Gas Phase H/D Exchange of Sodiated Amino Acids: Why Do We See Zwitterions?

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The gas-phase interaction of sodiated amino acids and sodiated amino acid methyl esters with various deuterium donors is investigated by combining results of H/D exchange reactions with those from density functional theory and molecular dynamics calculations. Discrepancy between experimentally and theoretically obtained structures for sodium cationized amino acids is explained by deuterium donor caused perturbation of the most stable amino acid conformation. Detailed study of H/D exchange mechanism on sodiated amino acids shows that the H/D exchange reaction is preceded by a multistep quasi-isoenergetic transition (perturbation) from a charge solvated to zwitterionic structure in the amino acid. Although the computation refers to the system $AlaNa^+$ and D_2O, these mechanisms apply to all amino acids, except those where a functional side-chain group takes part in the perturbation process. The suggested perturbation mechanism applies also for other deuterium donors such as CD_3OD or even ND_3 and indicates that a single water molecule suffices to convert the sodiated amino acid from charge solvated to zwitterionic form.

Gas-phase H/D exchange is one of the techniques for structural investigation of biomolecules [1–9]. Site-specific treatment of H/D exchange reaction on protonated amino acids provided evidence for their structure, which was found also to be in agreement with the calculated structure [1–9]. Complexation of amino acids with alkali metal ions stabilizes the amino acid gas-phase zwitterionic structure (ZW). Site-specific treatment of the H/D exchange reaction on sodiated amino acids is ideal for distinction between charge solvated (CS) and zwitterionic (i.e., salt bridged) structures. However, for sodiated amino acids, structural information may differ from that obtained by calculation [10, 11]. Because in the H/D exchange reaction sodiated amino acid interacts with a neutral molecule of deuterating agent, it is suggested that this interaction may perturb the conformation of sodiated amino acid and, thus, influence the experimental results and, thereby, the conclusions.

In a recent study, Cox et al. [10] proposed mechanisms for H/D exchange of sodiated glycine oligomers with ND_3. The exchange mechanism was studied by density functional theory (DFT) at the B3LYP/6-311++G** level in a way that reaction intermediates were determined and then connected with the proposed reaction coordinate. According to their proposed mechanism, perturbation of sodium cationized glycine from the CS to the ZW form is a result of H/D exchange of carboxylic hydrogen. The transition states and exact reaction energy profiles were not investigated. Also, it was not shown that the reaction follows the proposed reaction coordinate. If H/D exchange follows this mechanism through the first H/D exchange, the next one will take place in the ZW form of glycine. Exchange in the ZW form implies a different mechanism, which will probably yield different reaction rate constants for the remaining two hydrogens. Thus, the proposed mechanism predicts two reaction sites as identical and one to be different, which is clearly at odds with experimental data.

The purpose of this work is to answer the question in the title. It will be shown that experiment "designed" for structural investigation may change the structure of the analite. For that purpose, experimental (mass spectrometry) and theoretical (DFT and molecular dynamics) results were employed. Detailed description of the dynamic pathway for the H/D exchange reaction of sodiated alanine ($AlaNa^+$) with D_2O is given. $AlaNa^+$ is used as model compound for all amino acids, except for those in which a functional side-chain group takes part in the perturbation process. The proposed perturbation mechanism which converts a sodiated amino acid from CS to ZW form applies also for other deuterium donors such as CD_3OD and even ND_3. Furthermore, this mechanism shows that a single molecule of water is adequate for the conversion.

Table 1. Site specific H/D exchange reaction rate constants (in units of 10^{-11} cm^3 s^{-1} $molecules^{-1}$) for studied amino acids

Amino acid	$AlaNa^+$		$AspNa^+$		$CysNa^+$		$GluNa^+$	
Deuterium donor	CH_3OD	D_2O	CH_3OD	D_2O	CH_3OD	D_2O	CH_3OD	D_2O
k_1	20.5	1.5	4.6	3.6	1.6	0.4	3.5	3.1
k_2	20.5	1.5	1.1	0.5	1.6	0.4	1.6	0.9
k_3	20.5	1.5	1.1	0.5	1.6	0.4	1.6	0.9
k_4			1.1	0.5			1.6	0.9

Amino acid	$IleNa^+$		$LeuNa^+$		$ValNa^+$	
Deuterium donor	CH_3OD	D_2O	CH_3OD	D_2O	CH_3OD	D_2O
k_1	12	1.3	13.1	1.6	9.8	0.2
k_2	12	1.3	13.1	1.6	9.8	0.2
k_3	12	1.3	13.1	1.6	9.8	0.2

