Available online at www.sciencedirect.com
ScienceDirect
International Journal of Mass Spectrometry 255–256 (2006) 301–311
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Investigations of the clustering reactions of protonated amino acid esters by high pressure mass spectrometry and quantum chemical calculations

A. Simon, T.B. McMahon *

Department of Chemistry, University of Waterloo, Waterloo, Ont., Canada N2L3G1

Received 30 January 2006; received in revised form 1 May 2006; accepted 2 May 2006
Available online 27 June 2006

Abstract

The equilibria involved in the formation of the proton-bound dimers of the three simplest amino acid methyl esters (glycine, alanine and valine methyl esters) were characterized by means of pulsed ionization high pressure mass spectrometry (PHPMS) experiments and density functional theory (DFT) calculations. Our results would indicate that, within the temperature range employed in these experiments, the most stable proton-bound dimer conformers are formed in the case of glycine and alanine whereas a more entropically favoured isomer would dominate in the case of valine. Various possible isomers of each of the proton-bound dimer species have been investigated computationally each exhibiting a combination of inter- and intramolecular hydrogen bonding. A system of nomenclature for these various species is proposed. The possibility of structures exhibiting 'salt-bridge' interactions have also been explored, recognizing that such structures would necessarily result from highly energetic structural rearrangements.

Keywords: Amino acid ester; Clustering; High pressure mass spectrometry; Quantum chemical calculation; Structure and energetics; Thermochemistry

1. Introduction

The study of biomolecules in the gas phase has become of increasing interest in the past few years since the absence of solvent allows for insights to be gained into the factors influencing shapes, conformations, dynamics and functions of biomolecules and their intermolecular interactions [1]. Such studies have been made possible by the development of new ionization methods, most notably electrospray ionisation (ESI) [2] and matrix assisted laser desoprtion ionization (MALDI) [3], and new instrumentation techniques in mass spectrometry [4]. The large variety of combined techniques has thus allowed a significant amount of kinetic, thermochemical and structural data for biologically relevant compounds to be obtained during the last decade.

Intermolecular interactions within biological systems have been of particular interest. Two approaches for their investigation can be distinguished: (i) the 'top-down' approach, which involves working directly on large biological macromolecules, such as studies of protein–ligand interactions [5–7], for example; and (ii) the 'bottom-up' approach which involves the study of the properties of small systems which constitute real biological species in such a way as to permit their thermochemical and structural properties to model the macromolecular systems of which they are constituents. Meot-Ner pioneered this area in the early 1970s [8] in his initial studies of solvation and clustering reactions of protonated amino acids in the gas phase using the high pressure mass spectrometry technique in which he demonstrated their relevance in understanding interactions in biological systems. In the 1980s, he studied further the thermochemistry of intermolecular hydrogen bonds for model compounds of biological interest performing proton-transfer equilibria and clustering reaction experiments, [9,10] the acidities of biological hydrogen donors [11] and the solvation of ionic species in a protein-like environment [12].

More generally, the ion-neutral interactions involving amino acids have raised considerable interest. For example, the solvation of metallic cations by amino acids has been studied by both qualitative [13–17] and quantitative [18,19] collision induced dissociation experiments, as well as by accurate thermochemical calculations [20–23]. The solvation of protonated amino acids has also been studied by blackbody infrared radiative dissocia-

* Corresponding author. Tel.: +1 519 888 4591; fax: +1 519 746 0435.
E-mail address: mcmahon@uwaterloo.ca (T.B. McMahon).

1387-3806/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2006.05.001

tion (BIRD) experiments [24,25]. For example, this technique has also been applied to the investigation of the dissociation of a few amino acid proton-bound dimers, [26–29] however no high pressure mass spectrometry experiments have been performed on these systems since the work of Meot-Ner; nor have any extensive theoretical studies been performed.

The pulsed high pressure mass spectrometry (PHPMS) technique has played an extremely important role in gas phase ion thermochemistry in many ways. For example, it has contributed significantly to the construction of thermochemical ladders such as those used to obtain proton affinities and gas phase basicities [30,31]. Moreover, it is also well known to be a powerful tool to study ion-molecule association reactions, [30] providing valuable insights into the thermochemistry of solvation of ionic species [32–34] and allowing for thermochemical characterization of proton-bound dimers of small organic molecules [30,35]. Very few studies involving amino acids have been carried out to date by HPMS owing to their relatively low volatility [9,36]. In order to partially overcome this difficulty, methyl esters of amino acids have been employed as model systems.

In the present study, thermochemical data obtained from high pressure mass spectrometry equilibrium experiments for proton-bound dimers of the methylesters of the three simplest amino acids, glycine, alanine and valine, are presented. All experimental data were complemented by calculations at the density functional theory (DFT) level of theory, which provide valuable insights into the structures of the adducts.

2. Experiment

All experiments were carried out on a pulsed ionization high pressure mass spectrometer (PHPMS) built at the University of Waterloo, which has been described in detail previously [37]. Gas mixtures were prepared in a temperature-controlled 5 L stainless steel reservoir using methane as the bath gas at a pressure of 800–1000 Torr with 0.1–1% of added reactive species of interest. Sufficient mixing times were allowed to permit a homogeneous mixture to be obtained in thermal equilibrium with the gas handling system. This gas mixture was the bled into the ion source through a heated inlet line to a pressure of 5–6 Torr. Ionization was accomplished by a 10–500 μs pulse of 2 keV electrons focused into the ion source through a 200 μm aperture. Mass-selected ion temporal profiles were monitored using a PC-based multichannel scaler data acquisition system configured at 25–50 μs dwell time per channel, depending upon the persistence of the ion temporal profiles. A total of 250 channels were acquired using a duty cycle 10 ms longer than the residence time for the most persistent ion, which prevents pulse-to-pulse carry over in ion abundance. The results of 1000–2000 electron gun pulses were accumulated, dependent on the signal intensity. Such experiments are frequently particularly problematic because, as previously noted by Meot-Ner in his work on CH_3CO–Ala–OCH_3, [11] low volatility compounds may become absorbed on the walls of the gas handling system as well as the inlet to the ion source. In order to circumvent this problem, gas mixtures were flowed for a minimum of 1 h before data acquisition began in order to ensure that the sample concentrations in the ion source attained their nominal values.

