

Theoretical study of the gas-phase structures of sodiated and cesiated leucine and isoleucine: zwitterionic structure disfavored in kinetic method experiments

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The most stable charge-solvated (CS) and zwitterionic (ZW) structures of sodiated and cesiated leucine and isoleucine were studied by density functional theory methods. According to the Boltzmann distribution in gas phase, both forms of LeuNa^+ and IleNa^+ exist, but in LeuCs^+ and IleCs^+ , the ZW forms are dominant. Results for the sodiated compounds are consistent with the relationship found between decrease in relative stability of CS *versus* ZW form and aliphatic amino acid side chain length. The observed degeneracy in energy for IleNa^+ conformers is at odds with kinetic method results. Additional calculations showed that kinetic method structural determinations for IleNa^+ do not reflect relative order of populations in the lowest energy conformers. Since complexation of cationized amino acids into ion-bound dimers disfavors ZW structure by $\sim 8 \text{ kJ mol}^{-1}$, it is suggested that for energy close conformers of sodium-cationized amino acids, the kinetic method may not be reliable for structural determinations.

KEYWORDS: leucine; isoleucine; alkali metals; gas-phase structure; density functional theory; kinetic method

INTRODUCTION

Gas-phase alkali metal-cationized amino acids and the stability of their gas-phase zwitterionic (ZW) structures are intensively studied. Present theoretical calculations show that gas-phase alkali metal-cationized amino acids^{1–11} with the exception of arginine^{3,12} and proline^{2,13} are more stable in the charge-solvated (CS) form. However, looking at relative stabilities of ZW and CS forms (expressed by their energy difference, $\Delta_{\text{ZW-CS}}$) of sodium-cationized aliphatic amino acids ($\text{GlyNa}^+ - \Delta_{\text{ZW-CS}} = 11.7 \text{ kJ mol}^{-1}$, $\text{AlaNa}^+ - \Delta_{\text{ZW-CS}} = 5.0 \text{ kJ mol}^{-1}$, $\text{ValNa}^+ - \Delta_{\text{ZW-CS}} = 3.8 \text{ kJ mol}^{-1}$),^{4,7,10} a decrease in energy differences which follows the aliphatic side chain enlargement can be observed. This trend gives a reason for this study, i.e. to theoretically analyze the CS and ZW structures of IleNa^+ and LeuNa^+ and their relative stabilities.

Mass spectrometrical determinations of gas-phase structures of alkali metal-cationized amino acids mainly result from ion mobility,¹⁴ gas-phase H/D exchange¹⁵ and kinetic method¹⁶ experiments. However, in most cases, these results are not conclusive. Ion mobility is frequently limited because

CS and ZW structures can have very similar cross sections.⁴ The gas-phase exchange experiments have shown that regardless of the deuterium reagent, only ZW forms of sodium-cationized amino acids are probed.^{11,17,18} Study of H/D exchange mechanism suggested that prior to H/D exchange reaction, there is a quasi-energy-free transition from CS to ZW structure in the amino acid.¹⁸ As yet, kinetic method experiments produced the majority of results, which were also in agreement with theoretical predictions.^{5,19}

Wytttenbach *et al.*⁴ reported an interesting correlation between the stability of the gas-phase ZW/CS structures of alkali ion-cationized amino acids and their proton affinities (PA). On the basis of this correlation, it was suggested that sodium-cationized amino acids with proton affinity above $\sim 910 \text{ kJ mol}^{-1}$ predominately have ZW structures. But, in the suggested correlation, the possibility of the extra coordination site for sodium cation provided by the functional side chain group was ignored, and it was later demonstrated that this prediction is at odds with theoretical and kinetic method results.^{5,19}

The ZW conformations of sodium-cationized amino acids have a proton attached to the most basic site, and if probed in a kinetic method experiment, linear correlation between sodium ion affinities (ΔH_{Na}) and PA is expected.²⁰ Kish *et al.*²⁰ analyzed 17 amino acids by the kinetic method and found poor correlation ($R^2 = 0.601$) between corresponding affinities. It was concluded that poor correlation suggests

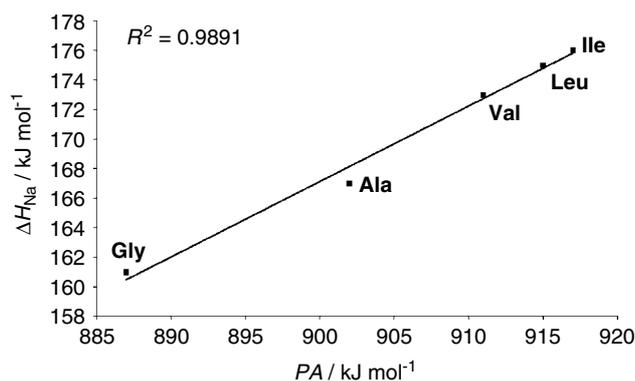


Figure 1. Correlation plot of the proton affinities versus sodium ion affinities of aliphatic amino acids. Dots represent experimental data from Ref. 20 and full line represents their least-square fit.