Methods

Experimental Methods

All amino acids (Ala, Asp, Cys, Glu, Ile, Leu, Val) and amino acid methyl esters (AlaOMe, LeuOMe, ValOMe) were obtained from Fluka (Buchs, Switzerland). The deuteration reagent D_2O (99.8%) was from Aldrich (Milwaukee, WI) and CD_3OD (99.8%) was from Cambridge Isotope Laboratories (Andover, MA). MALDI samples were prepared with a standard dried-droplet procedure using 2,5-dihydroxybenzoic acid (DHB) as matrix. Two consecutive 337 nm laser pulses from a nitrogen laser (VSL 337 NSD, LSI Laser Science, Newton, MA) were used to produce gas-phase samples. The H/D exchange experiments were performed in a 3 T Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (Extrel FTMS 2001, Madison, WI). Stabilized reagent gas pressure used in the exchange experiments was $2.67–1.33{\cdot}10^{-5}$ Pa at ambient temperature of 300 K. The H/D exchange experiments and determination of the site-specific reaction rate constants were performed by using the earlier described procedure [7]. Repetitive H/D exchange experiments indicate a relative standard deviation of up to 30% for the reported site-specific rate constants.

Computational Methods

Density functional theory (DFT) and G3(MP2) [12] theory calculations were carried out by using GAUSSIAN 03 [13] program package on the computer cluster at the Ruder Boskovic Institute. Initial search of minima on potential energy surface (PES) were performed by using B3LYP functional with 6-31G* basis set. Obtained geometries were additionally reoptimized at the B3LYP/6-311++G** level. Each stationary point (minimum on the potential energy surface) was tested by a vibrational analysis. The structures of the transition states were obtained by QST2 and QST3 optimization. Transition states structures were tested by verifying that one of the harmonic frequencies is imaginary, and by IRC analysis for the reaction pathways. Obtained energies of reactants and reaction intermediates were not corrected for zero point energy. Basis set superposition error (BSSE) was not calculated because it was assumed that relative energies of studied sodiated structures are independent of BSSE.

Since DFT gives only stationary structures, descriptions of reaction system sodiated amino acid-deuterium donor were supplemented with molecular dynamics (MD) simulations [4]. Molecular dynamics simulations were performed using MacroModel software [14] with AMBER* all atom force field. Several different MD simulations were performed depending which effect was studied. Most of the simulations were performed at 300 K with no SHAKE algorithm. MD simulations were carried out for 200 ps with time step of 1.5 fs.

Results and Discussion

H/D Exchange Reactions

To test the mechanism and increase the number of investigated sodiated amino acids in H/D exchange the gas-phase reactions of $AlaNa^+$, $AspNa^+$, $CysNa^+$, $GluNa^+$, $IleNa^+$, $LeuNa^+$, and $ValNa^+$ with CD_3OD and D_2O were analyzed and site-specific exchange reaction rate constants were determined, Table 1. No exchange of hydrogens was observed in $AlaOMeNa^+$, $LeuOMeNa^+$, and $ValOMeNa^+$.

Experimental results indicate all studied sodiated amino acids to be present in their ZW forms, at odds with available theoretically determined structures [15–20]. Present findings, together with earlier results [10, 11] indicate that in H/D exchange experiments only the ZW form of sodiated amino acid is probed. To answer the question why is that so, gas-phase interaction of sodiated amino acid with a deuterium donor was analyzed theoretically. For practical reasons (computational time and resources), the computational study aimed to explain the mechanism of H/D exchange was carried out for the model system $AlaNa^+$ and D_2O. In all calculations, the molecule of D_2O was mimicked with H_2O. However, because CD_3OD and D_2O exhibit

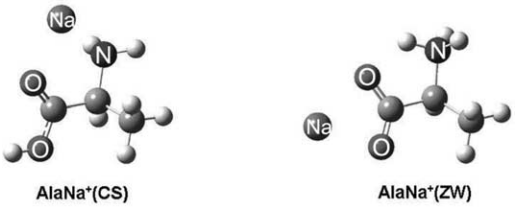

Figure 1. The most stable conformers of $AlaNa^+$ in a CS and ZW form at the B3LYP/6-311++G**.

similar reactivities in experiments, and because both participate only with a single deuterium exchange per reactive encounter, the mechanism also applies for CD_3OD.

Interaction of $AlaNa^+$ and D_2O

H/D exchange reaction begins with molecules D_2O and $AlaNa^+$ approaching each other. It is expected that $AlaNa^+$ is in its energetically most favorable conformation [7, 21]. Theoretical B3LYP/6-311++G** calculations [16, 19, 20] have shown as the most stable conformation of $AlaNa^+$ a structure, $AlaNa^+$(CS), depicted in Figure 1, in accordance with our calculations. This most stable conformation of $AlaNa^+$ is in a charge solvated form while the most stable zwitterionic form, $AlaNa^+$(ZW), is less stable by 5.2 kJ mol^{-1}, Figure 1.

