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Gas Phase Structure of Sodiated Amino Acids Probed by H/D Exchange Reactions*

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Taking into account the difference in structure of a sodiated gas phase amino acid in its zwitterionic and charge solvated forms, gas phase structures of sodiated histidine, lysine, phenylalanine, proline, tryptophan and tyrosine were probed by H/D exchange reactions. Experiment results obtained from the site-specific reaction rate constants indicated the zwitterionic structure for all the sodiated amino acids studied. In contrast, B3LYP calculations for sodiated lysine, along with the already presented theoretical results for other amino acids studied, show the charge solvated form to be stable, except for proline which is in the zwitterionic form. Absence of H/D exchange in sodiated methyl esters of phenylalanine and histidine, along with the discrepancy between experimental and theoretical data for the amino acids studied, indicates that H/D exchange occurs only when sodiated amino acids are in the zwitterionic form.

INTRODUCTION

In solutions and crystals, amino acids appear in the zwitterionic (ZW) form; however, the zwitterionic form is not stable in the gas phase and appears in the more stable neutral structure. Nevertheless, the zwitterionic form of amino acids in the gas phase can be additionally stabilized by addition of an alkali metal cation.

It has been shown both experimentally[1,2] and theoretically[2,3] that some gas phase amino acids, such as arginine and proline, when cationized with some alkali metal ions emerge more stable in the zwitterionic form. Recently published theoretical studies[4,5] point out that sodiated asparagine, cysteine, histidine, phenylanine, serine, tryptophan and tyrosine exist in the gas phase in the charge solvated (CS) form.

In accordance with the different structure of a sodiated amino acid in its zwitterionic and charge solvated forms, Figure 1, we assume that it may be possible to distinguish these two structures by applying the site-specific treatment[6,7] of the gas phase H/D exchange in Na^+ cationized amino acids. As a result of such treat-

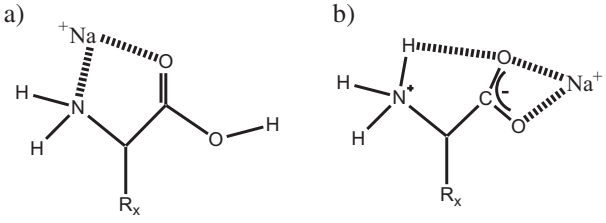


Figure 1. Gas phase structure of Na^+ cationized amino acid: a) charge solvated form; b) zwitterionic form.

* Dedicated to Dr. Edward C. Kirby on the occasion of his 70th birthday.

ment, we might determine whether the sodiated amino acid is in a zwitterionic form during the H/D exchange. By determining the site-specific reaction rate constants of the charge solvated form, we would get two identical reaction sites and a distinct one. In the case of the ZW structure (Figure 2b), which has three equivalent reaction sites, the outcome of the treatment will be three identical reaction rate constants whilst the CS structure (Figure 1a) with two identical and one distinct reaction site will correspondingly yield two equivalent and one different reaction rate constant.

Hence, during isotopic exchange on a sodium cationized amino acid, an interaction of the amino acid with the neutral molecule of the deuterium donor occurs; this can result in additional alteration of the sodium cationized amino acid conformation. It has been shown[8] that the conclusions on the structure based on the results of H/D exchange in sodium cationized glycine with ND_3 differ from those obtained theoretically.

Using H/D exchange, we have examined the following sodiated amino acids: histidine (His), lysine (Lys), phenylalanine (Phe), proline (Pro), tryptophan (Trp) and tyrosine (Tyr) and sodiated methyl esters of histidine (HisOMe) and phenylalanine (PheOMe). CD_3OD, D_2O and D_2S were used as exchange agents. Experimental results have been compared with theoretically derived structures.