The PHPMS technique permits the measurement of the equilibrium constant K_{exp} for an ion-molecule reaction as a function of temperature. This ion-molecule reaction might be, for example, either a proton exchange reaction, Eq. (1), or an association reaction, Eq. (2). From a knowledge of the partial pressures of the neutral species A and/or B, and the equilibrium ion intensity ratio, a Van't Hoff plot of $\ln(K_{exp})$ versus $1/T$ can be constructed from which the enthalpy and entropy changes may be determined from the slope and intercept, respectively, Eq. (3).

$$AH^+ + B \leftrightarrows A + BH^+ \quad (1)$$

$$AH^+ + B \leftrightarrows AHB^+ \quad (2)$$

$$\ln(K_{eq}) = -\frac{\Delta_r H^0}{RT} + \frac{\Delta_r S^0}{R} \quad (3)$$

In the case of ion-molecule association reactions, the comparison of the experimental $\Delta_r H^0$ and $\Delta_r S^0$ values with those obtained from *ab initio* calculations can give interesting insights into the structures of the adducts [35]. This approach has been employed to examine the equilibria of formation of the proton-bound dimers of the amino acid methyl esters H_2N–CH(R)–$COOCH_3$ (AaMe) with R=H (glycine (GlyMe)), R=CH_3 (alanine (AlaMe)) and R=$CH(CH_3)_2$ (valine (ValMe)), Eq. (4).

$$AaMeH^+ + AaMe \leftrightarrows (AaMe)_2H^+ \quad (4)$$

The methyl esters of the amino acids were obtained by base treatment of the corresponding commercial hydrochloride salts followed by extraction into methylene chloride and evaporation to dryness.

3. Calculations

All electronic structure calculations were performed using the hybrid Hartree-Fock/DFT method B3LYP [38] implemented in the Gaussian 98 series of programs [39]. At the outset of these calculations, it was noted that the computational data determined for the enthalpy changes for the formation of proton-bound dimers of the amino acid esters were very sensitive to the size of basis set and level of calculation employed. In order to determine what level of calculation, at the least computational cost, might best reproduce energetics for the formation of proton-bound dimers of amine species, a series of test calculations was performed on the energetics of formation of the proton-bound dimer of ammonia and one form of the proton-bound dimer of the methyl ester of glycine. The results of those calculations are summarized in Table 1 for both the key hydrogen bond distances and the energetics of proton-bound dimer formation. As can be seen from these data, the strong hydrogen bonds formed between a protonated amine and its neutral counterpart are not symmetric at any of the levels of theory employed. One of the N–H bond distances is consistently considerably shorter than the other in the N–$H^+\cdots$N hydrogen bond and these distances do not change appreciably as the level of

Table 1
Enthalpies and entropies of formation of the proton-bound dimers of ammonia $(NH_3)_2H^+$ and glycine methyl ester $(GlyMe)_2H^+$ calculated using the structures optimized at the B3LYP level of theory with three different basis sets

	6-31G(d)		6-31G(d,p)		6-31 + G(d,p)		6-311 + G(2d,2p)//6-31 + G(d,p)	
	$(NH_3)_2H^+$	$(GlyMe)_2H^+$)	$(NH_3)_2H^+$	$(GlyMe)_2H^+$)	$(NH_3)_2H^+$	$(GlyMe)_2H^+$)	$(NH_3)_2H^+$	$(GlyMe)_2H^+$)
N–H$^+$ (Å)	1.15	1.08	1.19	1.09	1.15	1.09		
H–N (Å)	1.52	1.70	1.46	1.67	1.51	1.68		
ΔH^0 (kcal mol^{-1})	−33.1	−31.4	−33.8	−31.5	−28.9	−28.0	−26.8	−26.2
ΔS^0 (cal mol^{-1} K^{-1})	30.4	−39.9	−29.2	−40.0	−29.4	−38.2		

The isomer DiGly3 was used for the calibration of $(GlyMe_2)H^+$.

calculation is changed. For example, these two unequal bond distances are found to be 1.15 Å versus 1.51 Å in $(NH_3)_2H^+$ and 1.09 Å versus 1.68 Å for $(GlyMe)_2H^+$ in the geometries optimized at the 6-31+G(d,p) basis set level. As can be seen from the data in Table 1, these distances do not change appreciably as the level of calculation is changed but it is noteworthy that for $(NH_3)_2H^+$, as p polarization orbitals are added, the two N–H distances become less different, even though the computational thermochemical values are essentially unaffected. However, as diffuse functions are added the distances revert effectively to the 6-31G(d) values. In contrast, the enthalpy changes associated with formation of the intermolecular, strong hydrogen bond vary significantly with the level of theory with the best agreement between computation and experiment [46] being obtained for the ammonia proton-bound dimer with single point energy calculations using the 6-311 + G(2d,2p) basis set on geometries optimized with a 6-31 + G(d,p) basis set. Accordingly, all equilibrium geometries were optimized at the B3LYP level of theory using the 6-31 + G(d,p) basis set. Frequency calculations based on the harmonic oscillator approximation were computed at the B3LYP/6-31 + G(d,p) level using these B3LYP/6-31 + G(d,p) optimized geometries. Single point calculations at the B3LYP/6-311 + G(2d,2p) level using the B3LYP/6-31 + G(d,p) optimized geometries were then carried out to obtain improved accuracy for thermochemical quantities.