According to the Boltzmann distribution at 300 K, approximately 10% of $AlaNa^+$ population should be in ZW form, which raises the question whether the experiment probes only this part of total $AlaNa^+$ population. Adding the zero point energy correction yields additional stabilization of CS form by 0.4 kJ mol^{-1} while anharmonic effect at the B3LYP/6-311++G** reduces the difference between CS and ZW form by 0.4 kJ mol^{-1}. For more accurate results, G3(MP2) computational protocol was applied. The energy difference between CS and ZW form obtained with G3(MP2) calculations is 12 kJ mol^{-1}, which strongly favors CS structure. Confirmation that all $AlaNa^+$ ions undergo H/D exchange can be found in the experimental results, e.g., time plot of the experimental intensities, Figure 2. The experimental decay of the reactant in an experiment which probes only 10% of the ion population with the rest unreactive certainly will look different from that in Figure 2. On the basis of these facts, it can be concluded that almost all $AlaNa^+$ ions are in CS form and all $AlaNa^+$ population undergoes H/D exchange. Further support that CS form of $AlaNa^+$ is initially formed comes also from vibrational spectra of $GlyNa^+$ [22], which favored CS form of $GlyNa^+$.

Molecular dynamics simulations of collisions of $AlaNa^+$(CS) with D_2O from various directions have shown that 3 to 7 ps is needed for D_2O to reach the cation, Figure 3a. The structure depicted in Figure 3a is also the most stable conformation of $AlaNa^+$-D_2O reaction complex. When D_2O approaches the carboxylic

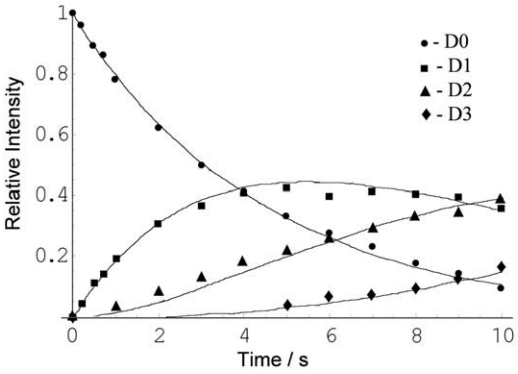

Figure 2. Time plot of the experimental intensities and the corresponding theoretical fit obtained with site-specific treatment of the H/D exchange of $AlaNa^+$ with D_2O. D0, D1, D2, and D3 represent the relative intensities of $AlaNa^+$ in which 0, 1, 2, and 3 hydrogen atoms have been replaced by deuterium.

group it spends there 5 ps and then shifts towards the sodium cation, Figure 3b (vide infra).

Figure 3a shows that there is no possibility for H/D exchange in the most stable reaction complex conformation, $AlaNa^+$(CS)-D_2O, because D_2O is fixed in the proximity of sodium cation. Sodium cation complexation also excludes amino group and carboxyl oxygen as possible sites for stabilization of D_2O and H/D exchange. However, experimental results for $AlaNa^+$ point to three equally fast exchanging sites attributable to protonated α-amino group, and consequently to the existence of $AlaNa^+$ in the ZW form. One concludes that before H/D exchange, the structure of $AlaNa^+$ is perturbed from the CS to the ZW form, with deuterium donor acting as a catalyst. After perturbation, a new reaction complex $AlaNa^+$(ZW)-D_2O is formed and it undergoes the H/D exchange.

The Perturbation Reaction

B3LYP/6-311++G** potential energy profile together with optimized structures for the D_2O catalyzed pertur-

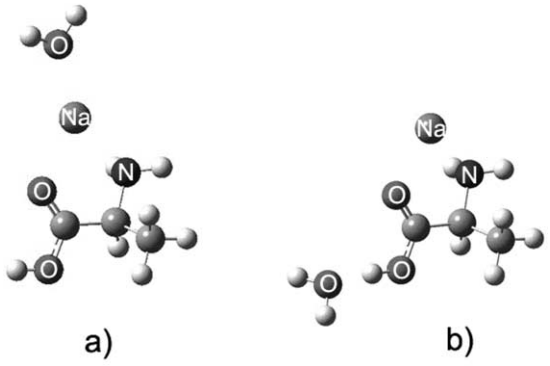

Figure 3. (**a**) The most stable conformation of the reaction complex $AlaNa^+$(CS)-D_2O. (**b**) Reaction complex **CSK1** obtained by B3LYP/6-311++G** optimization of the situation which arises from approach of D_2O towards the carboxylic group.