METHODS

Experimental Methods

All amino acids (His, Lys, Phe, Pro, Trp and Tyr) and amino acid methyl esters (HisOMe and PheOMe) were obtained from Fluka (Buchs, Switzerland). The deuteration reagent D_2O (99.8 %) was from Aldrich (Milwaukee, USA) and CD_3OD (99.8 %) was from the Cambridge Isotope Laboratories (Andover, USA). H/D exchange experiments were performed in a 3 T Fourier transform ion cyclotron resonance (FTICR) mass spectrometer with a Nicolet 1280 data station (Extrel FTMS 2001, Madison, USA) equipped with a nitrogen laser (VSL 337 NSD, LSI Laser Science, Newton, USA) emitting at λ = 337 nm.

MALDI samples were prepared with a standard dried-droplet procedure using 2,5-dihydroxybenzoic acid (DHB) as matrix. Stabilized reagent gas pressure used in the exchange experiments was $2.67–1.33 \cdot 10^{-5}$ Pa at ambient temperature of 300 K. Reagent gas pressure was measured with a Bayard-Alpert type ionization gauge using parameterized response factors for calibration.[9] H/D exchange experiments and determination of the site-specific rate constants were performed by the procedure mentioned in an earlier publication.[10] An interactive program for the site-specific rate constants determination was written in Mathematica 4.0 (Wolfram Research, Champaign, USA). Repetitive H/D exchange experiments indicated a relative standard deviation of up to 30 % for the reported site-specific rate constants. Assuming that ion gauges were properly calibrated, the major source of error in the site-specific rate constants were the pressure measurements. It is worth mentioning that the ratios between the exchange rates for the reaction sites in each experiment remained unaffected.

Computational Methods

Density functional theory (DFT) calculations were carried out using the GAUSSIAN 03 (Ref. 11) program package on the computer cluster of the Ruđer Bošković Institute. Initial search for the minima on the potential energy surface (PES) were performed using the B3LYP functional[12] 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, which was also used to obtain the zero point vibrational energy (ZPVE). Basis set superposition error (BSSE) was not calculated because it was assumed that relative energies of the studied sodiated structures were independent of BSSE.

RESULTS AND DISCUSSION

$HisNa^+$, $LysNa^+$, $PheNa^+$, $ProNa^+$, $TrpNa^+$ and $TyrNa^+$ and $HisOMeNa^+$ and $PheOMeNa^+$ were produced *via* MALDI and were allowed to react with CD_3OD, D_2O and D_2S for various reaction delay times. H/D exchange of three, five, three, two, three and three hydrogen atoms in $HisNa^+$, $LysNa^+$, $PheNa^+$, $ProNa^+$, $TrpNa^+$ and $TyrNa^+$, respectively, was observed with CD_3OD and D_2O but not with D_2S. No hydrogens were exchanged in $HisOMeNa^+$ and $PheOMeNa^+$ with CD_3OD, D_2O and D_2S. The H/D exchange reactions were analyzed and site-specific exchange rate constants were determined (Tables I and II).

TABLE I. Site-specific gas phase H/D exchange rate constants ($\times 10^{-11}$ cm^3 s^{-1} molecule^{-1}) of sodiated amino acids with CD_3OD

Amino acid	$HisNa^+$	$LysNa^+$	$PheNa^+$	$ProNa^+$	$TrpNa^+$	$TyrNa^+$
k_1	1.15	3.8	1.12	1.65	2.21	3.83
k_2	1.15	3.8	1.12	1.65	2.21	3.83
k_3	1.15	3.68	1.12	–	2.21	3.83
k_4	*	3.68	–	–	*	*
k_5	–	3.68	–	–	–	–

* H/D exchange not observed

TABLE II. Site-specific gas phase H/D exchange rate constants ($\times 10^{-11}$ cm^3 s^{-1} molecule^{-1}) of sodiated amino acids with D_2O

Amino acid	$HisNa^+$	$LysNa^+$	$PheNa^+$	$ProNa^+$	$TrpNa^+$	$TyrNa^+$
k_1	0.34	1.07	0.16	0.44	1.38	1.32
k_2	0.34	1.07	0.16	0.44	1.38	1.32
k_3	0.34	0.95	0.16	–	1.38	1.32
k_4	*	0.95	–	–	*	*
k_5	–	0.95	–	–	–	–

