^{-8} mbar, depending upon the magnitude of the exchange rate constants. Reaction times up to 2000 s. were used to examine the slowest reactions observed. Mass spectra were then taken at a series of controlled delay times spaced at relatively short intervals in the early reaction period to much longer intervals later in the reaction sequence when overall relative intensities were changing much more slowly. In another experiment, G_5Na^+ and G_7Na^+ were isolated together and allowed to react with ND_3 in order to derive

relative rate constants and check the consistency of the above experiments. Kinetics experiments were also carried out for A_2Na^+ , A_3Na^+ and A_5Na^+ to provide a comparison with the G_nNa^+ species.

In order to derive approximate rate constants for the H/D exchange reactions, the kinetic data were analyzed as $\log_{10}(\text{relative intensity})$ vs. time plots and the limiting slopes of intensity of the decaying species were analyzed assuming first order kinetics. An example is shown in Figure S1 for Ala_3Na^+ . It is evident that the intensities do not exhibit perfectly first order decay for which there are two reasons. The first of these concerns the initial decay of the perprotio species which exhibits a gradually increasing decay slope as a result of the fact that the ions initially arrive in the FTICR cell with excess kinetic energy. As collisional cooling occurs, resulting from the pulse of Ar gas, the ions are relaxed to a thermal distribution and the logarithmic intensity profile becomes a linear decrease. At the longer times, as exhibited in all of the cases shown, the penultimate exchange species also does not show simple first order kinetics. This is the result of the fact that, even though the ND_3 reagent itself is isotopically quite pure (>99%), H/D exchange occurs on the walls of the vacuum system such that a total of ~10% of ND_2H and/or NDH_2 is present which results in back exchange preventing the exchange sequence going to completion. Finally, due to the difficulty in controlling the pressure of ND_3 in the FTICR cell for the long periods of time (up to 2-3 hours) required to collect the kinetic data for each species examined, there are occasionally slight discontinuities (see Fig. S5 for example). For these reasons the absolute rate constants can only be considered to be accurate to roughly $\pm 30\%$.

Structures were energy-minimized at the B3LYP/6-31G(d) level. Vibrational frequencies were calculated at the same level to assess the minimum or saddle point nature of the extrema found. Transition states were identified by inspecting the transition mode associated with the single imaginary frequency, and in some cases intrinsic reaction coordinate calculations were carried out in order to check which energy minima are connected by this transition structure. Final energetics computed at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d) level turned out to be partly incompatible with experimental results for G_3Na^+ . The computational level was then improved to MP2(full)/aug-cc-pVTZ//MP2(full)/6-31G(d) and it is the results obtained at this level which will be described below. However the MP2 level becomes intractable for G_6Na^+ and

larger peptides. Thus B3LYP results were used for the limited computations of the larger peptides described below. All calculations were carried out using the Gaussian09 suite of programs³¹.

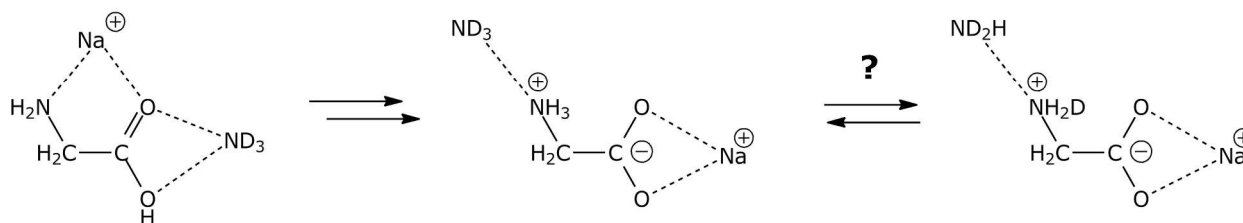
Results and Discussion

A. Kinetic Data

Even though this study is primarily concerned with polyglycines, it proved of interest simultaneously to examine the results for some polyalanines as well, in order to better understand the mechanisms involved in the H/D exchange reactions. The gas phase hydrogen-deuterium exchange reactions of a series of polyglycines (G_nNa^+ , $n=2-8$) as well as three polyalanines (A_nNa^+ , $n=2,3,5$) have been examined and the rate constants for these reactions are summarized in Table 1. Particular features for each of the polypeptides studied are summarized below. For completeness and context, the work of Beauchamp and co-workers for sodiated glycine GNa^+ ²¹ is also presented, although analogous experiments have not been carried out in the present work.

GNa^+

Beauchamp and co-workers previously carried out H/D exchange experiments for GNa^+ with ND_3 ²¹ which are described as exhibiting three fast exchanges, where “fast” for their purposes implied a rate constant greater than $10^{-10} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ while “slow” was taken to mean a rate constant between 10^{-12} and $10^{-10} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$. These authors also proposed a mechanism for the gas phase H/D exchange of sodiated glycine with ND_3 which, as shown below, involves transformation of this ion from a charge solvated to a zwitterionic form within the complex with ND_3 . In the lowest energy structure proposed for this complex, Na^+ is symmetrically coordinated to the two oxygens of the carboxylate moiety and ND_3 is associated *via* strong hydrogen bonding with a hydrogen of the ammonium functionality of the zwitterionic glycine.



As such, the ammonium-ND₃ combination bears a strong resemblance to the situation found for H/D exchange between ammonia and protonated glycine in which it is observed that more than one H/D exchange event could occur within a single encounter complex such that 1, 2 and 3 exchanges were observed to be occurring to a certain extent simultaneously^{16,17}. Unlike the situation for protonated glycine and protonated oligomers of glycine¹⁷ however, H/D exchange of sodiated glycine appears to occur via sequential events to exchange the three labile hydrogens. In an analogous study, Rozman *et al.*¹⁵ observed three sequential exchange events for a series of sodiated amino acids (Ala, Asp, Cys, Glu, Ile, Leu, Val) with either D₂O or CH₃OD. For reaction of both D₂O and CH₃OD with the sodiated complex of alanine, as well as the other amino acids studied, the reactions are reported as having identical (within experimental error) rate constants for each sequential exchange. The computational work carried out by Rozman *et al.*¹⁵ suggests that a relatively low energy pathway does exist, in the presence of D₂O, to transform the most stable charge solvated complex to its zwitterionic analogue which lies 5.2 kJ mol⁻¹ higher in energy. As in the case of the mechanism proposed by Beauchamp and co-workers, the absence of multiple exchanges within a single encounter with D₂O appears to be incompatible with a zwitterionic mechanism for ND₃ and the intermediates necessarily involved. Thus the kinetic data would seem to imply that a mechanism other than that implicating a zwitterionic complex might be involved. As shown below, the pattern of H/D exchange rates for GNa⁺ represents a unique case in that the second and third exchanges are relatively facile whereas those for G₂Na⁺ and AlaNa⁺ are either non-existent or extremely slow.

G₂Na⁺ and A₂Na⁺

Experiments carried out by Cox *et al.* for G₂Na⁺¹⁴ are reported to yield one fast and two slow exchanges. Although no transition states were located on their proposed potential energy surface, these authors suggested that while H/D exchange may occur rapidly at the carboxylic

acid site, the exchange of the terminal amine hydrogens must be the result of more extensive rearrangement to a complex of higher energy thus leading to slower exchange reactions. Even so, it might have been expected that once such a complex structure was accessed, more than one exchange could occur within the complex proposed, which is not the situation observed. In contrast, analogous experiments carried out in this work revealed only one fast exchange for G_2Na^+ , with no evidence for the two subsequent exchanges.

The H/D exchange results obtained in the present work for the two smallest sodiated peptides studied, G_2Na^+ and A_2Na^+ , are shown in Figure S1 in which both experiments were carried out simultaneously in the FTICR cell. On the time scale shown here, the rate constants for the first H/D exchanges are seen to be identical and to occur effectively at roughly one-third the ion-molecule collision rate with ND_3 . However, an examination of the longer time data reveals that, while the second and third H/D exchanges for G_2Na^+ are not observable at all, those for A_2Na^+ do occur, albeit with rate constants more than 100 times less than that of the first exchange reaction.

These experimental observations are consistent with a single, first H/D exchange which occurs at the C-terminal carboxyl hydrogen. The slow, or absent, exchanges at the amine terminus would suggest that the sodiated diglycine, in its most stable configurations, cannot easily bring the C- and N-terminal groups in close enough proximity to permit a facile hydrogen exchange. It is possible however, that the complexation with ND_3 can bring about an exchange by acting as a bridging group between the termini. That being said however, if this does occur, multiple exchanges might also be expected. These concerns for the viability of a zwitterionic mechanism for sodiated glycine and diglycine also apply equally well to the observations of H/D exchange for the larger polyglycines and polyalanines. Another important observation is that, for the sodiated esters of the peptides in question, it is found that rigorously zero exchange is observed.

G_3Na^+ and A_3Na^+

The H/D exchange data for G_3Na^+ are shown in Figure 1 and those for A_3Na^+ are shown in Figure S2. In agreement with the data obtained by Beauchamp and co-workers²¹ for G_3Na^+ , the first exchange is observed to be fast, occurring at essentially the ion-molecule collision rate,

followed by two very slow successive reactions. In contrast, the second and third exchanges for sodiated trialanine are much faster than those of sodiated triglycine, by roughly one order of magnitude which is somewhat analogous to the results obtained for the two dipeptides above. This may be either a purely energetic effect based on a methyl substituent effect or a subtle structural effect provoked by the greater steric demand of the methyl group. The H/D exchange reactions of the sodiated methyl ester of G_3 , $G_3\text{OMeNa}^+$, showed rigorously zero exchange, even at reaction times >500 s. This important result thus implies that the carboxylic acid hydrogen is key to the initiation of any H/D exchange process and that reaction at the N-terminus does not occur independently of the involvement of the C-terminal carboxylic acid proton. This observation of three exchanges for the sodiated triglycine, plus the complete absence of reaction for the methyl ester, led naturally to the suggestion of ND_3 assisted proton transport from the C-terminal carboxylic acid to the N-terminal amine leading to a zwitterionic structure¹⁴. In this case, contrary to that observed for sodiated diglycine, the peptide chain is sufficiently long and flexible that plausible structures can be generated in which ND_3 acts as a bridge between the C- and N- termini. Beauchamp and co-workers¹⁴ suggested such a mechanism and constructed a partial potential energy surface in which possible minima were identified computationally, however no attempt to locate transition states was made. As described below, a computational investigation of this proposed mechanism, as well as an alternative, has been carried out in the present work with identification of the relevant transition states.