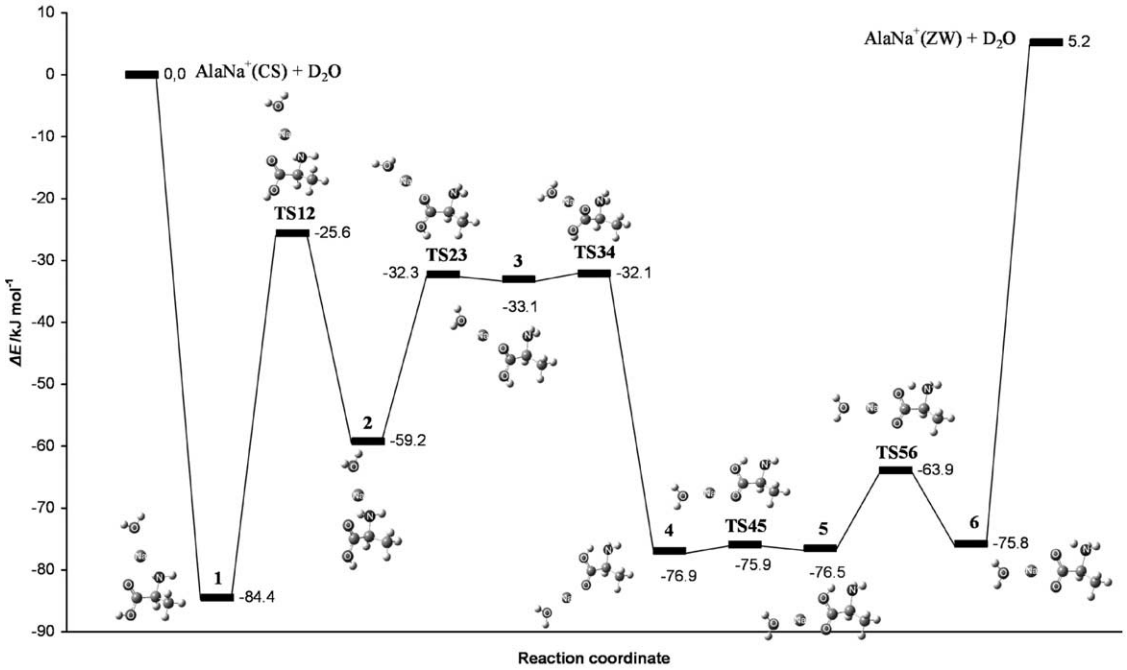

Figure 4. Schematic representation of the potential energy profile for the D_2O catalyzed perturbation of $AlaNa^+$ structure from CS to ZW form. The relative energies are calculated at the B3LYP/6-311++G** level of theory.

bation of $AlaNa^+$ structure from CS to ZW form is shown in Figure 4.

The reaction begins with a rotation of carboxylic group's hydrogen by 180° leading to minimum **2** via transition-state **TS12**. It is followed by a shift of triply coordinated sodium in **2** to a doubly coordinated in intermediate **3**. The D_2O molecule accompanies and coordinates the sodium cation during the transition. Unfortunately, attempts to identify corresponding transition-state failed, probably because of the very low potential energy barrier between transition-state **TS23** and reaction intermediate **3** of only 0.8 kJ mol^{-1}. Structural similarity between **TS23** and intermediate **3** is a consequence of a very shallow potential energy surface. Transition-state **TS23** (Figure 4) is found by QST3 optimization. Additional vibration analysis characterized this structure as a second-order saddle point with two imaginary frequencies. Animated vibration of one (16.03i cm^{-1}) corresponds to displacement of Na^+ and D_2O along the reaction coordinate, and it tends to lead in the direction of reaction intermediates **2** and **3**. Animation of the other imaginary frequency (38.78i cm^{-1}) corresponds to rotation of D_2O around the O-Na axis. We trust that obtained transition-state **TS23** well approximates the actual transition-state.

The isomerization from **3** to **4** occurs via transition-state **TS34**, and it is a result of a torsion around C–Cα axis. The next step is a migration of sodium cation from one side of the carboxylic group to the middle, through the transition-state **TS45**, which is only 0.6 to 1 kJ mol^{-1} higher in energy than intermediates **4** and **5**. Zwitterionic intermediate **6** is then formed by proton transfer from carboxylic to α-amino group of reaction intermediate **5**. Dissociation of **6** forms ion $AlaNa^+$(ZW).

Hence, perturbation of the $AlaNa^+$ from the CS to the ZW form seams to be a five step reaction with D_2O acting as a catalyst. The reaction energy barrier is overcome by initial complexation of reactants $AlaNa^+$ and D_2O, which yields 84.4 kJ mol^{-1}. It is important to notice that a single molecule of water is sufficient to change conformation of sodiated amino acid. These findings also support assumptions made by Hoyau et al. [23, 24] that binding of an additional ligand (D_2O or ND_3) to $GlyNa^+$ would provide enough energy for CS-ZW isomerization.