4. Results and discussion

The experimental Van't Hoff plots for the association reactions of $AaMeH^+$ with AaMe (Aa=Gly, Ala and Val) are reported in Fig. 1. The thermochemical data derived from those Van't Hoff plots are summarized in Table 2. The enthalpy changes for formation of the protonated dimers range from −27.1 kcal mol^{-1} for Gly to −20.8 kcal mol^{-1} for Val. While these values fall within the range of what might normally be expected for typical intermolecular bond strengths measured for proton-bound homodimers of simple amines ($\Delta H^0 \approx -23$ kcal mol^{-1}), [40] it is unusual that there should be such a large difference in binding energetics between molecules within a homologous series. For these three amino acid esters the intermolecular bond strength decreases as the size of the alkyl side chain increases with a decrease of 1.2 kcal mol^{-1} from Gly to Ala, and a further drop of 4.9 kcal mol^{-1} from Ala to Val. Simultaneously the measured entropy changes range from −33.0 cal mol^{-1} K^{-1} for Gly to −23.4 cal mol^{-1} K^{-1} for Val. The entropy change observed for Ala of −31.4 cal mol^{-1} K^{-1} is similar to that found for Gly however. The more favorable entropy change of 10 cal mol^{-1} K^{-1} observed for Val relative to Gly, combined with the 6.1 kcal mol^{-1} less favorable enthalpy change for formation of the larger protonated homodimer, thus strongly suggest that two different structural motifs might be exhibited in these species. In particular, the data for Val would indicate that a more loosely bound but entropically less restricted dimer may be formed.

The thermochemical data obtained in this work for the association reactions leading to protonated dimer formation are near the expected order of magnitude for formation of proton-bound

Table 2
Enthalpy and entropy changes of the association reactions $AaMeH^+ + AaMe \leftrightarrows (AaMe)_2H^+$ obtained from PHPMS experiments for Aa=Gly, Ala and Val

Aa	$AaMe + AaMeH^+ \leftrightarrows (AaMe)_2H^+$	
	ΔH^0 (kcal mol^{-1})	ΔS^0 (cal mol^{-1} K^{-1})
Gly	−27.1	−33.0
Ala	−25.7	−31.4
Val	−20.8	−23.4

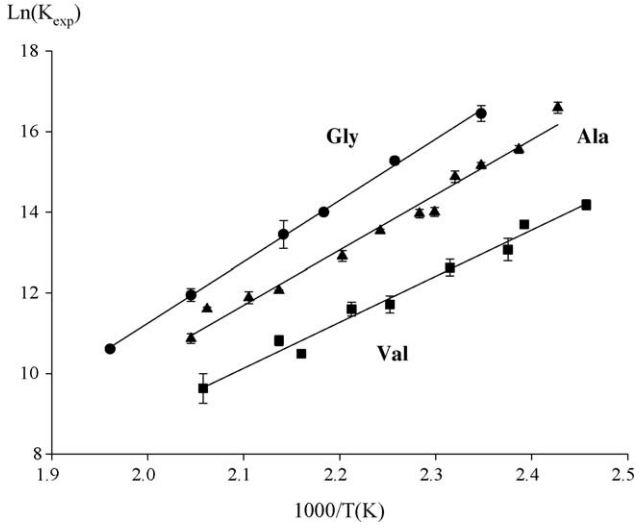


Fig. 1. Van't Hoff plots for the association reactions of protonated amino acid esters ($AaMeH^+$) with the corresponding neutral molecules (AaMe) to give proton-bound dimers ($(AaMe)_2H^+$). The experimental points (circles for Aa=Gly, triangles for Aa=Ala and squares for Aa=Val) are fitted by linear regressions represented by solid lines.

dimers of alkyl amines and might also seem to present a logical evolution with the size of the alky side chain. That is, as the alkyl group size increases, possible steric hindrance and restrictions of internal rotations of the alkyl groups lead to a weakening, and lengthening, of the intermolecular bond to relieve these effects. However, previous studies of clustering reactions of protonated amino acids by PHPMS were carried out by Meot-Ner in the early 1980s [36] and the enthalpy and entropy changes of the association reactions leading to the proton-bound dimers of glycine and proline yielded values of ΔH^0 of −31 and -29 ± 2 kcal mol^{-1} and of ΔS^0 of −33 and -32 ± 5 cal mol^{-1} K^{-1} [36]. Surprisingly, only small differences were observed between these two amino acid systems, even though proline has a rigid cyclic side chain which binds also to the amine nitrogen giving rise to a secondary amine.

Binding energies for the proton-bound dimers of glycine and alanine were also deduced from blackbody infrared radiative dissociation experiments with the aid of master equation modeling by Williams and co-workers [29]. A value of 1.15 ± 0.05 eV (26.5 ± 1.2 kcal mol^{-1}) was obtained for each of these amino acids. In a separate BIRD study, these authors also determined that the binding energy for the proton-bound dimer of the methyl ester of glycine was similar to that for the proton-bound dimer of glycine itself (1.10–1.15 ± 0.05 eV (25.3–26.5 ± 1.2 kcal mol^{-1})) [28]. From the similarity of the data for glycine and glycine methyl ester the authors concluded that salt-bridge structures were not formed in these association reactions.

Most recently, the association reaction of protonated glycine with glycine has been examined in our own laboratory using PHPMS experiments [41] and values of ΔH^0 of -27.7 ± 1 kcal mol^{-1} and of ΔS^0 of -33 ± 5 cal mol^{-1} K^{-1} were obtained. These data are in excellent agreement with those obtained by Williams and co-workers using the BIRD technique [29]. Similarly, the data reported in the present study for glycine methyl ester can be seen to be in excellent accord with the BIRD data [28]. Also significantly, the values obtained are much closer to those expected for proton-bound dimers of alkyl amines.