CS structure of sodium-cationized amino acids. But, if we correlate only proton and sodium ion affinities of aliphatic amino acids (with no extra coordination site), a good correlation ($R^2 = 0.989$) can be found (Fig. 1). Furthermore, if the ZW stability limit⁴ (amino acids with proton affinity above 910 kJ mol^{-1}) is applied, this could mean that in the gas phase, the presence of ZW forms of LeuNa^+ and IleNa^+ should not be neglected.

The above finding provided additional motivation for this work in which structures of IleNa^+ and LeuNa^+ were analyzed by means of the density functional theory (DFT). Additionally, in order to test kinetic method results, sodium-bound dimers of Ile were theoretically analyzed and their relative stabilities were compared with those of the monomers.

COMPUTATIONAL METHODS

DFT calculations were performed using the GAUSSIAN 03 program package²¹ on the computer cluster at the 'Ruđer Bošković' Institute. Initial search of minima on potential energy surface (PES) was performed by using B3LYP functional^{22,23} with 6-31G* basis set. The obtained geometries were additionally reoptimized at the B3LYP/6-311++G** level. The cesiated structures were studied at the B3LYP/LanL2DZ level. Harmonic vibrational analyses have been performed in order to characterize each stationary point as a minimum on the PES; these results were also used to obtain zero point vibrational energy (ZPVE). The basis set superposition error (BSSE) was not calculated because it was assumed that relative energies of the studied structures are independent of BSSE.

RESULTS AND DISCUSSION

In order to identify the most stable conformers of CS and ZW sodium-cationized Ile and Leu, we used the strategy of a similar conformer. In this approach, the starting structures were built from the most stable conformations of the studied alkali metal-cationized amino acids (glycine, alanine and valine).^{4,6,7,10,18} Special care was taken for modeling a coordination site of the cation, which involves either the lone pair electrons of carbonyl oxygens in the ZW or

those of carbonyl oxygen and the amine nitrogen in the CS structure. Aliphatic side chains in both forms (CS and ZW) of cationized amino acids were placed in different positions with steric strain taken into consideration. These geometries were then optimized at the B3LYP/6-31G* level and then reoptimized at the B3LYP/6-311++G** level to obtain final energy and relative stabilities. The lowest-energy gas-phase CS and ZW structures of LeuNa^+ and IleNa^+ are shown in Fig. 2. The corresponding B3LYP/6-311++G** energies, ZPVE-corrected energies (E_{ZPVE}) and relative stabilities of conformers are listed in Table 1.

For both amino acids, the CS structure in which Na^+ is coordinated by carbonyl and amino group lone pair electrons appears to be energetically more stable. In the less stable ZW form, the positive charges of sodium cation and protonated amino group are separated by the negative charge of the carboxylic group. The energy differences between CS and ZW structures are very small (practically degenerate), and from the presented data, it is obvious that the relative stability decreased when compared with glycine, alanine and valine (*vide supra*). It was suggested¹⁹ that one reason for stabilizing the salt bridge (ZW) structure is the linearity of the $+ - +$ charges (H-O-Na angle). However, all aliphatic amino acids have pretty much the same H-O-Na angle ($\sim 180^\circ$). So, additional stabilization of ZW forms may result from $-\text{NH}_3^+$ group stabilization by aliphatic (electron donor) side chains in agreement with the link between decrease in relative energies and length of aliphatic side chain (*vide supra*). From values in Table 1 and according to the Boltzmann distribution at 300 K, one should expect the existence of CS and ZW forms of studied sodium-cationized amino acids in the gas phase. On the other hand, experimental results¹⁹ predict the existence of only CS forms of both sodium-cationized amino acids. In these experiments, to compare the relative stability of alkali metal (M^+) - cationized amino acid conformations, J. M. Talley *et al.*¹⁹ used the kinetic method.