$G_4\text{Na}^+ - G_8\text{Na}^+$

The H/D exchange data for the series of peptides, $G_4\text{Na}^+ - G_8\text{Na}^+$ are shown in Figures 2 and S3-S5. The tetrapeptide is the first of this series of polyglycines to show a significant rate constant for the second and third exchanges. The $G_4\text{Na}^+$ exchange data in fact very closely resemble those observed for the $A_3\text{Na}^+$ system with the second and third exchange rate constants representing a significant fraction of that for the first exchange (see Table 1). The $G_5\text{Na}^+$ rate constants for the second and third reactions are roughly twice as large as those for $G_4\text{Na}^+$. The kinetic data for H/D exchange in $G_6\text{Na}^+$ exhibit a slight decrease in rate constants. This represents the first instance in the series of increasing oligomer size in which the overall reaction rate constants for the sequential H/D exchanges show a significant decrease relative to

the preceding members of the series. The kinetic data for G_7Na^+ show a further, and more dramatic, decrease in the rate constants for the series of H/D exchange reactions. The decrease, by a factor of 5 or more in each case, would appear to be a clear indication that some change, probably structural, has occurred to impede either unimolecular or bimolecular H/D exchange. The kinetic data for G_8Na^+ show an increase in rate constants which are roughly similar to those observed for G_6Na^+ . Finally, when the carboxylic acids in G_6Na^+ and G_7Na^+ were derivatized as esters, no exchange reaction was observed with ND_3 . The same observation was reported previously¹⁴ for G_3Na^+ and G_5Na^+ . This again demonstrates that the carboxylic hydrogen of the C-terminus must be involved in the H/D exchange mechanism.

Examination of the trends in Table 1 indicates that the first reaction occurs for these peptides at rates which are within a factor of 3-4 of the collision rate ($\sim 10^{-9} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$), except for G_7Na^+ . This suggests that the latter peptide bears some strong differences with all others. One obvious hypothesis is that its structure is different and less prone to facile proton exchange with ND_3 ²⁴. Variations among other peptide sizes, although non negligible, are not large enough to infer qualitative differences. It may also be seen that the second reaction is slower than the first by a factor of 2-3 for all G_nNa^+ with $n=4-8$, and that the third reaction is slower than the second by approximately the same factor. This might be inferred to be due to statistical effects since H/D exchange becomes less likely as the number of exchangeable H atoms diminishes. This is in sharp contrast with the second and third reactions of G_2Na^+ (not observed at all) and of G_3Na^+ (much slower than the first). Since there is no significant qualitative difference between the most stable structures of G_2Na^+ , G_3Na^+ and G_4Na^+ ^{25-28,30} this suggests that peptide size has a different influence on the first reaction than on the second and third reactions.

Also highly meaningful is the observation of strictly successive exchange reactions, with no sign of multiple H/D exchanges in a single bimolecular encounter of G_nNa^+ with ND_3 . This is in significant contrast to the results observed for reactions of protonated oligoglycines with ND_3 ^{16,17}, in which several “simultaneous” exchanges were observed. As discussed below, this observation helps to rule out several conceivable mechanisms for H/D exchange.

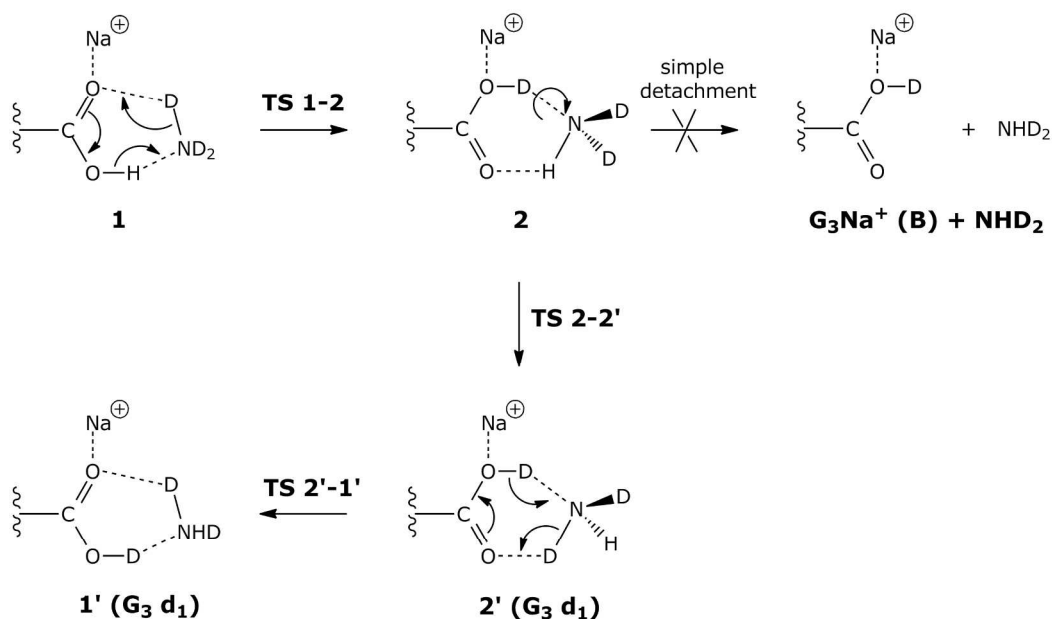
B. Computational Structural and Energetic Data

G_3Na^+

Considerable work has previously been carried out to explore the potential energy surface for G_3Na^+ and several stable minima have been described²⁵⁻²⁸. As such, G_3Na^+ represents a valuable starting point for the detailed understanding of the H/D exchange processes observed here, at least for the smaller oligomers. Briefly, for G_3Na^+ , the most stable structures all involve sodium chelation with the three carbonyl oxygens, with an additional possible structure involving the terminal amine nitrogen as a fourth binding site. Although all other structures identified lie sufficiently higher in energy that they need not be considered as contributing to a statistical population for this species alone, some of these could prove to be relevant to the interpretation of the present H/D exchange studies of complexation with ND_3 . For example, it is observed that, although three sequential H/D exchanges are observed for the smaller sodiated polyglycines (except G_7Na^+) and polyalanines, when the C-terminal OH is esterified, rigorously zero H/D exchange is seen. This implies that the C-terminal OH is implicated in all exchange processes, including those at the amine terminus. Although higher in energy, several of the G_3Na^+ structures involve folding of the G_3 backbone in such a way as to bring about a direct interaction between the carboxyl and amino termini or, at least to bring them close enough together that an incoming ND_3 molecule can construct a hydrogen bonding bridge between them. Structures of this type proved to be essential to effect H/D exchange at the amine terminus.

Since G_3 consists of too few basic heteroatoms and too restrictive a geometry to effectively saturate sodium coordination, binding of ND_3 to G_3Na^+ can occur at either Na^+ or carbonyl and/or amine sites, acting as a hydrogen bond donor or acceptor or both. In particular it can bind to the C-terminal carboxyl OH, providing a starting point for H/D exchange since, as noted above, esterification experiments demonstrate that the carboxyl group must be intimately involved in the mechanism.

Since, in all cases, the first H/D exchange is faster than the remaining two, it is logical to suppose that the simple mechanism shown in Scheme 1 is the first step in the overall H/D exchange process. Here, ND_3 interacts with the carboxyl terminus only, maintaining G_3Na^+ in its

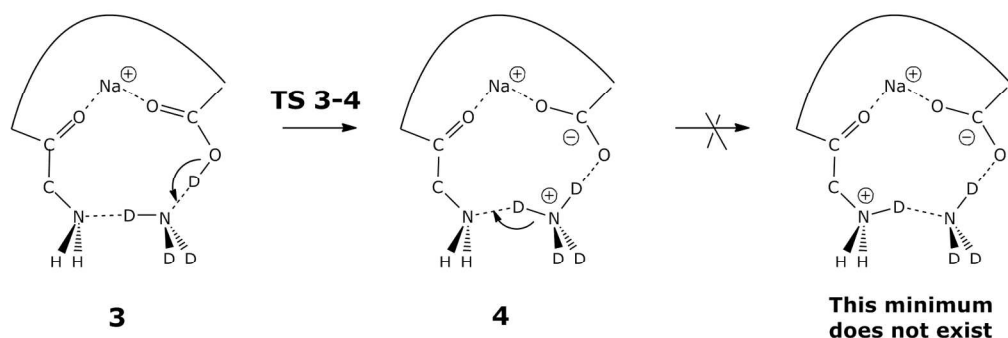


Scheme 1

most stable conformation (named A). Deuterium transfer from ND_3 to the carbonyl oxygen and hydrogen transfer from the carboxyl O-H to ND_3 can occur concertedly via a 6-membered ring transition state with a small calculated energy barrier of 23.3 kJ.mol^{-1} . While this mechanism accomplishes H/D exchange, upon loss of ND_2H the resulting G_3Na^+ species formed would require that the Na^+ be bound to a hydroxyl oxygen rather than the more energetically favorable carbonyl oxygen. This would then result in a less stable structure (named B) than the initial G_3Na^+ rendering the overall process endothermic by 30.8 kJ.mol^{-1} and thus unlikely to occur (see Figure 3). Alternatively, a rotation of ND_3 within the intermediate complex may occur, with a calculated barrier of 20.8 kJ.mol^{-1} . Deuterium transfer from ND_2H to the carbonyl oxygen and from the carboxyl to the N leads back to the most stable structure. From it, loss of ND_2H results in an essentially thermoneutral reaction with Na^+ bound to the carbonyl oxygen and the carboxyl OH replaced by an OD. The net result of the above mechanism of concerted double transfer thus leads back to the most stable structure of G_3Na^+ with a single O-H hydrogen exchanged for a deuterium. This mechanism is especially appealing since it involves energy barriers which, as shown in Figure 3, are well below the energy reservoir provided by initial association of ND_3 and G_3Na^+ . This rather facile exchange process thus precedes the

remaining two involving the amine terminal hydrogens which must involve an alternative mechanism and which, nevertheless, still involve the C-terminal carboxyl hydrogen. The typical, dramatically reduced, rates of exchange for the second and third exchange reactions, relative to the first, are therefore only compatible with a different mechanism being operative for the subsequent steps.