It is of interest to note that, in agreement with the BIRD experiments, the two PHPMS experiments from our laboratory lead to an only slightly lower value for the enthalpy change for formation of the proton-bound dimer of the methylester of glycine (−27.1 kcal mol^{-1}) relative to that obtained for formation of the proton-bound dimer of glycine itself (−27.7 kcal mol^{-1}). This slight difference will be discussed below in light of the computational data.

Although a considerable amount of theoretical work has been conducted over the last 30 years to improve the description of intermolecular hydrogen bonds, [43] relatively fewer efforts have been directed toward the study of cationic hydrogen bonds [42,44]. In particular, relatively few attempts have been made to elucidate the bonding in adducts involving protonated amino acids and their derivatives. An important exception to this has been the work of Williams and co-workers who carried out a computational analysis of proton-bound dimer structures of amino acids as an aid to the interpretation of the results of their BIRD experiments [45].

In order to probe the energetics of interaction in the amino acid methyl ester proton-bound dimers computationally, it is necessary to obtain the energetics of the individual neutral (AaMe), protonated monomer ((AaMe)H$^+$) and proton-bound dimer ((AaMe)$_2$H$^+$) species. The calculated energetics are based, in the first instance, on the most stable structures found for each species. All geometry optimizations were performed at the B3LYP/6-31 + G(d,p) level of theory. The final geometries of the most stable conformers for the neutral amino acid esters (GlyMe, AlaMe and ValMe), protonated monomers (GlyMeH$^+$, AlaMeH$^+$ and ValMeH$^+$), and proton-bound dimers (DiGly1, DiAla1 and DiVal1), are reported in Fig. 2. The calculated enthalpy and entropy changes for the association reactions involving the most stable forms of the neutral and protonated amino acid esters to give these most stable protonated dimers are summarized in Table 3. The thermochemical data obtained from the PHPMS experiments are also given in this Table for comparison purposes.

The enthalpy changes calculated for the association reactions leading to protonated dimer formation at the B3LYP/6-311 + G(2d,2p)//B3LYP/6-31 + G(d,p) level are in excellent agreement with the experimental data in the cases of glycine and alanine (Table 3). The enthalpy change for the protonated dimer of the methyl ester of glycine was taken to be −26.7 kcal mol^{-1} based on the formation of the most stable protonated dimer conformer (DiGly1) is in excellent agreement with the experimental value of −27.1 kcal mol^{-1} from the PHPMS data, with a difference between theory and experiment of only 0.4 kcal mol^{-1}. The comparison between the values of the enthalpies at the B3LYP/6-31 + G(d,p) and at the B3LYP/6-311 + G(2d,2p)//B3LYP/6-31 + G(d,p) levels of theory shows that increasing the basis set size decreases the enthalpy change by 1.3–1.6 kcal mol^{-1} (Table 3), leading to an improvement in the match between experimental and theoretical results. This mirrors the behavior described above for calculations of the

Table 3
Enthalpy and entropy changes of the association reactions AaMeH$^+$ + AaMe ⇆ (AaMe)$_2$H$^+$ calculated at the B3LYP/6-31 + G(d,p) level of theory

Adduct	Theoretical		Experimental	
	ΔH^0 (kcal mol^{-1})	ΔS^0 (cal mol^{-1} K^{-1})	ΔH^0 (kcal mol^{-1})	ΔS^0 (mol^{-1} K^{-1})
DiGly1	−28.2 (**−26.7**)	−32.6	−27.1	−33.0
DiAla1	−27.5 (**−25.9**)	−36.3	−25.7	−31.4
DiVal1	−26.5 (**−25.2**)	−34.8	−20.8	−23.4

Single point calculations were performed at the B3LYP/6-311 + G(2d,2p)//B3LYP/6-31 + G(d,p) to improve the energetics (given in bold). The corresponding experimental values are reported in the last two columns for comparison.