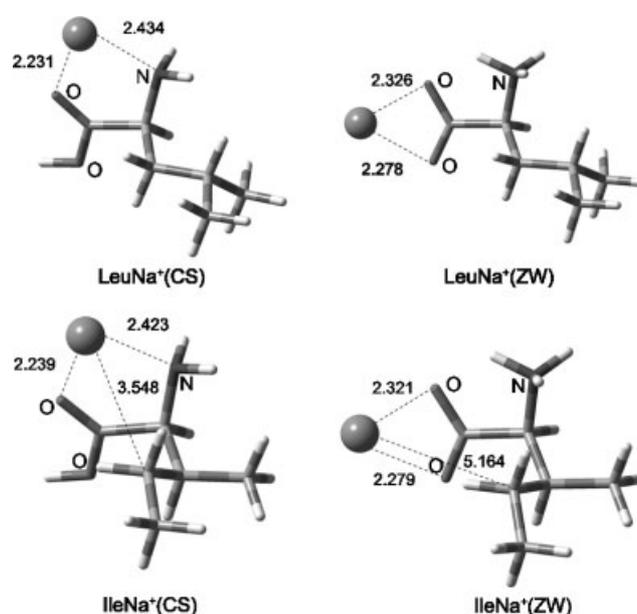


Figure 2. The most stable conformers of LeuNa^+ and IleNa^+ in a CS and ZW form at B3LYP/6-311++G**.

Table 1. Energies, ZPVE-corrected energies (both in E_h) and relative stabilities (in kJ mol^{-1}) of IleNa^+ and IleNa^+ conformers computed at the B3LYP/6-311++G** level

Amino acid Conformer	IleNa^+			IleNa^+		
	CS	ZW	$\Delta_{\text{ZW-CS}}$	CS	ZW	$\Delta_{\text{ZW-CS}}$
E	-603.986528	-603.985421	2.9	-603.98455	-603.984429	0.3
E_{ZPVE}	-603.791762	-603.790789	2.6	-603.78969	-603.789449	0.6

The kinetic method is based on dissociation of an ion-bound dimeric cluster. If the gas-phase amino acid is in the CS form, its methyl ester will bind M^+ more strongly as a consequence of electron-donating properties of the methyl group.¹⁹ Dissociation of this dimer will yield more abundant population of amino acid methyl ester- M^+ . A more abundant population of amino acid - M^+ will be formed by dissociation of the [amino acid(ZW)- M -amino acid methyl ester]⁺ dimer. The ZW form of amino acid - M^+ binds M^+ more strongly because of ionic interactions between COO^- and M^+ .

Still, the mere fact that more ions of methyl ester are formed does not necessarily mean that gas-phase CS amino acid - M^+ was more stable and/or most abundant. In the kinetic method, there is a dissociation of a FAB-, MALDI- or ESI-formed M^+ -bound dimer, and one should take care to see whether the most stable dimer probed by kinetic method is actually formed from the most stable M^+ -cationized amino acids and their methyl esters. Previous energy comparisons of dimers *versus* monomers reported disfavor of ZW structure relative to CS upon complexation but no reversal of energy ordering in case of lithiated glycine.¹ Similar comparison for lithiated proline showed energy reversal and the ZW structure was disfavored.¹⁹ To test this possibility for the energy-equivalent gas-phase conformers of IleNa^+ , calculations for Ile-Na-IleOMe^+ were carried out.

Sodium-bound clusters of $\text{Ile}(\text{CS})\text{-Na-IleOMe}^+$ and $\text{Ile}(\text{ZW})\text{-Na-IleOMe}^+$ were formed from the most stable conformations of corresponding amino acids and their methyl esters. Those clusters were then optimized at the B3LYP/6-311++G** level to obtain the final energies and relative stabilities. The most stable conformations are shown in Fig. 3, while corresponding B3LYP/6-311++G** energies, ZPVE-corrected energies (E_{ZPVE}) and relative stabilities of clusters ($\Delta_{\text{ZW-CS}}$) are listed in Table 2.

In the most stable sodium-bound cluster of Ile and IleOMe, the Ile is in the CS form. The energy difference between $\text{Ile}(\text{CS})\text{-Na-IleOMe}^+$ and $\text{Ile}(\text{ZW})\text{-Na-IleOMe}^+$ is 8.1 kJ mol^{-1} . Additional stabilization of the $\text{Ile}(\text{CS})\text{-Na-IleOMe}^+$ dimer is probably the result of better coordination/solvation of sodium cation with aliphatic (electron-donating) side chains (Fig. 3). In the $\text{Ile}(\text{CS})\text{-Na-IleOMe}^+$ cluster, the sodium cation is coordinated by two aliphatic side chains, while in $\text{Ile}(\text{ZW})\text{-Na-IleOMe}^+$ because of the ZW nature of interaction, sodium is coordinated by only one side chain.