As already mentioned, the same mechanism can be applied for CD_3OD, (vide supra) and, we believe, also for interaction of sodiated amino acid and ND_3. There are no obvious reasons why ND_3 in the perturbation reaction will not exhibit the same reactive pattern as D_2O.

The exchange mechanism proposed by Cox et al. [10], which requires carboxylic hydrogen to complete first exchange and perturbation, is additionally supported by lack of H/D exchange in sodiated amino acid methyl esters.

However, we believe that sodiated amino acid methyl esters do not exchange with deuterium donor for the same reasons as $AlaNa^+$(CS), depicted in Figure 3a. Perturbation of sodiated amino acid methyl ester in a zwitterionic-like structure is less probable because the additional methyl group on the carboxylic site (also a very important coordination site) poses steric hindrance and inhibits the ability for conformational change. Also,

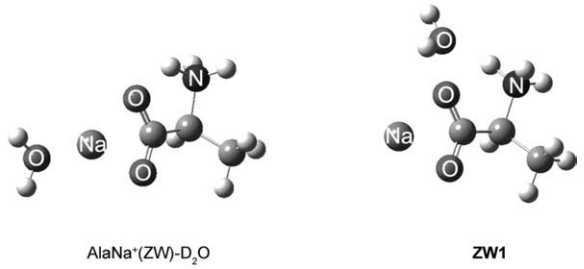

Figure 5. Complexes $AlaNa^+(ZW)$-D_2O and **ZW1** are results of B3LYP/6-311++G** optimization of the two stable sites according molecular dynamics simulations.

all our attempts to stabilize the multiply hydrogen bonded reaction complex between the zwitterion-like structure of $AlaOMeNa^+$ and D_2O were unsuccessful. During optimization, D_2O moved either toward sodium cation or away from carbonyl oxygen.

After formation of $AlaNa^+(ZW)$ due to the energetic barrier [20] of 71 kJ mol^{-1}, there is a low probability for back isomerization to $AlaNa^+(CS)$, however, reverse D_2O catalyzed perturbation is possible (vide infra).

H/D Exchange of $AlaNa^+(ZW)$ with D_2O

Again, collisions of $AlaNa^+(ZW)$ and D_2O from various directions were simulated by molecular dynamics calculations. The results show that within 3 ps D_2O stabilizes at two sites, Figure 5. Optimization of structure $AlaNa^+(ZW)$-D_2O gives the energetically most stable complex of $AlaNa^+(ZW)$ and D_2O, with no possibility for H/D exchange.

From the aspect of H/D exchange, more interesting is a structure where D_2O is close to the $-NH_3^+$ group. The interaction brings D_2O into position which corresponds to starting H/D exchange intermediate **ZW1**, Figure 5. The B3LYP/6-311++G** potential energy profile and optimized structures for the three-step H/D exchange reaction are shown in Figure 6.

The mechanism starts with concerted double transfer of hydrogen/deuterium within **ZW1**. In the transition-state **TS-ZW12** one of $-NH_3^+$ group hydrogens is transferred to D_2O while, simultaneously, one of the deuteriums moves to the carbonyl oxygen. To eliminate dissociation or longer stabilization of reaction intermediate **ZW2**, molecular dynamics simulations started from this point. Simulations have shown that within 3 ps HDO reaches a position in minimum **ZW3** where it partakes in sodium cation solvation. Animated vibration of imaginary frequency of transition-state **TS-ZW23** corresponds to the displacement of HDO towards **ZW2** and **ZW3**. Position of HDO is stable in **ZW3** and further dynamics do not suggest any significant movement except oscillation around C–Cα axis. Finally, in intermediate **ZW3**, transfer of the deuteron occurs. Here, the deuteron transfer is identical to the proton transfer in the perturbation reaction of sodiated amino acid from its CS to ZW form. Once minimum **ZW4** is formed, the reaction complex dissociates into products $AlaNa_{D1}^+$ and HDO, where D1 represents the particular hydrogen atom that has been replaced by deuterium.

Although IRC analysis was carried out for reaction pathways, our attempts for IRC analysis of the reaction pathway from transition-state **TS-ZW23** to reaction