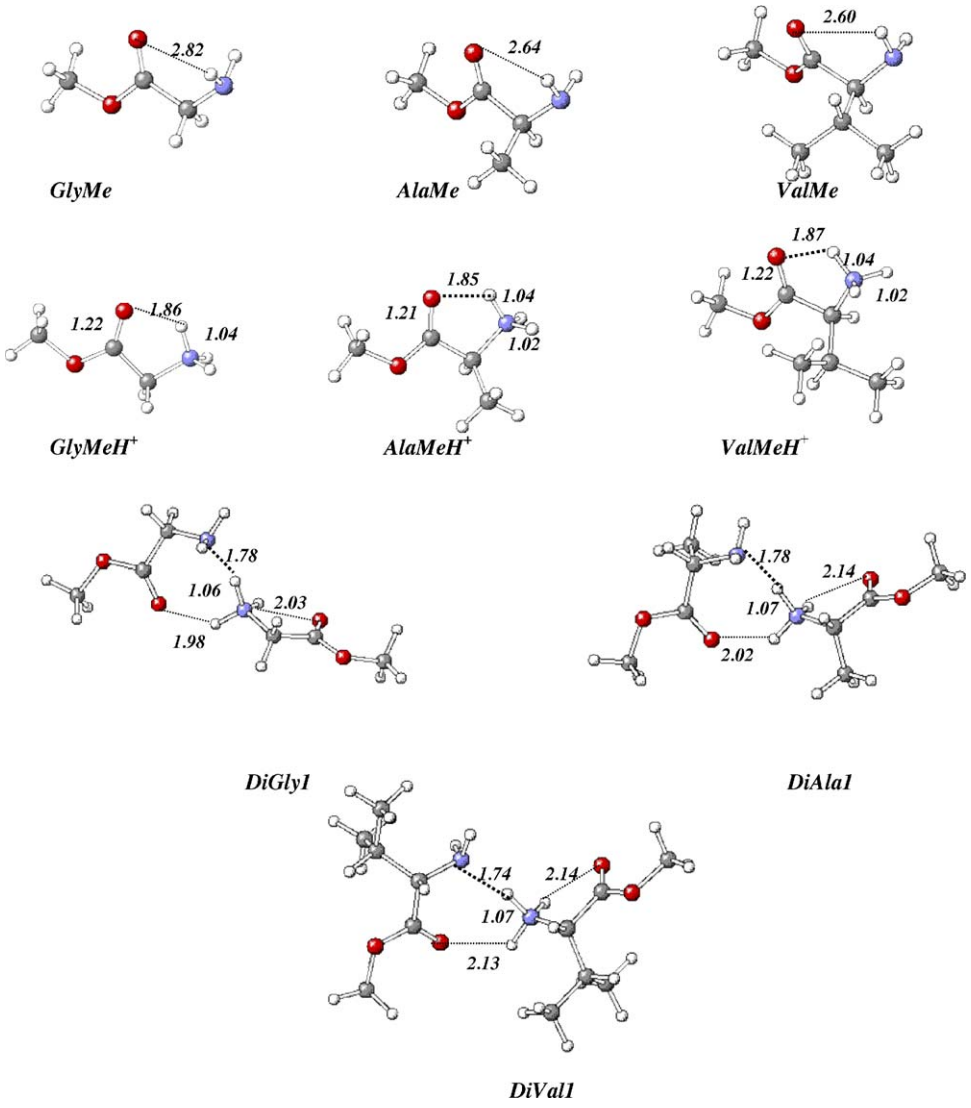


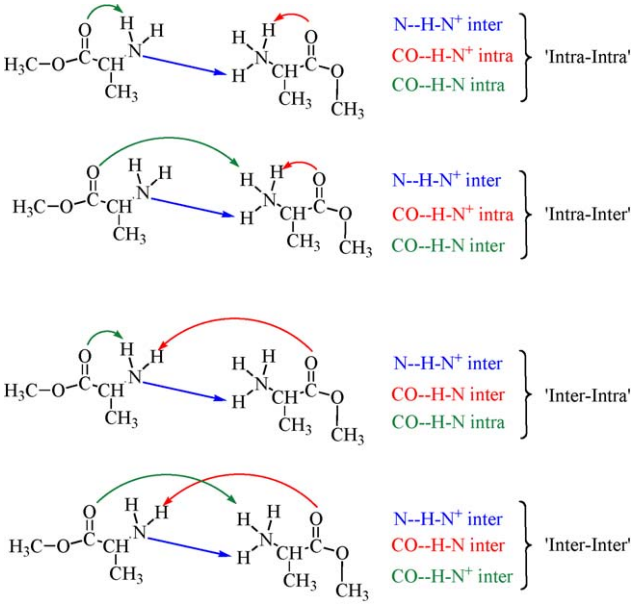
Fig. 2. Most stable conformers for the monomers, protonated monomers and proton-bound dimers of the methylesters of glycine (GlyMe, $GlyMeH^+$ and DiGly1), alanine (AlaMe, $AlaMeH^+$ and DiAla1), valine (ValMe, $ValMeH^+$ and DiVal1), optimized at the B3LYP/6-31 + G(d,p) level of theory.

energetics of proton-bound dimer formation in NH_3 which led to the choice of basis set and level of theory for this work. The effects of the basis set on the energetics will be discussed below. The agreement between the calculated and experimental entropy changes was found to be very good also with a difference of only 0.4 cal mol^{-1} K^{-1} in the case of the proton-bound dimer of glycine where a value of −32.6 cal mol^{-1} K^{-1} at the B3LYP/6-31 + G(d,p) level of theory is found versus −33.0 cal mol^{-1} K^{-1} from PHPMS experiments. In the case of the methylester of alanine however, a difference of 4.9 cal mol^{-1} K^{-1} was found between calculated and experimental entropy change values (−36.3 and −31.4 cal mol^{-1} K^{-1}, respectively). The increase of the discrepancy between the experimental and calculated entropy values when the alkyl side chain increases in size from H (glycine) to CH_3 (alanine) might be explained by the involvement of a hindered rotation of the CH_3 group in the latter case since it is well known that *ab initio* calculations do not adequately consider restrictions of internal rotations due to the harmonic approximation and the assumption that vibrational and rotational modes are not coupled [35]. Although the comparison between the calculated and experimental thermodynamic values indicate that the most stable proton-bound dimer conformers of glycine and alanine methyl esters would form, this does not seem to be the case for the methylester of valine (Table 3). The formation of the most stable proton-bound dimer conformer DiVal1 was found to be exothermic by −25.2 kcal mol^{-1} with respect to the reactants $ValMeH^+$ and ValMe at the B3LYP/6-311 + G(2d,2p)//B3LYP/6-31 + G(d,p) level of theory while the enthalpy change for this association reaction obtained from the PHPMS experiments was considerably less favorable at −20.8 kcal mol^{-1} (Table 3). Similarly, the entropy change calculated at the B3LYP/6-31 + G(d,p) level of theory of −34.8 cal mol^{-1} K^{-1} was not in good agreement with the value obtained from the PHPMS experiments of −23.4 cal mol^{-1} K^{-1}. Such pronounced differences between calculations and experiments are not expected and this led to the conjecture that the enthalpically most favorable isomer might not necessarily be the only form of the proton-bound dimer of

valine methyl ester present in the temperature range employed. As shown previously, at high ion source temperatures, the formation of less strongly bound, but entropically more favorable, isomers can be favored.