The expected population ratio of those two dimers based on Boltzmann distribution at 300 K is 99.6:0.4 in favor of $\text{Ile}(\text{CS})\text{-Na-IleOMe}^+$. On the other hand, the population ratio of gas-phase CS and ZW forms of IleNa^+ is 57.8 to 42.2,

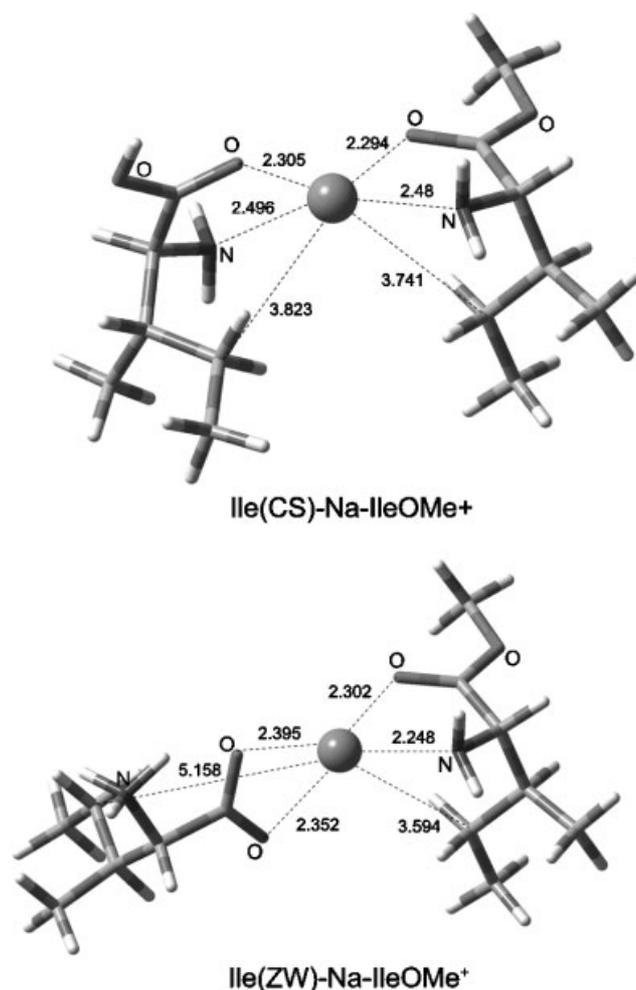


Figure 3. Dimers $\text{Ile}(\text{CS})\text{-Na-IleOMe}^+$ and $\text{Ile}(\text{ZW})\text{-Na-IleOMe}^+$ obtained by the B3LYP/6-311++G** optimization.

Table 2. The B3LYP/6-311++G** energies, ZPVE-corrected energies (in E_h) and relative stabilities (in kJ mol^{-1}) of $\text{Ile}(\text{CS})\text{-Na-IleOMe}^+$ and $\text{Ile}(\text{ZW})\text{-Na-IleOMe}^+$ dimers

Dimer	$\text{Ile}(\text{CS})\text{-Na-IleOMe}^+$	$\text{Ile}(\text{ZW})\text{-Na-IleOMe}^+$	$\Delta_{\text{ZW-CS}}$
E	-1085.17264	-1085.169555	8.1
E_{ZPVE}	-1084.755863	-1084.752879	7.8

respectively. Although, because of the nature of the theoretical treatment (error of the method), the calculated Boltzmann distribution should be considered only as approximate, the following trend is evident: population ratios of the CS and ZW forms of IleNa^+ based on the dissociation of most stable Ile-Na-IleOMe^+ dimers do not represent actual population

Table 3. Interatomic distances (in Å) in the LeuCs⁺, IleCs⁺, Ile(CS)-Cs-IleOMe⁺ and Ile(ZW)-Cs-IleOMe⁺ calculated on the B3LYP/LanL2DZ level of theory

Bond	LeuCs ⁺ (CS)	LeuCs ⁺ (ZW)	IleCs ⁺ (CS)	IleCs ⁺ (ZW)	Ile(CS)-Cs-IleOMe ⁺	Ile(ZW)-Cs-IleOMe ⁺		
Cs-N	3.43	–	3.381	–	3.475	3.441	–	3.453
Cs-O (N side)	2.987	3.156	3.009	3.146	3.08	3.058	3.229	3.081
Cs-O	–	3.078	–	3.09	–	–	3.153	–
Cs-C (β -C ethyl group)	–	–	4.266	5.844	4.369	4.336	5.93	4.226