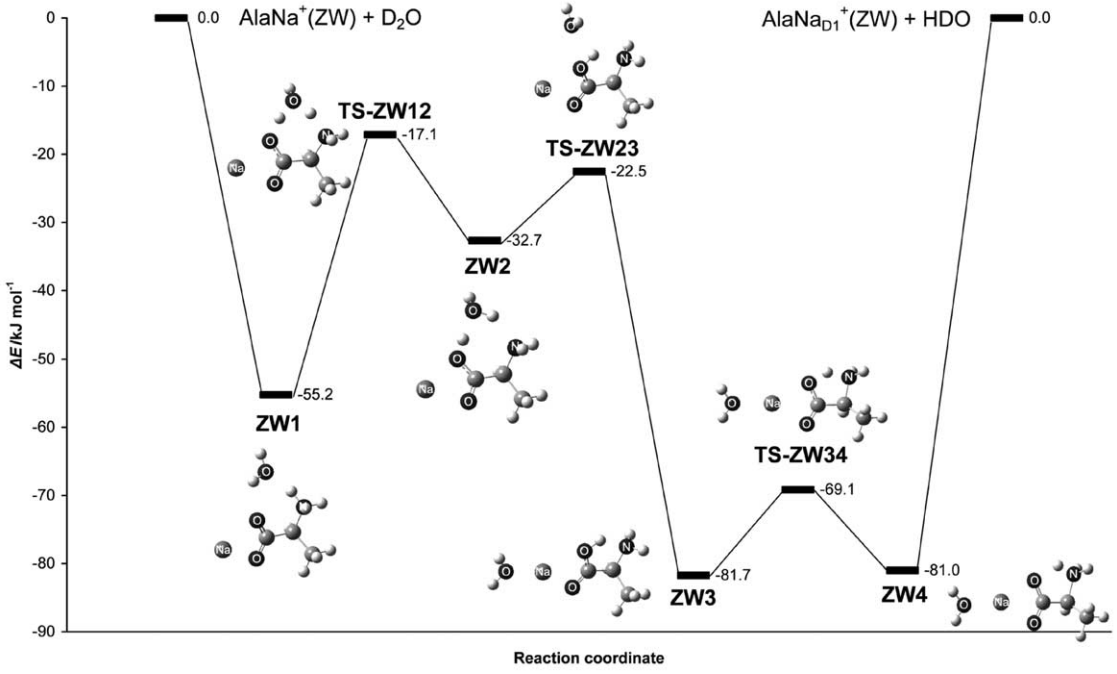

Figure 6. Potential energy profile for the H/D exchange reaction of $AlaNa^+(ZW)$ with D_2O. The relative energies and optimized structures were obtained at the B3LYP/6-311++G** level of theory.

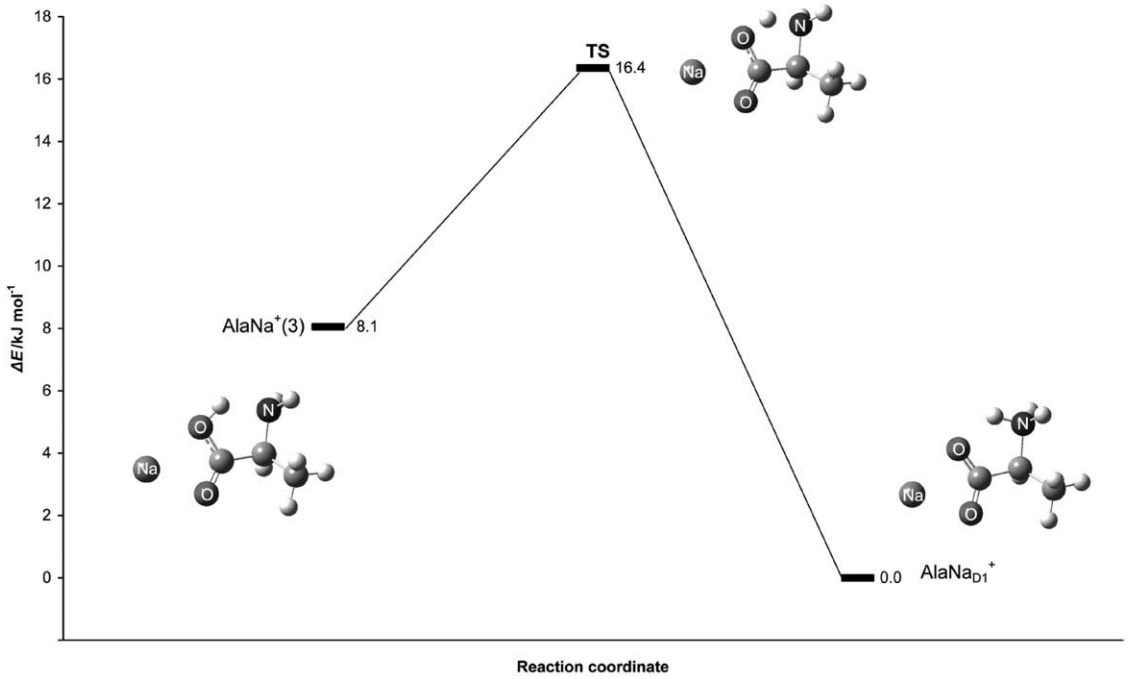

Figure 7. Schematic representation of the potential energy profile for the reaction of the deuteron transfer in $AlaNa^+(3)$. The relative energies are calculated at the B3LYP/6-311++G** level of theory.