As noted above, the calculated energetics presented in Table 3 are based upon the most stable structures for each of the AaMe, $AaMeH^+$ and $(AaMe)_2H^+$ species shown in Fig. 2. These were, by no means, the only possible stable structures found for these species. For the monomers, a conformational search was conducted in which rotation about the $C_{\alpha'}$-C_β axis was performed. In the case of the protonated monomers, each of the most stable structures presents a similar intramolecular N–$H^+\cdots$O=C stabilizing interaction involving the carbonyl oxygen, resulting in a lengthening of the N–H bond by 0.02 Å (1.04 Å versus 1.02 Å in the neutral). Similarly, all of the most stable proton-bound dimers DiGly1, DiAla1 and DiVal1 were found by a systematic search considering all different possible conformers with either N–$H^+\cdots$N or N–$H^+\cdots$O=C as the dominant interaction. In addition, a variety of associated secondary hydrogen bond interactions were considered. As expected, all of the most stable dimer structures found exhibit analogous conformations in which the N–$H^+\cdots$N hydrogen bond is the primary interaction, linking an acidic ammonium ion proton to the most basic, amine, site of the neutral moiety. All possible secondary N–H$\cdots$O=C intra- and intermolecular interactions have also been considered and these are depicted in Scheme 1. Depending upon the nature of these interactions, the various conformers are denoted inter–inter, intra–inter, inter–intra or intra–intra as shown in Scheme 1.

The first 'Inter-' or 'Intra-' of each pair indicates whether the interaction between the carbonyl oxygen of the protonated monomer takes place with a hydrogen of either the protonated amino group (intra-) or a hydrogen of amino group of the neutral monomer moiety (inter-). The second '-Inter' or '-Intra' of each pair designates whether the interaction between the carbonyl oxygen of the neutral monomer takes place with either a hydrogen of the amino group of the monomer moiety (-intra) or a hydrogen located on the protonated amino group (-inter). Thus, each of the most stable proton-bound dimers, DiGly1, DiAla1 and DiVal1, exhibits an interaction of the intra–inter type with three hydrogen bonds evident involving the ammonium ion protons. In order of increasing strength of interaction, one of these interacts with the amine nitrogen lone air of the neutral moiety, another with the carbonyl oxygen lone pair of the neutral moiety while the third maintains an, albeit weaker, stabilizing intramolecular N–$H^+\cdots$O interaction with the carbonyl oxygen lone pair of the protonated moiety. The primary hydrogen bonding interaction is the N–$H^+\cdots$N bond with intermolecular bond distances of 1.78 Å in both DG1 and DAla1 and 1.74 Å in DVal1. As noted above in the discussion of choice of a computational method, it is noteworthy that even though these species are homodimers, they are not symmetric [42] with covalent N–H^+ bond lengths of 1.06 Å for DiGly1 and 1.07 Å for both DiAla1 and DiVal1. Within this series, the intermolecular N–$H^+\cdots$O interaction exhibits somewhat of an increase from 2.03 Å for DiGly1 to 2.14 Å for both DiAla1 and DiVal1. This is similarly the case for the intramolecular N–$H^+\cdots$O interaction where the distances are 1.98, 2.02, and 2.13 Å for the proton-bound dimers of glycine, alanine and valine methylesters, respectively. These interactions, designated by Meot-Ner as 'secondary interactions', have been shown to play an important role in the additional stabilization of proton-bound dimers when the monomer presents more than one basic site [9]. In the cases of DiGly1, DiAla1 and DiVal1, the lengthening of this intermolecular hydrogen bond distance reflects a weakening of the secondary interaction, which is consistent with a possible increase in steric hindrance, leading to the slightly decreasing dimer bond strengths within this homologous series of esters (Table 3).



Scheme 1. Conformations investigated and proposed nomenclature.

While the computational thermochemical data above pertain to the most stable structures obtained for both reactants and products, it is, however, to be expected that, even for such small size biological systems, there may be a large number of conformers of relatively low lying energy, especially in the case of the dimer species. This was confirmed during the series of calculations that were performed to search for the most stable conformers of the proton-bound dimers of the methylesters of glycine, alanine and valine. The final minima resulting from these optimizations are reported in Figs. 3–5 for the proton-bound dimers of glycine, alanine and valine methyl esters, respectively. The relative enthalpies and entropies of all local minima with respect to the neutral and protonated monomers AaMe and $AaMeH^+$ are also reported in Tables 4–6 for Gly, Ala and Val, respectively. Conformers DiGly1–DiGly6 and DAla1–DAla6 all contain the primary N–$H^+\cdots$N interaction as the strongest bonding mode and differ from one another only in the nature of the secondary N–$H^+\cdots$O interactions. Isomers DiGly1–DiGly5 all lie within 0.5 kcal mol^{-1} in enthalpy, with the calculated entropy changes with respect to monomer and protonated monomer ranging from −31.9 to −38.2 cal mol^{-1} K^{-1}. It should be noted however that, for the reasons noted above, these relative entropy changes must be considered to be quite approximate. On the basis of