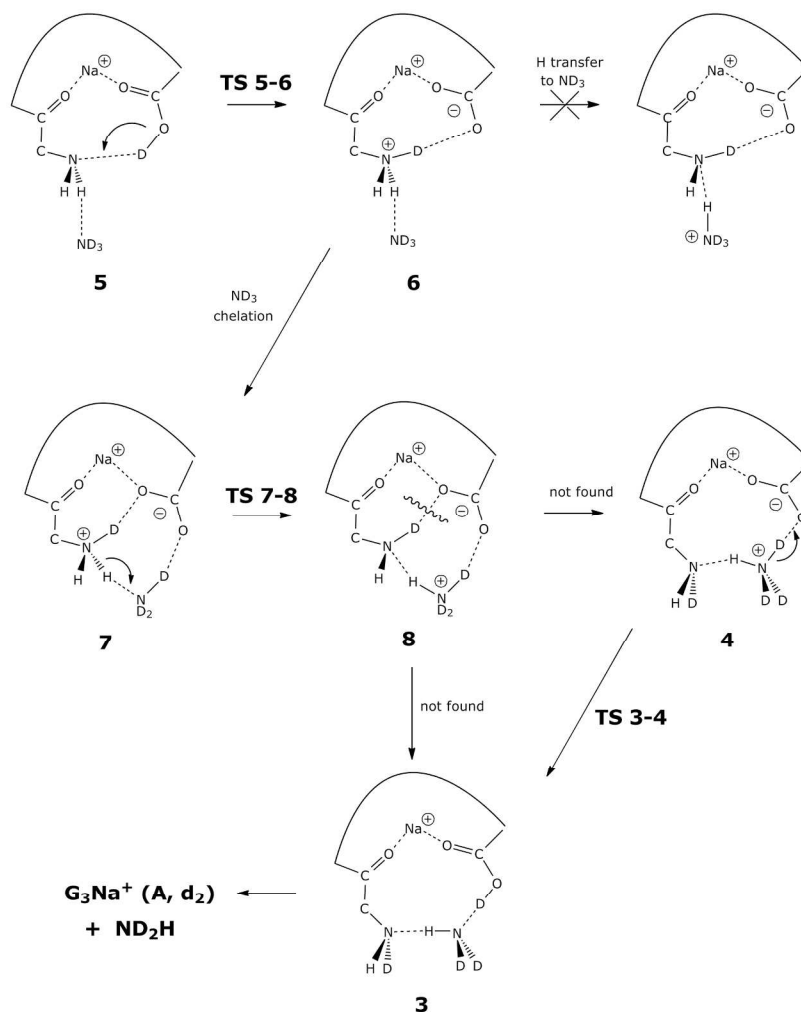
An obvious possibility for a different mechanism following this first exchange is a process *via* which a complexed ND_3 can effect deuterium exchange with one of the amino hydrogens. Such an intramolecular hydrogen exchange, illustrated in Scheme 2, had been previously suggested¹⁴. Presuming that the first exchange has already occurred this results in a zwitterion like structure in which ND_4^+ is hydrogen (deuterium) bonded to both the the amine terminus and the carboxylate oxygen not bound to sodium. The single transfer of deuterium to the amine would effectively generate a salt bridge structure which, following rotation about C-N bond of the amine terminus, could lead to back transfer of a single hydrogen and, upon dissociation, yield an H/D exchange at the amine terminus. If this occurred, it would also be possible to conceive of two sequential H/D exchanges due to a rotation of the new ND_2H moiety within a single collision complex, which would then appear as a multiple exchange in the kinetics observed. This double exchange was not observed and, consistent with this experimental finding, it was impossible to locate such a structure as a minimum on the potential energy surface. It is very likely that this is due to the fact that the distance between the ammonium and carboxylate charges would be too large in such a structure for it to be a stable minimum. The failure of this suggested mechanism, which could have explained all three exchanges, leads to the conclusion that the second and third H/D exchange reactions require a completely different mechanism to be consistent with all of the experimental observations.



Scheme 2

A plausible mechanism, which is, in fact, a variation of the previous one, is shown in Scheme 3 with the associated energy profile displayed in Figure 4. Here, an initial structure **5** of somewhat higher energy (20 kJ mol^{-1}) than that of **3** is accessed. The ND_3 is hydrogen bonded to one of the two free hydrogens of the amine. A multi-step process then occurs in which the positive and negative charges are maintained in direct interaction throughout. It starts with a deuteron transfer from the carboxyl oxygen to the amine nitrogen, leading to **6**. A structural shift then occurs whereby ND_3 binds to both the carboxylate and the ammonium termini (**7**). This enables proton transfer from the N terminus to ND_3 while maintaining favorable charged site interactions (**8**).

From **8**, the hydrogen bond between the carboxylate oxygen bound to sodium and the amine hydrogen can be broken resulting in a ND_3H^+ ion simultaneously hydrogen bonded to both the other carboxylate oxygen and the amine terminus nitrogen. This structure is, in effect, the second intermediate proposed by Beauchamp (**4**, see Scheme 2). A transition state to form this structure from **8** was not found explicitly but it was clear from numerous attempts that it was very close in energy to **8** in a very flat portion of the potential energy surface in which the transition from **7** to **8** to **4** can proceed smoothly. As shown in Figure 4, a very low lying transition state, **TS 3-4**, connects **4** to **3**, which, upon dissociation gives rise to a second incorporation of deuterium into the system. The fact that this sequence proceeds smoothly energetically downhill without significant barriers mitigates against the reverse sequence of reactions occurring which could, in principle lead to a third deuterium incorporation within a single reaction event.



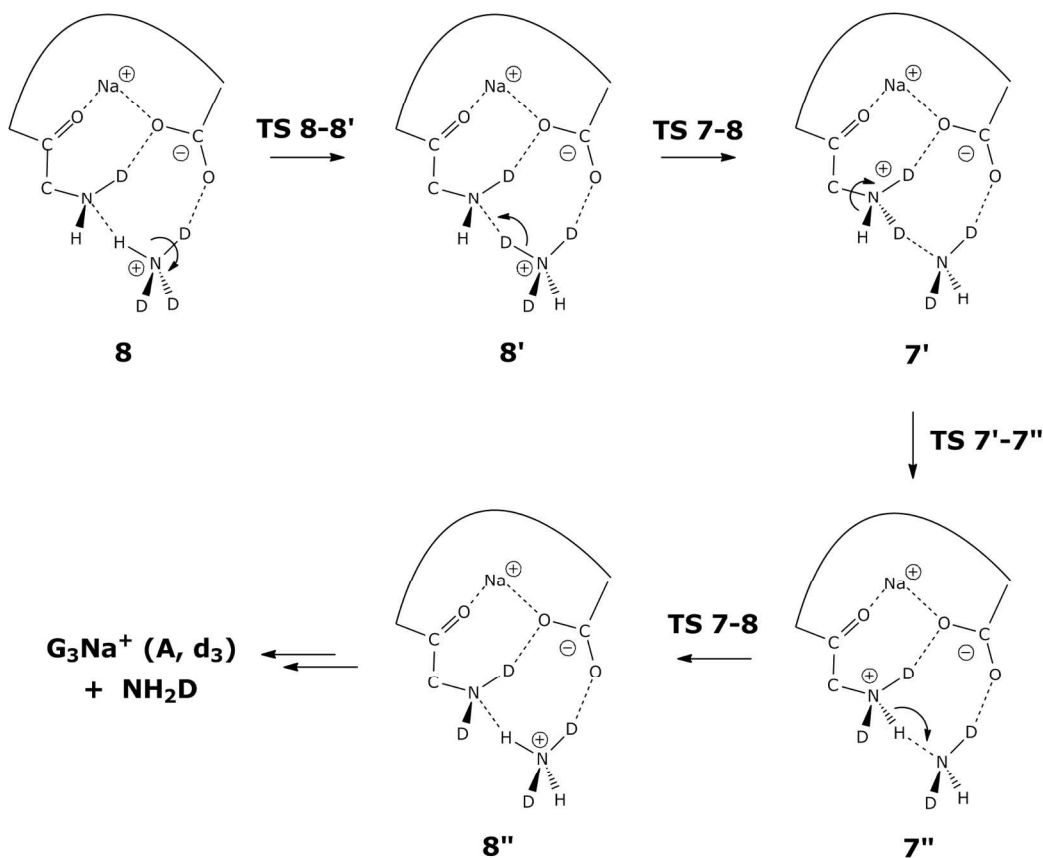
Scheme 3

The possibility was also considered that a unimolecular transfer of the carboxyl D in **5** to the amine terminus followed by a back transfer of H to carboxyl oxygen could occur without the participation of ND_3 . This potential process was explored computationally and neither the transition state nor the resulting salt bridge complex were found to be stable without ND_3 present.

A further pathway was also explored which could in principle effect two H/D exchanges in a single reaction event, as shown in Scheme 4 and Figure 5. In this mechanism, rotation about the ammonium N-D---O linkage, via a transition state **TS 8-8'**, transforms **8** into an equivalent structure **8'** which is now prepared for deuterium transfer to the amine terminus.²⁹ This can

occur via **TS 7-8** to produce **7'**. In order to complete the full deuteration of the amine terminus, **7'** is transformed, via transition state **TS 7-7''**, into an equivalent structure **7''** in which it is the N-H bond which interacts with ND_2H , preparing for H^+ transfer in a last step. After this transfer, structure **8''** can proceed as previously described (see Scheme 3) until dissociation of NDH_2 , yielding a G_3Na^+ in which all three labile hydrogens have been replaced by deuterium. If this mechanism were operative it could effectively incorporate two deuteriums into the system in a single reaction event. The fact that all of the H/D exchange kinetics experiments show that the second and third exchanges are rigorously sequential, rather than simultaneous, demonstrates that this mechanism is unlikely. A comparison of the potential energy profiles shown in Figures 4 and 5 makes it clear why the reaction proceeds by the simpler path predominantly if not exclusively.

Various other mechanisms in which the ND_3 interacts only with the N-terminal part of the peptide were also envisaged. Proton transfer from the first amide N-H to the terminal amine using ND_3 as a relay, either in two steps with intermediate formation of an amidate/ ND_3H^+ complex, or in a concerted manner via a 7-membered ring transition state, were found to involve very high energy barriers. This is consistent with the observation that C-terminal esterified G_nNa^+ , with exchangeable hydrogens exclusively on the N-terminal amine, do not exchange at all with ND_3 .