intermediate **ZW3** were unsuccessful. Because of that and since the energy difference between minima **ZW3** and **ZW4** is very small (0.7 kJ mol^{-1}), one should take into account possible dissociation of **ZW2** or **ZW3**. Dissociation of **ZW2** or **ZW3** is a formation of minimum $AlaNa^+(3)$. Minimum $AlaNa^+(3)$ can either collide with another molecule of D_2O and form reaction complex **ZW3** or rearrange into a minimum $AlaNa^+_{D1}$ by deuteron transfer from carbonyl oxygen to amino nitrogen, Figure 7. Thus, dissociation of minimum **ZW2** or **ZW3** results in formation of final product $AlaNa^+_{D1}$.

Confirmation of such reaction path is found in the

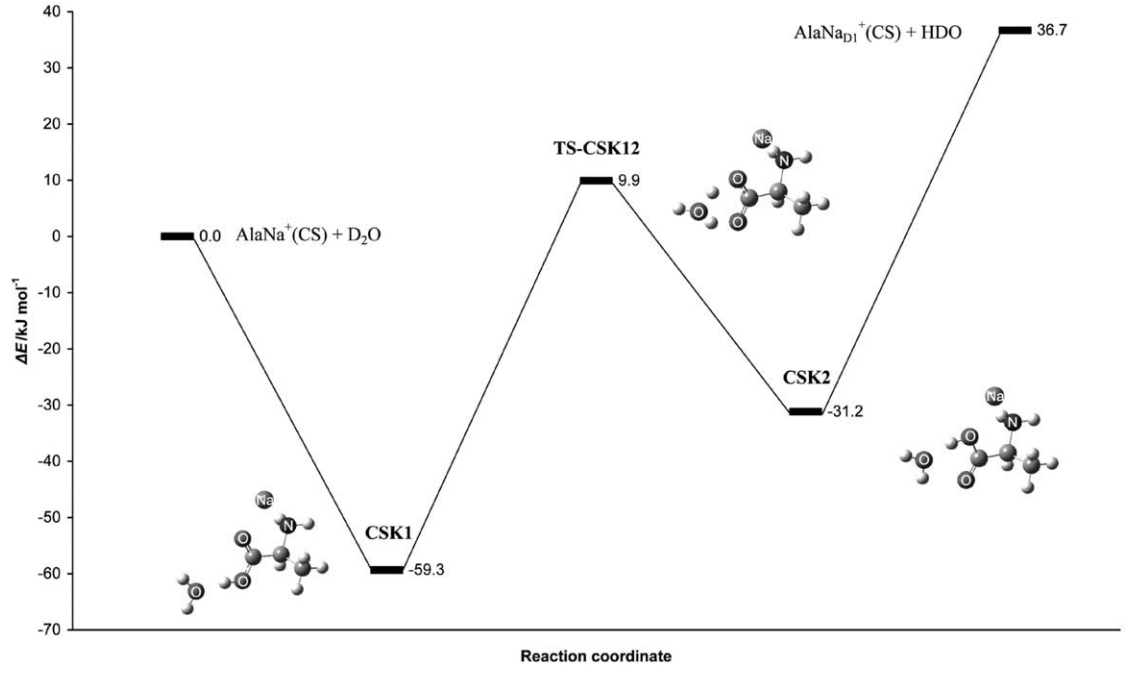

Figure 8. Schematic potential energy diagram for the H/D exchange mechanism of carboxylic hydrogen via "flip-flop" mechanism. The relative energies are calculated at the B3LYP/6-311++G** level of theory.

experimental values of reaction rates constants. Reaction rate constants for exchange of hydrogens of the α-amino group in sodiated amino acids are up to 10 times higher from those in the protonated ones [5–7, 9]. Fast exchanges on $-NH_3^+$ group suggest absence of rotation of -COOD group around C–Cα axis and confirm transfer of HDO toward its position in minimum **ZW3** [9]. Rotation around C–Cα axis would slow down the reaction, and observed reaction rates will be lower and more like those for protonated amino acids. Similar behavior, i.e., fast exchanging hydrogens of the amino group can be found in experiments with molecules where the carboxylic group is rigid, e.g., for $GlyGlyH^+$ [6].

Although subtle, support for this H/D exchange mechanism can be found in the Arrhenius equation. Since it is a mechanism with zero activation energy, the reaction rate constant needs to have a value of the pre-exponential factor. Theoretical values of pre-exponential factors for bimolecular gas-phase reaction with zero activation energy are in the range of 10^{-9} to 10^{-11} $cm^3\ s^{-1}$ $molecule^{-1}$, i.e., values are pretty much consistent with obtained H/D exchange site-specific reaction rate constants.