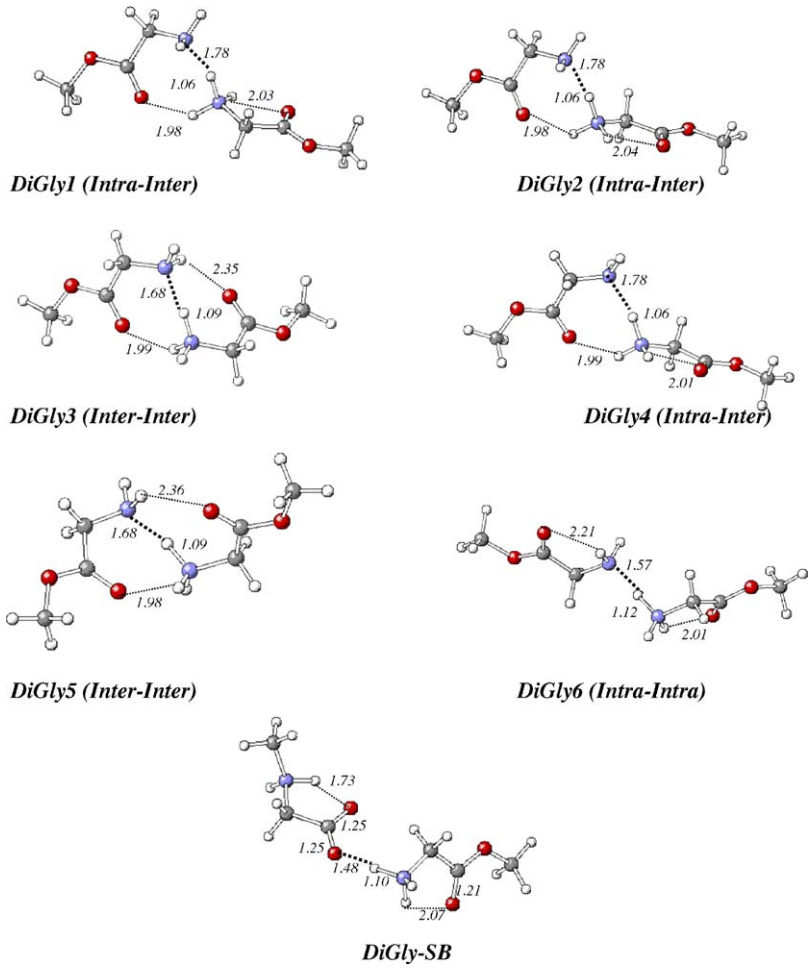


Fig. 3. Most stable conformers for the proton-bound dimers of the methylester of glycine optimized at the B3LYP/6-31 + G(d,p) level of theory.

these calculated energetics, it is not possible to exclude the potential presence of any of these species at the conditions of temperature and pressure found in the high pressure ion source. Conformer DiGly6, which does not exhibit any secondary intermolecular interaction (intra–intra isomer), is higher in energy by 5.1 kcal mol^{-1} with respect to DiGly1. This thus demonstrates the relative importance of this secondary N–H$^+\cdots$O interaction in the stability of such complex proton-bound dimers, as previously suggested by Meot-Ner [9]. The comparison between the values of the enthalpies at the B3LYP/6-31 + G(d,p) and at the B3LYP/6-311 + G(2d,2p)//B3LYP/6-31 + G(d,p) levels of theory shows that increasing the basis set has a large influence

Table 4
Relative enthalpies and entropies of the most stable conformers of the proton-bound dimers $(GlyMe)_2H^+$ calculated at the B3LYP/6-31 + G(d,p) level of theory

		GlyMe + GlyMeH$^+$ ⇆ $(GlyMe)_2H^+$	
		ΔH^0 (kcal mol^{-1})	ΔS^0 (cal mol^{-1} K^{-1})
Exp.		−27.1	−33.0
Calc.	DiGly1	−28.2 (**−26.7**)	−32.6
	DiGly2	−28.1 (**−26.6**)	−35.5
	DiGly3	−28.0 (**−26.2**)	−38.2
	DiGly4	−28.2 (**−26.7**)	−31.9
	DiGly5	−28.0 (**−26.2**)	−38.2
	DiGly6	−23.0 (**−21.6**)	−30.6
	DiGly-SB	−32.7 (**−31.6**)	−38.7

Single point calculations were performed at the B3LYP/6-311 + G(2d,2p)//B3LYP/6-31 + G(d,p) to improve the energetics (given in bold). The experimental values are presented in the first row for comparison.

Table 5
Relative enthalpies and entropies of the most stable conformers of the proton-bound dimers $(AlaMe)_2H^+$ calculated at the B3LYP/6-31 + G(d,p) level of theory

		AlaMe + AlaMeH$^+$ ⇆ $(AlaMe)_2H^+$	
		ΔH^0 (kcal mol^{-1})	ΔS^0 (cal mol^{-1} K^{-1})
Exp.		−25.7	−31.4
Calc.	DiAla1	−27.5 (**−26.3**)	−36.3
	DiAla2	−26.3 (**−25.2**)	−35.8
	DiAla3	−26.7 (**−24.9**)	−36.7
	DiAla4	−26.0 (**−24.7**)	−36.7
	DiAla5	−27.0 (**−25.8**)	−34.6
	DiAla6	−27.0 (**−25.3**)	−38.7
	DiAla7	−22.0 (**20.6**)	−32.6
	DiAla-SB	−32.8 (**−31.8**)	−38.1

Single point calculations were performed at the B3LYP/6-311 + G(2d,2p)//B3LYP/6-31 + G(d,p) to improve the energetics (given in bold). The experimental values are presented in the first row for comparison.