Scheme 4

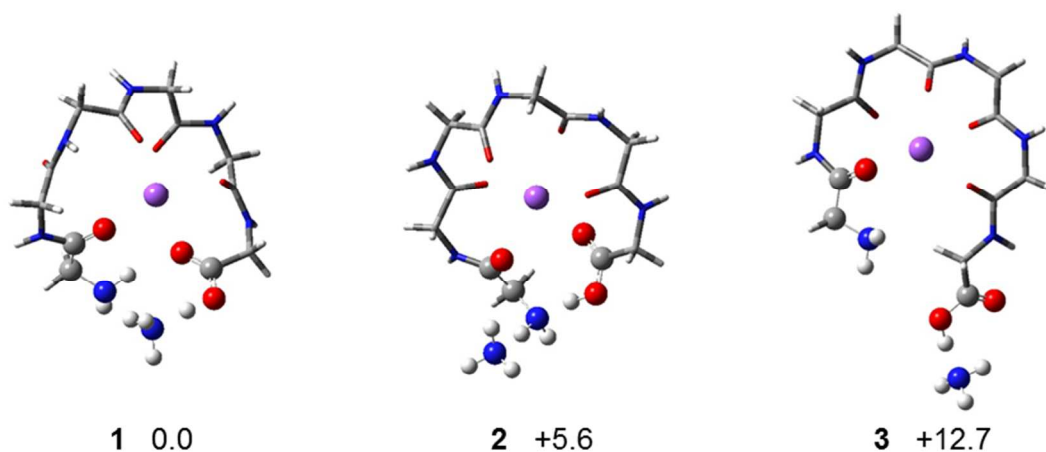
G₄Na⁺ and G₅Na⁺

The potential energy surfaces for the mechanisms to explain the C-terminal and N-terminal hydrogen-deuterium exchanges in G₄Na⁺ and G₅Na⁺ can be considered to be analogous to that described for G₃Na⁺ above since the lowest energy structures are quite similar to those of the tripeptide^{24,25,28}. In each of these cases the C-terminal hydrogen remains relatively exposed and thus readily available for exchange with ND₃ via the same mechanism proposed for C-terminal exchange of G₃Na⁺ and, significantly, the rate constants observed for the first exchange remain quite high, near the collision rate constant (see Table 1). A significant difference however is that, as the peptide chain length increases, there is also an increased flexibility in the peptide backbone which also increases the probability that the C- and N-termini can become intramolecularly coordinated, which also increases the probability that the second and third exchanges can occur. This is manifested by the increase of more than an order

of magnitude in the rate constants for the second and third exchanges of the sodiated tetrapeptide relative to the tripeptide analogue (see Table 1). Similarly, for the pentapeptide, an even more accessible intramolecularly hydrogen bonded structure will be present and the expected additional increase in rate constant for the second and third exchanges is indeed observed.

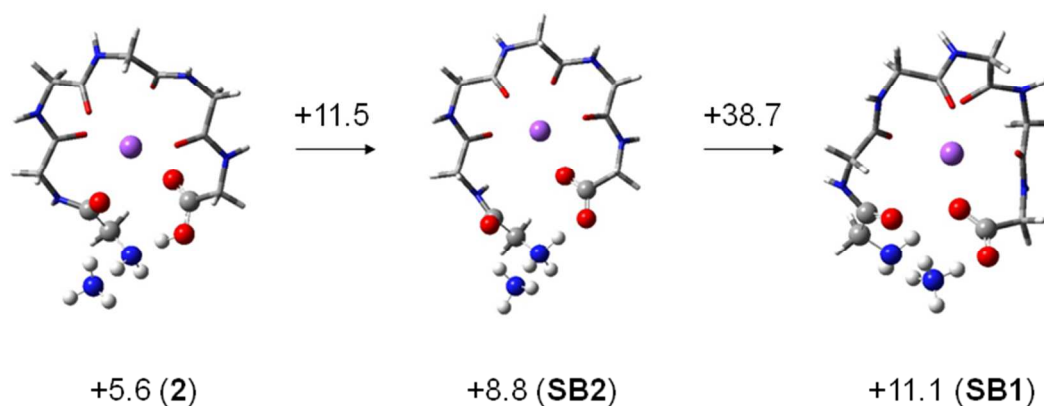
$G_6-Na^+ - G_8-Na^+$

Adding a sixth Gly residue makes the peptide large enough to wrap around Na^+ , coordinating through all six of its oxygens *and* establishing a O-H---N hydrogen bond between its C and N termini in the lowest energy structure. G_6 forms a very approximately planar backbone, with peptidic carbonyl oxygens pointing alternatively above and below the average plane²⁴. Interaction of ND_3 with such a structure may be established without significantly disrupting it. The most stable such conformation found (see **1** below) involves ND_3 as a hydrogen bond acceptor from the C terminus and hydrogen bond donor to the N terminus, while maintaining an interaction between the termini, however through an N-H---O hydrogen bond. Slightly less stable (+5.6 $\text{kJ}\cdot\text{mol}^{-1}$) is another structure, (**2**), involving a hydrogen bond from the amino terminus of G_6 to ND_3 and O-H---N interaction between the termini. Finally the C terminus can be rotated away from Na^+ yielding a structure in which it can interact directly with ND_3 . This structure **3** is 12.7 $\text{kJ}\cdot\text{mol}^{-1}$ higher in energy than **1**. In the lowest energy structure, complexation of G_6Na^+ by ND_3 brings a stabilization energy of 38.7 $\text{kJ}\cdot\text{mol}^{-1}$.



The first H/D exchange reaction might have been expected to proceed at the C terminus in a mechanism analogous to that described for G_3Na^+ . Since structure **3** is 26.0 $\text{kJ}\cdot\text{mol}^{-1}$ more stable than the isolated reactants, and assuming a barrier of ca. 20-25 $\text{kJ}\cdot\text{mol}^{-1}$ in analogy to that

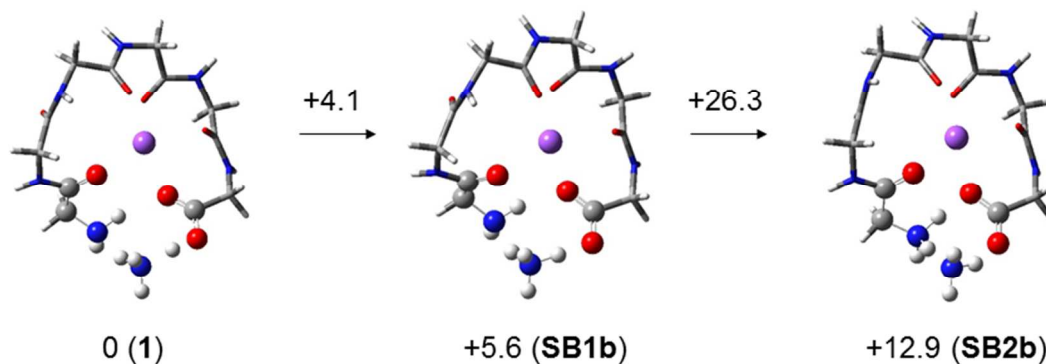
computed for G_3Na^+ , it may be that this reaction cannot proceed efficiently. Thus other mechanisms were sought. The first of these, described below in Scheme 5, starts from **2** with proton transfer from the C to the N terminus yielding a salt bridge (**SB2**). This structure involves breaking the CO^1 interaction with sodium, which is compensated for to some extent by the stabilization gained by a hydrogen bond between CO^1 and ND_3 . This would in principle be followed by a second proton transfer from the N terminus to ND_3 yielding a second salt bridge (**SB1**) in which ND_3H^+ bridges the carboxylate and amino termini. However the computed barrier for this step is $38.7 \text{ kJ}\cdot\text{mol}^{-1}$ above the most stable structure **1**, which is the same energy as that for the separated reactants. Therefore this mechanism is unlikely to be operative.



Scheme 5

The second mechanism (Scheme 6) starts from structure **1** in which ND_3 bridges between the N and C termini. It proceeds through two proton transfers from the C terminus to ND_3 and then from ND_3H^+ to the N terminus, forming two successive salt bridges **SB1b** and **SB2b**. It maintains full Na^+ coordination and has ND_3 or ND_3H^+ bridging throughout, yielding a particularly favorable energy profile. However direct deuterium transfer from the N to the C terminus in **SB2b** could not be characterized, probably because it would require dissociating ND_2H to regenerate **1**. H/D exchange then requires permutation of hydrogen and deuterium atoms through rotation about single bonds. Rotation of ND_2H around the N-H---N axis in **SB2b** results in the replacement of a N-H---O by a N-D---O linkage with a barrier of 18.8 kJ mol^{-1} . Upon

reversal of the pathway described, from **SB2B** to **SB1B** and then to **1**, allows for back transferring D instead of H to the C terminus in the last step. The highest energy barrier of 26.3 kJ.mol⁻¹ makes this a viable mechanism. Rotation of ND₃H⁺ around the N-H---O axis in **SB1b** could also be envisaged, however this would involve an energy barrier of 42.1 kJ.mol⁻¹ which makes this mechanism impossible.



Scheme 6

The above mechanism accounts for one H/D exchange at the carboxylic position, therefore another process must occur to allow for two additional steps. After detachment of ND₂H, proton scrambling within G₆Na⁺ can occur. It is found that in much resemblance with the mechanism described for the C-terminus reaction of G₃Na⁺ described above (see Scheme 1), with the N terminus now playing the role of the exchanging amine, double hydrogen transfer between the carboxyl and amino groups can proceed. This effectively amounts to exchanging the carboxyl D with one of the amino H's. The energy requirement for this step is computed to be 34.6 kJ.mol⁻¹, 4.1 kJ.mol⁻¹ below the energy of separated reactants. From this, reaction with a second ND₃ molecule allows for incorporation of a second D in the peptide at the C terminus, and the process can be repeated again to yield fully deuterated amino and carboxyl termini. The successive reaction rates are diminished by a statistical factor, leading to a second reaction being slower than the first by a factor of 2/3 and the third by a factor of 1/3 (still relative to the first). As can be seen from Table 1, the measured ratios are close to 1/3 and 1/10. The difference from the purely statistical factors may be due to the rate limiting step being the

internal H/D scrambling within the peptide, whose barrier is computed to be only 4.1 kJ.mol^{-1} below the energy of separated reactants.

There appears to be a break in the series at G_7Na^+ for which the rate constant of the first H/D exchange reaction is smaller than for all other peptide sizes by at least an order of magnitude. This is most likely due to a structural change in this case, leading to a significantly different mechanism which requires a higher energy pathway, either to effect H/D exchange or to transform the peptide into a conformation more analogous to those of smaller peptides, from which similar exchange mechanisms can start. The most stable structure found for G_7Na^+ by a combination of quantum chemical computations and IRMPD spectroscopy²⁴ is indeed different from those of all other peptide sizes (see Figure 7). In this structure, the carboxyl terminus donates a hydrogen bond to CO^2 , while the N and C termini remain remote from each other. Searches for a simple path to transform this structure into one in which the C and N termini interact directly were unsuccessful.