Table 4. Energies, ZPVE-corrected energies (both in E_h) and relative energies (in kJ mol⁻¹) of LeuNa⁺ and IleNa⁺ conformers computed at the B3LYP/LanL2DZ level

Amino acid Conformer	LeuCs ⁺			IleCs ⁺		
	CS	ZW	Δ_{ZW-CS}	CS	ZW	Δ_{ZW-CS}
E	-461.385707	-461.395198	-24.9	-461.383545	-461.394439	-28.6
E_{ZPVE}	-461.19128	-461.200837	-25.1	-461.189487	-461.199784	-27

Table 5. The B3LYP/LanL2DZ energies, ZPVE-corrected energies (in E_h) and relative energies (in kJ mol⁻¹) of Ile(CS)-Cs-IleOMe⁺ and Ile(ZW)-Cs-IleOMe⁺ dimers

Dimer	Ile(CS)-Cs-IleOMe ⁺	Ile(ZW)-Cs-IleOMe ⁺	Δ_{ZW-CS}
E	-942.327405	-942.335825	-22.1
E_{ZPVE}	-941.910582	-941.919376	-23.1

ratios of the CS and ZW forms of IleNa⁺. In kinetic method experiments, Ile(CS)-Na-IleOMe⁺ dimers will be probed, and their dissociation will yield a more abundant population of the IleOMeNa⁺ and, thereby, leads to the conclusion that the CS form of IleNa⁺ is the dominant one.

DFT calculation showed here that kinetic method experiments for IleNa⁺ do not reflect relative populations of the lowest energy conformers. For IleCs⁺, the kinetic method gives the ZW form as the more stable.¹⁹ To further test the kinetic method results and reliability of DFT calculations, complexation of Ile with Cs⁺ was investigated. Conformations of IleCs⁺ and Ile-Cs-IleOMe⁺ were built in a such way that in the most stable conformations of IleNa⁺ and Ile-Na-IleOMe⁺, the sodium was replaced by the cesium cation. Structures were then optimized at the B3LYP/LanL2DZ level where the final energetic and relative stabilities were obtained (Tables 3, 4 and 5).

On the basis of the presented data, one can presume that in both, the stable conformation of IleCs⁺ and the more stable Ile-Cs-IleOMe⁺ dimer, Ile is in the ZW form. Dissociation of that dimer will yield the ZW form of IleCs⁺ as the stable one in agreement with predictions of being the more stable form of monomer. The theoretical result is also in accordance with the kinetic method experimental result.¹⁹ The relative energies again showed the IleCs⁺ ZW form to be disfavored but without reversal in energy ordering because of great energy differences between the CS and ZW forms. Additional stabilization of the CS dimer is probably a result of a better coordination/solvation of cesium cation by two aliphatic side chains (instead of one in ZW dimer). According to the previous^{1,19} and present 'tests' of kinetic

method reliability, it seems that complexation of cationized amino acids into ion-bound dimers favors the CS structure by ~ 8 kJ mol⁻¹.

CONCLUSIONS

DFT study of LeuNa⁺ and IleNa⁺ indicated a relationship between decrease in energy difference of ZW and CS forms and aliphatic side chain length. Results for IleNa⁺ showed energy degeneracy of CS and ZW conformers, which is consistent with predictions based on correlation between sodium ion and proton affinities and ZW stability limit. On the other hand, the energy degeneracy for CS and ZW conformers of IleNa⁺ is not consistent with kinetic method results. Additional calculations on the relative stability of Na⁺-bound dimers of Ile and IleOMe showed discrepancy between relative stabilities of naturally occurring gas-phase CS and ZW forms and those produced by dissociation of Ile-Na-IleOMe⁺.

Further study of LeuCs⁺ and IleCs⁺ showed ZW forms to be the more stable ones in agreement with relative stabilities of conformers obtained from simulated dissociation of Cs⁺-bound dimers of Ile and IleOMe. These results are in agreement with kinetic method experiments.

Finally, the most interesting conclusions are that complexation of cationized amino acids into ion-bound dimers disfavors ZW structure by ~ 8 kJ mol⁻¹ and that for conformers of sodium-cationized amino acids which are close in energy the kinetic method may not be reliable for structural determinations.

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