With the mechanism of H/D exchange established, the description of interaction between $AlaNa^+$ and D_2O is almost complete. To finish it, the situation that arises from the approach of D_2O towards the carboxylic group is now considered, Figure 3b. Examination of this complex addresses the possibility of H/D exchange of the carboxylic hydrogen. Experimental results do not indicate the presence of that hydrogen. H/D exchange at the carboxylic oxygen via proton transfer by formation of "hydronium" cation is very unfavorable, and our attempts to find such mechanism were unsuccessful. Although H/D exchange of this hydrogen is possible through a "flip-flop" mechanism (similar to that in protonated amino acids [2, 6], Figure 8, the potential energy profile for such exchange of carboxylic hydrogen shows that energy gained from the formation of the initial reaction complex **CSK1** is not sufficient to overcome the barrier of transition-state **TS-CSK12**. With this in mind, it can be concluded that for interaction between $AlaNa^+$(CS) and D_2O the perturbation reaction will be the favored process.

Conclusions

The major finding of this study and an answer to the question why we see zwitterions is that in a reaction system amino acid-deuterium donor H/D exchange is preceded by a multistep quasi-isoenergetic perturbation, which converts the sodiated amino acid structure from the CS to the ZW form. The proposed dynamic path for this deuteration reagent catalyzed perturbation is valid for all sodiated amino acids in which the functional side-chain group does not significantly partake in the perturbation. This perturbation mechanism is proposed to apply for D_2O and CD_3OD, and there is no reason why not also for ND_3 as deuteration reagents.

Acknowledgments

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Appendix

Absolute energies and relative stabilities of the computed structures.

Table 1. (with Figure 1) The B3LYP/6-311++G** and G3(MP2) energies (in E_h) and relative stabilities (in kJ mol^{-1}) of the most stable conformers of $AlaNa^+$ in a CS and ZW form

		$AlaNa^+$		
Model	Energy	CS	ZW	Δ_{ZW-CS}
B3LYP/6-311++G(d,p)	E	−486.011984	−486.009987	5.2
	${}^{H}E_{zpe}$	−485.901918	−485.899784	5.6
	${}^{ANH}E_{zpe}$	−485.903325	−485.901507	4.8
G3(MP2)	E(0 K)	−485.018313	−485.013976	11.4
	E(298 K, 10^5 Pa)	−485.009813	−485.005256	12.0

Table 2. (with Figure 4) The B3LYP/6-311++G** energies (in E_h) and relative stabilities of conformers (in kJ mol^{-1}) for the D_2O catalyzed perturbation of $AlaNa^+$ structure from CS to ZW form

Structure	E	Δ
$AlaNa^+$(CS) + D_2O	−562.470515	0
1	−562.502679	−84.4
TS12	−562.480253	−25.6
2	−562.493062	−59.2
TS23	−562.482800	−32.3
3	−562.483104	−33.1
TS34	−562.482739	−32.1
4	−562.499811	−76.9
TS45	−562.499420	−75.9
5	−562.499651	−76.5
TS56	−562.494855	−63.9
6	−562.499368	−75.8
$AlaNa^+$(ZW) + D_2O	−562.468518	5.2

Table 3. (with Figure 6) The B3LYP/6-311++G** energies (in E_h) and relative stabilities of conformers (in kJ mol^{-1}) for the H/D exchange reaction of $AlaNa^+$(ZW) with D_2O

Structure	E	Δ
$AlaNa^+$ (ZW) + D2O	−562.468518	0.0
ZW1	−562.489554	−55.2
TS-ZW12	−562.475044	−17.1
ZW2	−562.480962	−32.7
TS-ZW23	−562.477100	−22.5
ZW3	−562.499651	−81.7
TS-ZW34	−562.494855	−69.1
5	−562.499368	−81.0
$AlaNa_{D1}{}^+$(ZW) + HDO	−562.468518	0.0

Table 4. (with Figure 7) The B3LYP/6-311++G** energies (in E_h) and relative stabilities of conformers (in kJ mol^{-1}) for the reaction of the deuteron transfer in $AlaNa^+$(3)

Structure	E	Δ
$AlaNa^+$(3)	−486.006919	8.1
TS	−486.003757	16.4
$AlaNa_{D1}{}^+$	−486.009987	0.0

Table 5. (with Figure 8) The B3LYP/6-311++G** energies (in E_h) and relative stabilities of conformers (in kJ mol^{-1}) for the H/D exchange mechanism of carboxylic hydrogen via "flip-flop" mechanism

Structure	E	Δ
$AlaNa^+$(CS) + D2O	−562.470515	0.0
CSK1	−562.493120	−59.3
TS-CSK2	−562.466736	9.9
CSK2	−562.482390	−31.2
$AlaNa_{D1}{}^+$(CS) + HDO	−562.456545	36.7