Attachment of ND_3 to G_7Na^+ was investigated at several sites including Na^+ and both peptide termini. The most stable structure was found to involve interaction of ND_3 with $N-H^4$ as a hydrogen bond acceptor, keeping the most stable structure of G_7Na^+ . All attempts to find a structure, with limited deformation from the most stable, enabling ND_3 to bridge between the two peptide termini, were unsuccessful. A path for insertion of ND_3 into the $O-H^7---O=C^2$ bond was determined, with an energy barrier and a product lying 33.4 and 7.7 kJ.mol^{-1} higher in energy than the most stable structure, respectively. It is highly improbable that this structure can lead to viable H/D exchange between ND_3 and the C terminus; such a path was not found. In any case, the further two H/D exchanges which are experimentally observed cannot be explained from such a structure. It is therefore likely that G_7Na^+ must sample its conformational space until it reaches a conformation with direct interaction between the termini, preparing for H/D exchange reactions of one of the types described above. This extensive conformational restructuring is the likely cause of the dramatic rate reduction observed for G_7Na^+ relative to other peptide sizes. Thus, in this case, the H/D exchange rate difference may be considered as diagnostic of a structural difference.

The lowest energy structure of G_8Na^+ was found to involve direct interaction between its termini^{26, 27}. It is therefore expected that it can follow H/D exchange pathways which are similar to those described above for G_6Na^+ . The limited rate reduction relative to those of G_6Na^+ may be due to the collision rate constant being slightly smaller for G_8Na^+ . The rate reduction for the successive reactions of G_8Na^+ may be due to statistical scrambling.

For the peptides of increasing chain length it becomes progressively easier for the C- and N- ends of the peptide to interact and thus to facilitate this new mechanism. In particular, the most stable forms found for G_6Na^+ and G_8Na^+ contain such a hydrogen bond interaction already. The exception to this is clearly for G_7Na^+ in which it is found that the most stable form of the sodiated peptide is significantly different and it requires a higher energy pathway to transform the peptide into a conformation in which the necessary interaction with ND_3 can lead to exchange.

Summary

A consideration of the ensemble of the kinetic, structural and energetic data for the sodiated polyglycines leads to some considerable insight into the mechanisms of H/D exchange. For the smaller G_nNa^+ systems, where $n = 1, 2$ and 3 , the peptide backbone is sufficiently short that carboxyl and amine termini do not interact directly and a single H/D exchange *via* reaction with ND_3 can be effected by a mechanism, such as that outlined in Scheme 1, which can be expected to occur at or near the collision rate constant. This is indeed what is observed for these first three species with the single reaction being associated with the carboxyl terminal hydrogen. For G_2Na^+ no second or third exchanges are observed, indicating that no direct exchange with ND_3 can occur with the amine terminal hydrogens. In addition, the interaction of the carbonyl oxygens with Na^+ in the lowest energy configurations forces the system into an orientation in which the termini are unable to interact, even via bridging effected by addition of ND_3 . For GNa^+ the second and third exchanges are observed but the fact that they are sequential, rather than simultaneous, would mitigate against involvement of a zwitterionic structure. In the most stable structure the Na^+ is coordinated directly with the amine site which could sufficiently activate the amine hydrogens toward exchange with ND_3 . For G_3Na^+ extremely slow exchanges are observed which can be explained by a bridge created between

the carboxyl and amine termini by ND_3 . However the potential profile exhibited for these exchanges is complex and involves several relatively high energy transition states thus rendering the exchanges very slow.

For G_4Na^+ the most stable structure involves a species in which Na^+ is coordinated with all four carbonyl oxygens keeping the peptide chain extended in such a way that the carboxyl and amine termini are held far away from each other. However, a structure which is 12 kJ mol^{-1} higher in energy also exists in which the carboxyl hydrogen is hydrogen bonded to the amine terminus while maintaining interactions between all four carbonyl oxygens and the Na^+ . The pattern of the three rate constants exhibited might then suggest that the H/D exchange may be a combination of mechanisms such as that in Scheme 1 involving the most stable structure and a second mechanism, as described for G_6Na^+ , involving the hydrogen bonded structure. The fact that the second and third sequential exchanges are significantly faster than those with G_3Na^+ suggests that a mechanism involving the hydrogen bonded structure in interaction with ND_3 is quite favourable.

A similar argument likely pertains for G_5Na^+ where each rate constant is very approximately twice that in the tetrapeptide suggesting that a hydrogen bonded structure for the sodiated pentapeptide may be even closer in energy to the most stable structure.

In G_6Na^+ , the most stable structure is one in which an intramolecular hydrogen bond exists between the carboxyl and amine termini. A double proton transfer mechanism, completed by internal rotation of ND_2H relative to the peptide, can only explain one exchange at the C terminus. It is then necessary to invoke an intramolecular H/D exchange between the peptide termini, before further reactions with ND_3 can lead to incorporation of a second and then a third deuterium into the peptide, all at the C terminus. The lower rate constants are likely a reflection of the greater energetic cost of the latter step.

G_7Na^+ represents a special case in which the most stable structure involves an intramolecular hydrogen bond between the carboxyl terminal hydrogen and an amide carbonyl oxygen, keeping the carboxyl terminus well away from the amine terminus. In this case, the energetic cost of accessing the type of hydrogen bonded structure above necessary to effect the first, second and third exchanges is sufficiently high that there is a dramatic drop in the observed H/D exchange rate constants. In contrast, for G_8Na^+ the most stable structure is

analogous to that observed for G_6Na^+ and the pattern and magnitude of the H/D exchange rate constants are seen to be very similar as well.

It is also of significance to note that for G_4Na^+ through G_8Na^+ the pattern of rate constants is such that there is roughly a factor of 2-3 between each of the values of k_2 relative to k_1 and k_3 relative to k_2 . This would suggest a statistical partitioning of H and D among the available labile hydrogens involved at the reaction site for each exchange.

The fact that we see only three exchanges irrespective of peptide size is a clear indication that backbone amide hydrogens are not involved. If scrambling occurred between the termini and backbone protons, more than three exchanges would be expected to occur. Several sodiated peptides, e.g. VEVIPY and FLEEL [20] have been previously observed to exchange termini and side chain exchangeable protons, not backbone amide protons. The extent of exchange was found to be even less for sodiated RGD [19]. In one instance, sodiated bradykinin [32], to be added], exchange of all exchangeable protons was observed. It may be hypothesized that backbone exchange requires proton mobility, which is much more readily available in protonated than in sodiated peptides, but may become easier when several basic or acidic side chains are present, stabilizing salt bridges.

The difference in H/D exchange reactivity of sodiated and protonated oligoglycines [17] is striking, as triglycine was found to exchange all six exchangeable protons in reaction with ND_3 , including several exchanges following a single collision event. It is difficult to use the present results to draw conclusions about larger and more commonly used protonated peptides, as there appears to be no clear trend of H/D exchange extent and rate in protonated vs. sodiated small peptides. Williams et al. [20] found that sodium adduction sometimes increases the H/D exchange rate with D_2O , while decreasing it in other cases. Alkali ion adduction with dipeptides containing Arg was found to increase the rate and extent of H/D exchange with D_2O [21], an effect which was attributed to the stabilization of salt bridges by alkali cations. Other structural tools are required to make the link by H/D exchange behavior and peptide structure in the gas phase. While the present work made use of results from IRMPD spectroscopy, the development of ETD coupled to H/D exchange provides an attractive track for larger peptides and proteins [33, 34].

There has been little or no study on the influence of exchange reagent on H/D exchange of sodiated peptides in the gas phase. This is at variance with protonated peptides, see e.g. ref. 16 and 35. In this case the proton affinity difference between the peptide and the exchange reagent (e.g. D_2O , CH_3OD , CH_3COOD , ND_3) is an obvious factor, in addition to peptide structure. Similarly, the mechanisms we establish herein would have somewhat different energy profiles if D_2O were used instead of ND_3 , especially those for which intermediate protonation of the deuterating agent is required. This would affect the rates of certain mechanisms, possibly changing the most favorable mechanism in some cases.

Conclusion

The experimental and computational work described here give, for the first time, a coherent explanation to describe mechanisms and energetic for the occurrence of H/D exchange in sodiated peptides with a significant size range. The lack of exchange exhibited by the analogous esters of the same peptides, the absence of multiple exchanges within a single collision event and the pattern of reaction rate constants can all be explained with mechanisms which differ for the first vs. the second and third exchange for any given peptide size, and which also differ depending upon whether the peptide termini can interact directly without significant energy penalty. The understanding gained from this work provides a solid basis on which to interpret H/D exchange observations in similar situations involving sodiated peptides. In particular a slow first exchange should be indicative of a structure in which both termini are remote from one another and the C terminus is not easily accessible to the deuterated reagent.