* H/D exchange not observed

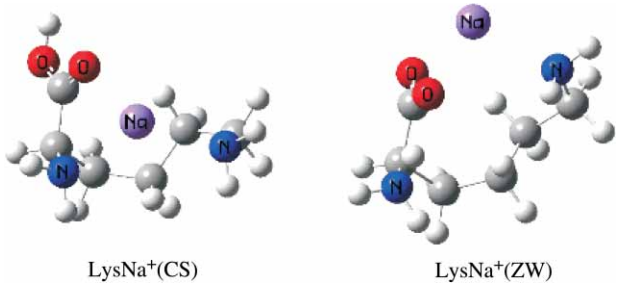
Absence of H/D exchange in sodiated amino acids with D_2S is in agreement with that already observed for protonated amino acids[10] and seems to be the result of weak hydrogen bonding within the reaction complex sodiated amino acid–D_2S. Formation of multiple hydrogen bonds within the reaction complex lower the barrier to the internal proton transfer[6] and therefore the energy gain through formation of a weak hydrogen bonded reaction complex with D_2S is not sufficient to overcome the barrier to isotopic exchange.

Results for $HisNa^+$, $PheNa^+$, $TrpNa^+$ and $TyrNa^+$ point to three equally fast exchanging sites. Two equivalent fast exchanging sites for $ProNa^+$ were found. Finally, $LysNa^+$ gives two and three equivalent sites, but with rate constants being almost the same.

The fast exchanging sites in $HisNa^+$, $PheNa^+$, $TrpNa^+$ and $TyrNa^+$ are attributed to the protonated α-amino group, thus proving the existence of the zwitterionic form of the sodium cationized amino acid. Lack of exchange of the remaining hydrogen in $HisNa^+$, $TrpNa^+$ and $TyrNa^+$ during the reaction time can be explained as a consequence of two partially positive charges in the zwitterionic form (Figure 1b). In collisions between the sodium cationized amino acid and deuteration reagent, the protonated α-amino group and sodium cation sites will attract the deuteration reagent and the remaining part of the molecule will stay intact. Absence of one independent site (which could be attributed to the carboxylic group) in the exchange results for $ProNa^+$ and $LysNa^+$ implies the zwitterionic structures of the latter.

It is also noteworthy that the exchange at the protonated α-amino group in sodiated amino acids is about 3 to 10 times faster than that in protonated ones,[10] except for sodiated proline where it is much the same.

Theoretical calculations[4,5] for sodium cationized forms of His, Phe, Trp and Tyr show the charge solvated form to be stable. For $ProNa^+$, it is shown[1] that the zwitterionic structure is dominant in the gas phase. Despite intensive theoretical work on sodium cationized amino acids, no results describing the gas phase structure of $LysNa^+$ were found. To fill the gap in the theoretical description of sodium cationized acids, a series of DFT calculations on $LysNa^+$ were undertaken. Lys is one of the most basic amino acids and it has a side chain with an NH_2 functional group. According to the studied[4,5] sodiated amino acids with a functional side chain, it is expected that the NH_2 group of Lys will provide an extra coordination site for a sodium cation, which will yield a charge solvation structure. Initial search for PSE was performed at the B3LYP/6-31G* level and the obtained structures were then reoptimized at the B3LYP/6-311++G** level. Conformers of sodiated lysine in charge solvated and zwitterionic forms are shown in Figure 2. The corresponding B3LYP/6-311++G** energies, ZPVE corrected energies (E_{ZPVE}) and relative stabilities of conformers ($\Delta_{ZW\text{-}CS}$) are listed in Table III.



$LysNa^+$(CS) $LysNa^+$(ZW)

Figure 2. Conformers of $LysNa^+$ in CS and ZW forms at the B3LYP/6-311++G** level.