Acknowledgements

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References

1. M. K. Green, C. B. Lebrilla, *Mass Spectrom. Rev.*, 1997, **16**, 53-71.
2. S. Osburn, V. Ryzhov, *Anal. Chem.*, 2013, **85**, 769-778.
3. C.S. Contreras, N.C. Polfer, A.C. Chung, J. Oomens, J.R. Eyler, *Int. J. Mass Spectrom.*, 2010, **297**, 162 – 169.
4. S. Kato, C.H. DePuy, S. Gronert, V. Bierbaum, *J. Amer. Soc. Mass Spectrom.*, 1999, **10**, 840-847.
5. J.C. Kleingeld, N.M.M. Nibbering, *Tetrahedron*, 1983, **39**, 4193-4199.
6. H.D.F. Winkler, E.V. Dzyuba, C.A. Schalley, *New. J. Chem.*, 2011, **35**, 529-541.
7. (a) D.R. Reed, S.R. Kass, *J. Amer. Soc. Mass Spectrom.*, 2001, **12**, 1163-1168. (b) Z. X. Tian, L. Lis, S. R. Kass, *J. Am. Chem. Soc.* 2008, **130**, 8-9.
8. Y. Hamuro, S.J. Coales, M.R. Southern, J.F. Nemeth-Cawley, D.D. Stranz, P.R. Griffin *Biomol. Tech.*, 2003, **14**, 171-182.
9. T.E. Wales, J.R. Engen *Mass Spectrom. Rev.*, 2006, **25**, 158-170.
10. Y. Huang, J.A. Marini, J. A. McLean, S.E. Tichy, D. H. Russell, *J. Am. Soc. Mass Spectrom.*, 2009, **20**, 2049-2057.
11. Y. Kang, P. Terrier, C. Ding, D.J. Douglas, *J. Am. Soc. Mass Spectrom.*, 2012, **23**, 57-67.
12. J. Pan, B.L. Heath, R.A. Jockush, L. Konermann, *Anal. Chem.*, 2012, **84**, 373-378.
13. S. Hoyau, J.-P. Pélicier, F. Rogalewicz, Y. Hoppilliard, G. Ohanessian, *Eur. J. Mass Spectrom.*, 2001, **7**, 303.
14. H. A. Cox, R. R. Julian, S.-W. Lee, J.L. Beauchamp, *J. Am. Chem. Soc.*, 2004, **126**, 6485-6490.
15. M. Rozman, B. Bertosa, L. Klasinc, D. Srzic, *J. Am. Soc. Mass Spectrom.*, 2006, **17**, 29-36.
16. S. Campbell, M.T. Rodgers, E.M. Marzluff, J.L. Beauchamp, *J. Am. Chem. Soc.*, 1995, **117**, 12840-12854.
17. B. E. Ziegler, T. B. McMahon, *J. Phys. Chem. A*, 2010, **114**, 11953 – 11963.
18. T. Wyttenbach, J.E. Bushnell, M.T. Bowers, *J. Am. Chem. Soc.*, 1998, **120**, 5098-5103.
19. T. Solouki, R.C. Fort, Jr., A. Alomary, A. Fattahi, *J. Am. Soc. Mass Spectrom.*, 2001, **12**, 1272-1285.
20. J.C. Jurchen, R.E. Cooper, E.R. Williams, *J. Am. Soc. Mass Spectrom.*, 2003, **14**, 1477-1489

21. L.A. Mertens, E.M. Marzluff, *J. Phys. Chem. A*, 2011, **115**, 9180-9187.
22. J. Chamot-Rooke, G. van der Rest, A. Dalleu, S. Bay, J. Lemoine, *J. Am. Soc. Mass Spectrom.*, 2007, **18**, 1405-1413.
23. P. Wang, C. Kapota, G. Ohanessian, C. Wesdemiotis, *J. Am. Soc. Mass Spectrom.*, 2007, **18**, 541-552.
24. O.P. Balaj, D. Semrouni, V. Steinmetz, E. Nicol, C. Clavaguéra, G. Ohanessian, *Chem. Eur. J.*, 2012, **18**, 4583-4592.
25. J. Ye, P. B. Armentrout, *J. Phys. Chem. A*, 2008, **112**, 3587 – 3596.
26. D. Semrouni, O.P. Balaj, F. Calvo, C.F. Correia, C. Clavaguéra, G. Ohanessian, *J. Am. Soc. Mass Spectrom.*, 2010, **21**, 728 – 738.
27. D. Semrouni, C. Clavaguéra, J.-P. Dognon, G. Ohanessian, *Int. J. Mass Spectrom.*, 2010, **297**, 152 – 161.
28. O.-P. Balaj, C. Kapota, J. Lemaire, G. Ohanessian, *Int. J. Mass Spectrom.* 2008, 269, 196–209.
29. It is important to recognize that rotation of ND₂H about the ammonium-amine bond in **8'** can lead to a transfer of hydrogen back to the C terminus leading eventually to the formation of a peptide singly deuterated at the N instead of the C terminus. If this were to occur, it could diminish the apparent rate of deuterium incorporation. However given that the rate of exchange at oxygen is nearly two orders of magnitude more rapid than that at nitrogen, the actual effect would be negligible.
30. M. M. Kish, C. Wesdemiotis, G. Ohanessian, *J. Phys. Chem. B* 2004, 108, 3086-3091
31. GAUSSIAN09 revision D.01, M. J Frisch et al., Gaussian, Inc., Wallingford CT, 2013.
32. M. A. Freitas, A. G. Marshall *Int. J. Mass Spectrom.*, 1999, **182/183**, 221 – 231.
33. K. D. Rand, S. D. Pringle, M. Morris and J. M. Brown, *Anal. Chem.* 2012, **84**, 1931.
34. K. D. Rand, M. Zehl, O. N. Jensen and T. J. D. Jørgensen, *Anal. Chem.* 2009, **81**, 5577.
35. O. Geller, C. Lifshitz *J. Phys. Chem. A* 2005, **109**, 2217 – 2222

Table 1 : Rate constants for the H/D exchange of oligomers G_nNa^+ and A_nNa^+ with ND_3 (all values given in $cm^3 molecule^{-1} s^{-1}$)

G_nNa^+	k_1	k_2	k_3
$n = 2$	4.0×10^{-10}	–	–
$n = 3$	1.4×10^{-9}	2.4×10^{-11}	9.2×10^{-12}
$n = 4$	7.3×10^{-10}	3.6×10^{-10}	1.3×10^{-10}
$n = 5$	1.1×10^{-9}	6.5×10^{-10}	2.6×10^{-10}
$n = 6$	5.5×10^{-10}	1.8×10^{-10}	5.2×10^{-11}
$n = 7$	6.8×10^{-11}	2.9×10^{-11}	1.2×10^{-11}
$n = 8$	3.1×10^{-10}	1.5×10^{-10}	7.1×10^{-11}
A_nNa^+			
$n = 2$	4.0×10^{-10}	3×10^{-12}	1×10^{-12}
$n = 3$	1.0×10^{-9}	5×10^{-10}	3×10^{-10}
$n = 5$	1.0×10^{-9}	1.0×10^{-9}	5×10^{-10}

Figure Captions

- Figure 1. Variation of relative ion intensities for sodiated triglycine as a function of reaction times with ND_3 at a pressure of 6.8×10^{-9} mbar; (a) Reaction times 0-12 s. (b) Reaction times 0-500 s.
- Figure 2. (a) Variation of relative ion intensities for sodiated hexaglycine as a function of reaction times with ND_3 at a pressure of 6.9×10^{-9} mbar.
(b) Variation of relative ion intensities for sodiated heptaglycine as a function of reaction times with ND_3 at a pressure of 2.8×10^{-8} mbar.
- Figure 3. Potential energy profile, corresponding to Scheme 1, for the first hydrogen-deuterium exchange of sodiated triglycine.
- Figure 4. Potential energy profile, corresponding to Scheme 3, for the second hydrogen-deuterium exchange of sodiated triglycine. The dashed boxes linking **6** to **7** and **8'** to **4** indicate that a transition state has not been located and is of undetermined energetic position.
- Figure 5. Potential energy profile, corresponding to Scheme 4, illustrating the internal rotations which must occur to effect the second and third hydrogen-deuterium exchanges.
- Figure 6. Potential energy profile for the first (carboxylic acid) hydrogen-deuterium exchange of sodiated hexaglycine.
- Figure 7. Lowest energy structures for sodiated hexa-, hepta- and octa- glycine.

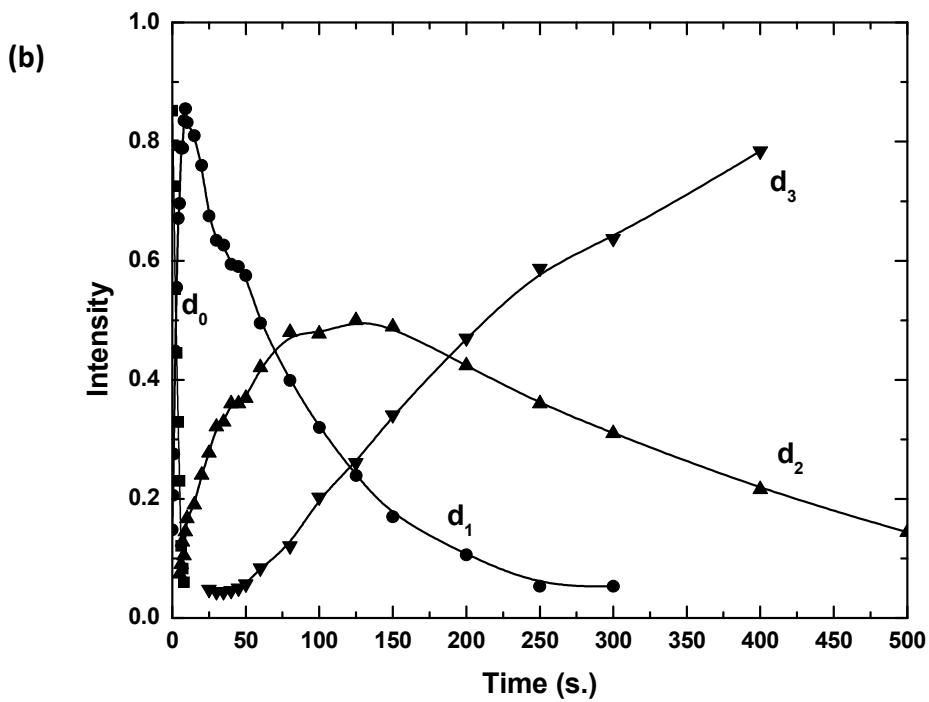
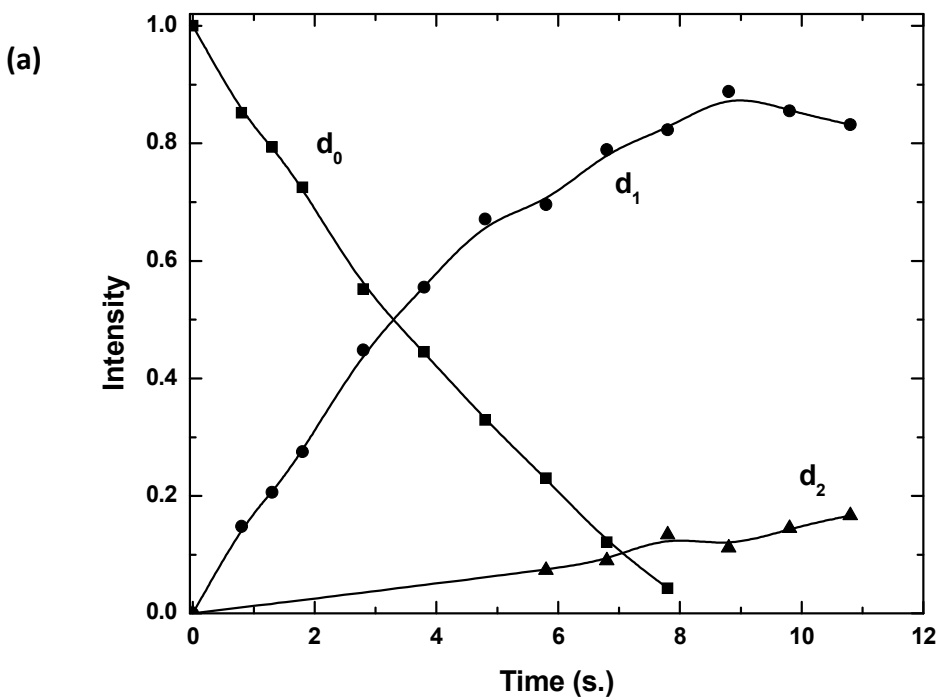
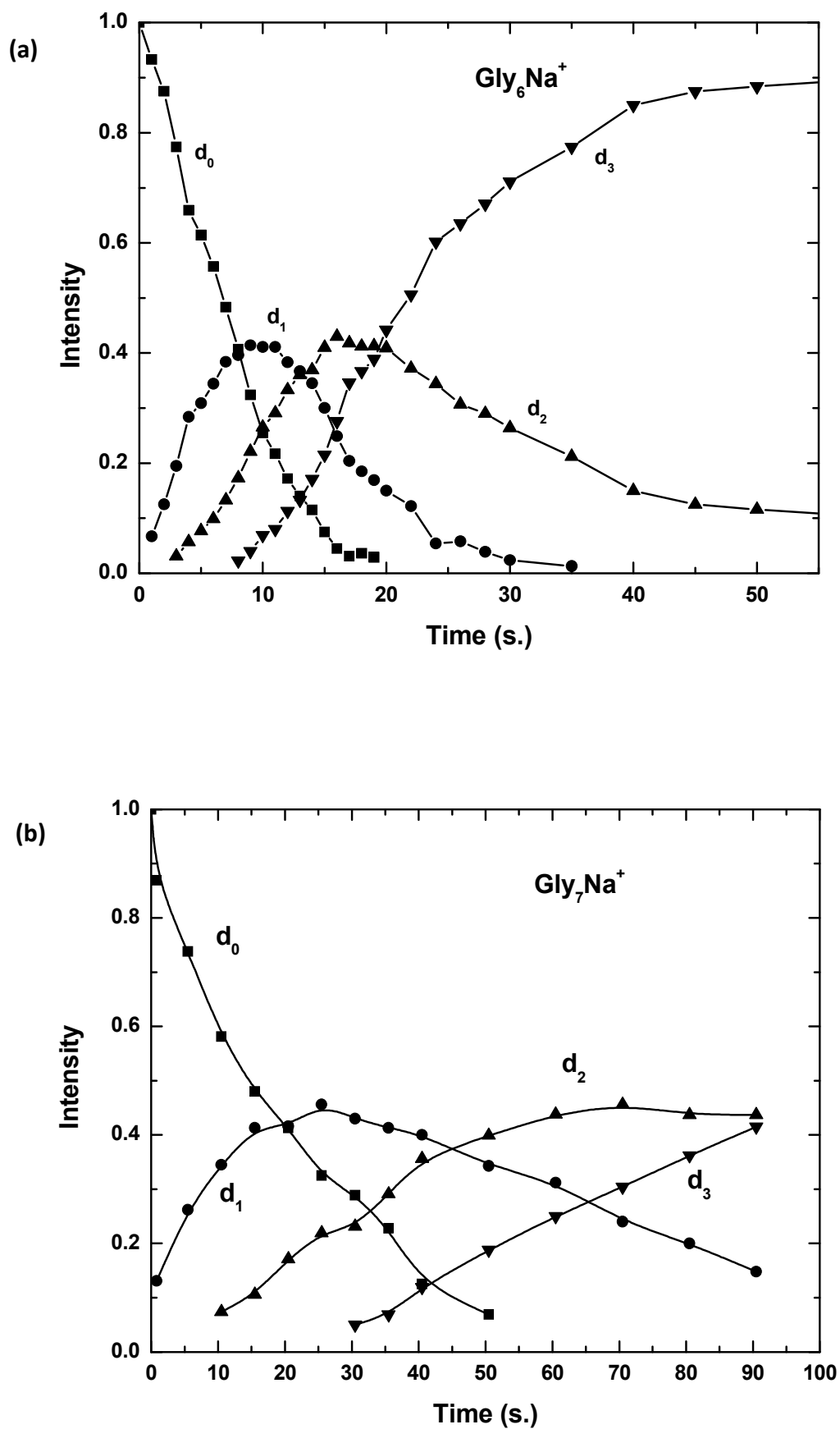