TABLE III. The B3LYP/6-311++G** energies, ZPVE corrected energies (in a.u.) and relative stabilities of conformers (in kJ mol^{-1}) of $LysNa^+$ in CS and ZW forms

Conformation	$LysNa^+$(CS)	$LysNa^+$(ZW)	$\Delta_{ZW\text{-}CS}$
E	–659.373014	–659.362645	27.22
E_{ZPVE}	–659.158115	–659.147668	27.42

On the basis of the presented data we presume that the stable conformation of $LysNa^+$ accordance is triply coordinated CS form, in which Na^+ is coordinated to lone pair electrons of the carboxylic and two amino groups. In the less stable zwitterionic form, $LysNa^+$ is also triply coordinated, but here with the use of the lone pair of the amino group and two electron pairs derived from the deprotonated carboxylic group. Possible reasons for $LysNa^+$(ZW) energy increase are the appearance of a four- instead of five-membered ring between Na^+ and COO^- group, and the decrease in negative charge at the carboxylic group that solvates the sodium cation. In both forms, the sodium cation is solvated by three electron pairs, but in the ZW form two lone pairs of the COO^- group combined with Na^+ compose a four-membered ring, whereas in the CS form one lone pair of carboxylic and one of the α-amino group together with sodium cation form a five-membered ring.

Presented theoretical results indicate that all the sodiated amino acids studied except proline are in the charge solvated form whereas experimental results for all of them indicate the zwitterionic form to be dominant. It seems that, with the exception of $ProNa^+$, H/D exchange experiments on sodiated amino acids do not probe the most stable ion structure. Similar discrepancy between theoretical and experimental results was already observed[8] in the gas phase H/D exchange reactions of sodiated glycine with ND_3. Although ND_3 has a different mechanism of exchange and can partake in multiple exchanges during a single collision *versus* CD_3OD and D_2O which participate only in a single, sequential, deuterium exchange,[13] one may conclude that in the gas phase exchange experi-

ments, regardless of which deuterium reagent partakes in the exchange, only the zwiterionic form of Na^+ cationized amino acid is probed. Further support to such a conclusion is the lack of H/D exchange in sodium cationized methyl esters ($HisOMeNa^+$ and $PheOMeNa^+$), which are unable to form zwitterionic structures.

CONCLUSIONS

Site-specific treatment of the gas phase H/D exchange on sodiated His, Lys, Phe, Pro, Trp, and Tyr with CD_3OD and D_2O indicated that all investigated sodiated amino acids were in the zwitterionic form. Lack of H/D exchange in sodiated amino acids with D_2S is a result of too weak hydrogen bonding within the reaction complex.

Results for $LysNa^+$ PES favor the CS structure in agreement with the earlier results[4,5] for $HisNa^+$, $PheNa^+$, $ProNa^+$, $TrpNa^+$ and $TyrNa^+$ which, except for $ProNa^+$, pointed to the same conclusion.

Inconsistency between experimental and theoretical data for the studied amino acids along with lack of H/D exchange on $PheOMeNa^+$ and $HisOMeNa^+$ indicate that only zwitterionic forms of sodiated amino acids are probed in H/D exchange experiments.

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SAŽETAK

Određivanje strukture natrijem kationiranih aminokiselina u plinskoj fazi pomoću H/D izmjene

Marko Rožman

Obzirom na različitu strukturu natrijem kationirane aminokiseline u zwitterionskoj formi od one u formi solvatiranoga naboja, moguće je promatrajući H/D izmjenu razlikovati te dvije strukture. Informacije dobivene određivanjem konstanti brzine kemijske reakcije na specifična mjesta u molekuli ukazuju da se promatrane natrijem kationirane aminokiseline: histidin, lizin, fenilalanin, prolin, triptofan i tirozin nalaze u zwitterionskoj formi. Nasuprot tome, B3LYP rezultati za natrijem kationirani lizin zajedno s već publiciranim rezultatima za ostale promatrane aminokiseline ukazuju da se iste u plinskoj fazi nalaze u formi solvatiranoga naboja, uz iznimku prolina koji se nalazi u zwitterionskoj formi. Izostanak H/D izmjene na natrijem kationiranim metilnim esterima histidina i fenilalanina zajedno s neslaganjem teorijskih i eksperimentalnih rezultata upućuje da u eksperimentima H/D izmjene do iste dolazi samo kada je natrijem kationirana aminokiselina u zwiterionskoj formi.