Figure 1



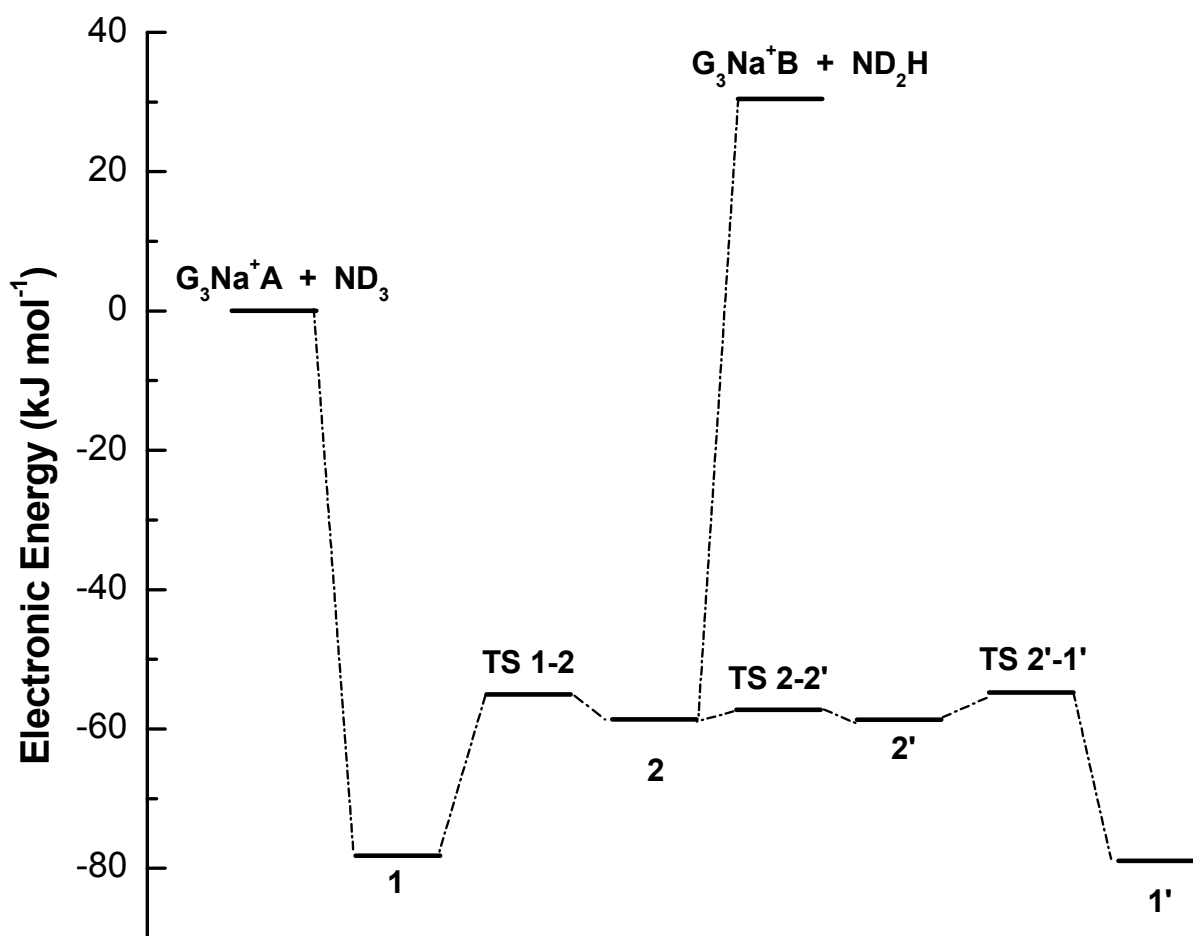


Figure 3

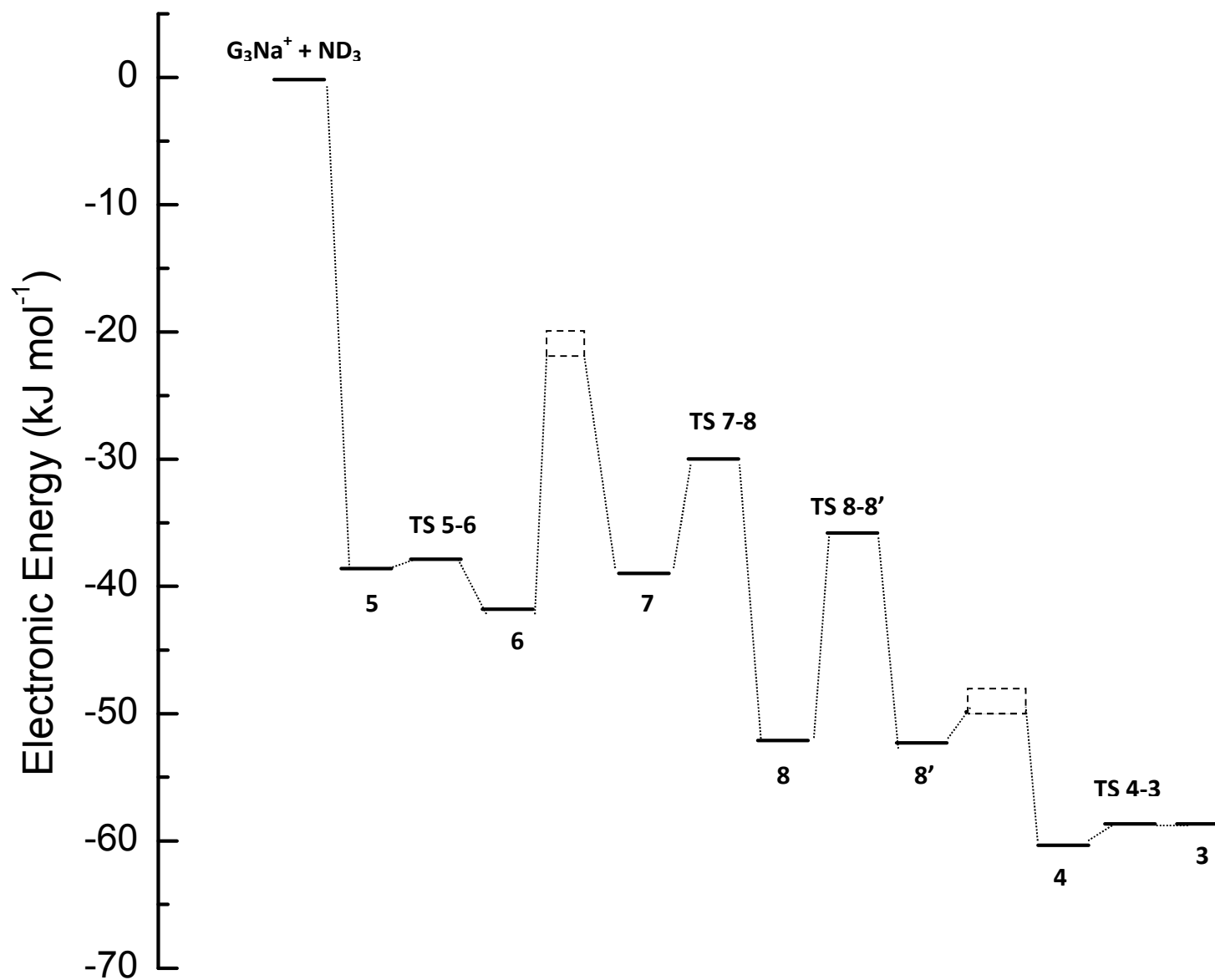


Figure 4

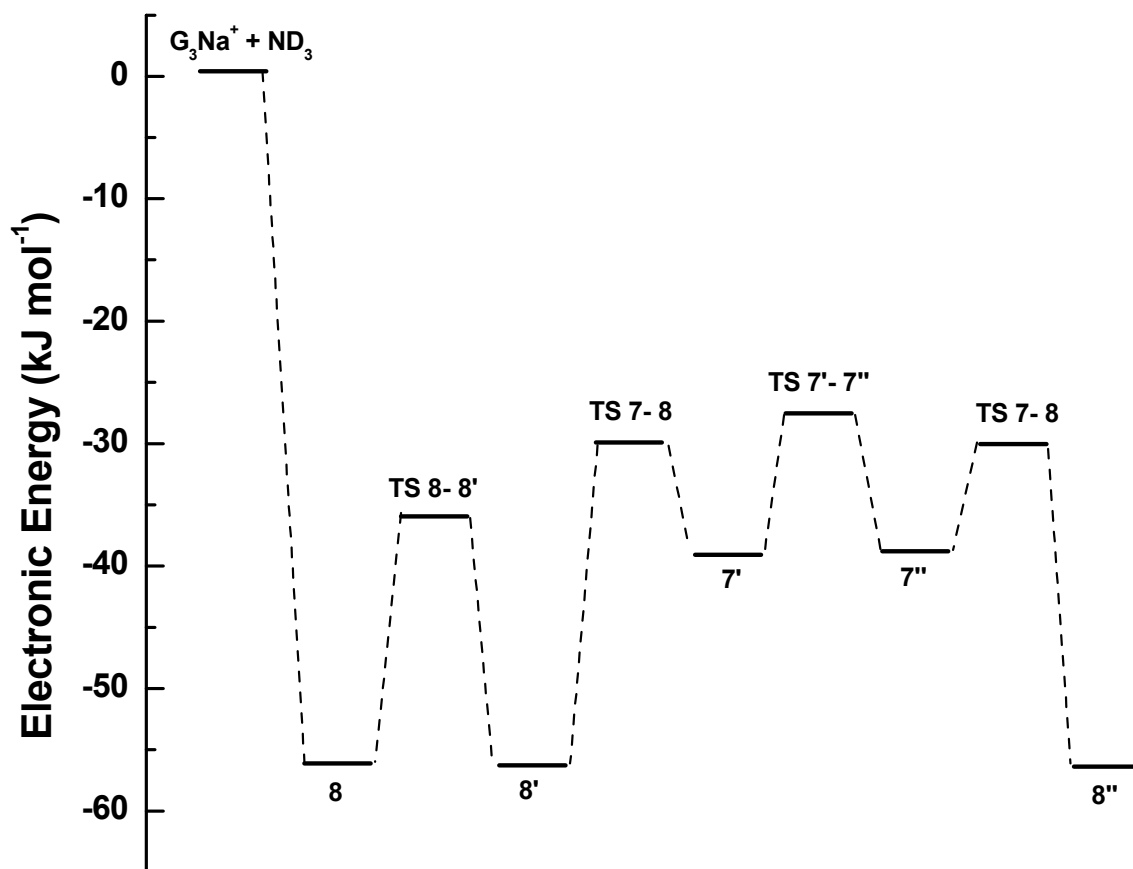


Figure 5

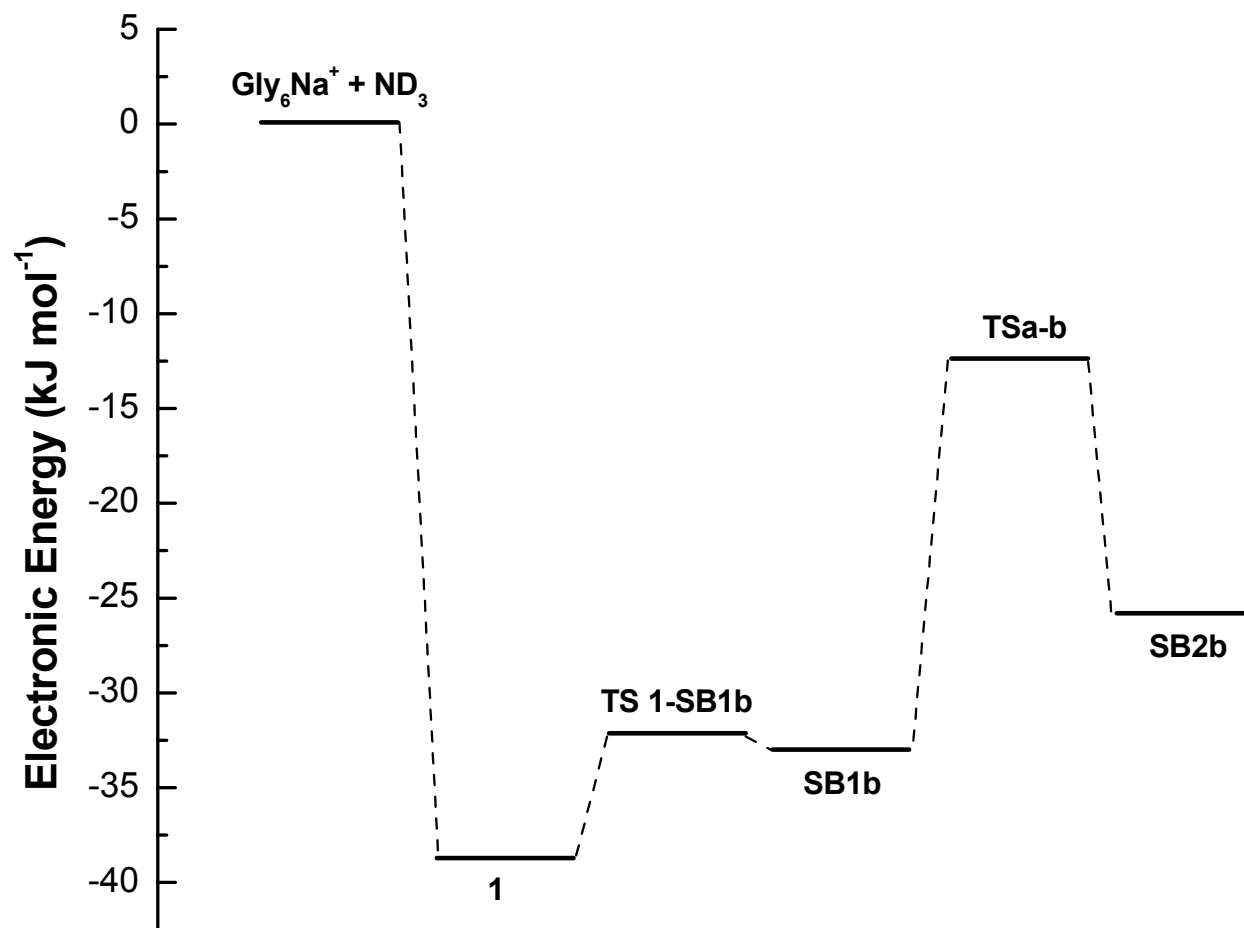


Figure 6

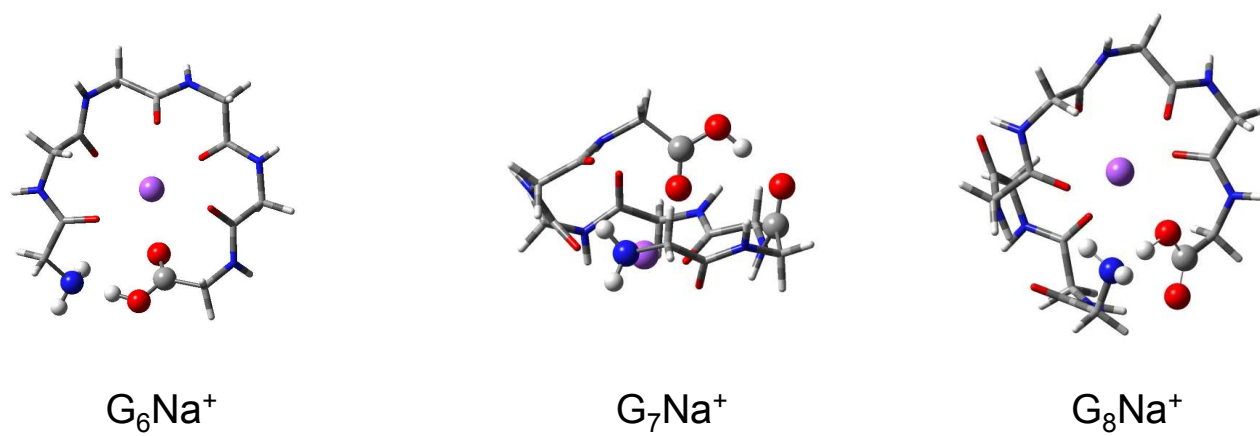


Figure 7