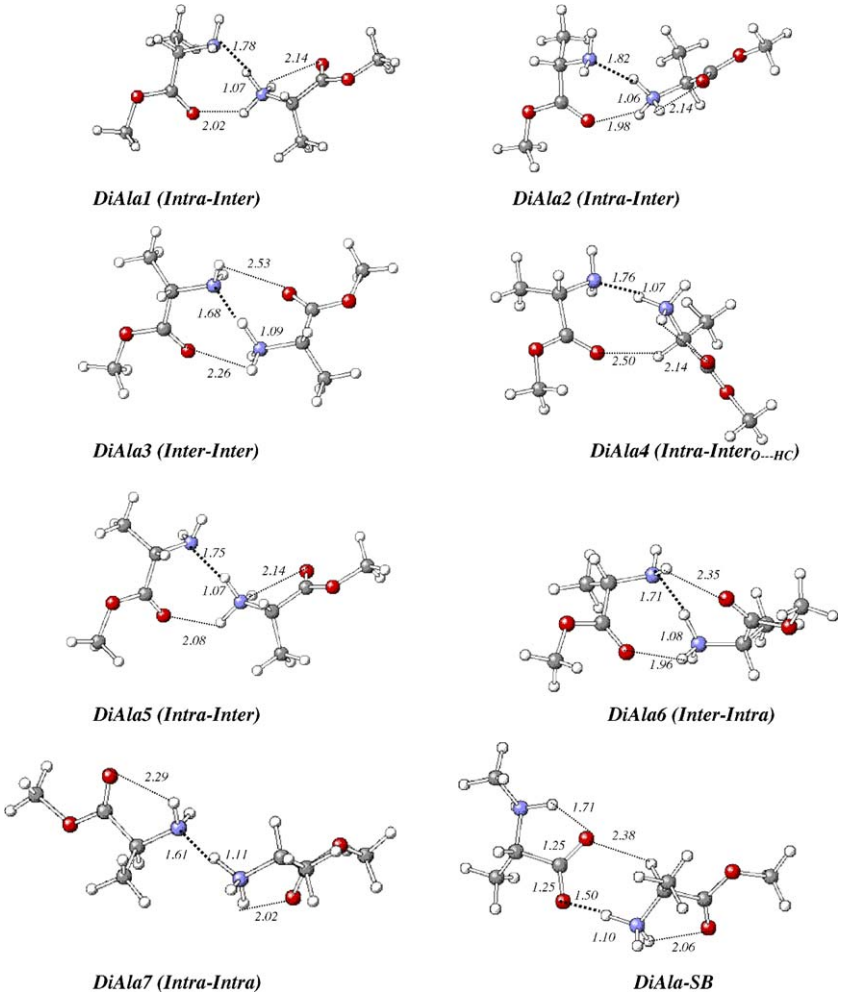


Fig. 4. Most stable conformers for the proton-bound dimers of the methylester of alanine optimized at the B3LYP/6-31 + G(d,p) level of theory.

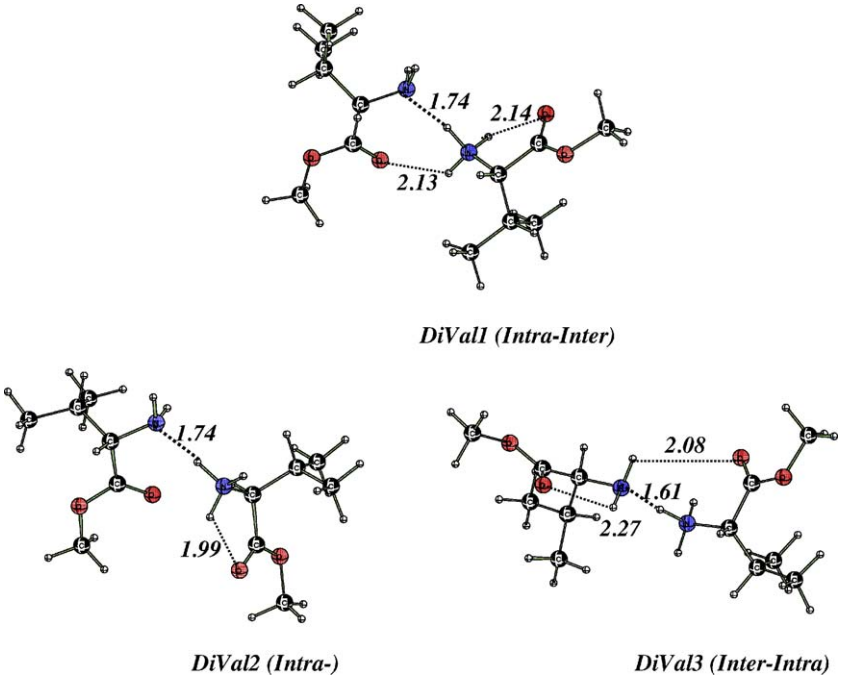


Fig. 5. Most stable conformers for the proton-bound dimers of the methylester of valine optimized at the B3LYP/6-31 + G(d,p) level of theory.

Table 6
Relative enthalpies and entropies of the most stable conformers of the proton-bound dimers $(ValMe)_2H^+$ calculated at the B3LYP/6-31 + G(d,p) level of theory

		$ValMe + ValMeH^+ \leftrightarrows (ValMe)_2H^+$	
		ΔH^0 (kcal mol^{-1})	ΔS^0 (cal mol^{-1} K^{-1})
Exp.		−20.8	−23.4
Calc.	DiVal1	−26.5 (**−25.2**)	−34.8
	DiVal2	−22.9 (**−21.8**)	−35.7
	DiVal3	−19.6 (**−18.4**)	−34.2

Single point calculations were performed at the B3LYP/6-311 + G(2d,2p)// B3LYP/6-31 + G(d,p) to improve the energetics (given in bold). The experimental values are presented in the first row for comparison.

on the energetics with an increase in basis set size leading to a decrease in the enthalpy change by 1.2–1.5 kcal mol^{-1}, which also corresponds to an improvement in the agreement between experimental and theoretical results.

The same approach was used to calculate the most stable conformers of the proton-bound dimer of the methyl ester of alanine. The optimized geometries are reported in Fig. 4 and the corresponding energetics in Table 5. As can be seen from the data in Table 5, the six isomers DiAla1–DiAla6 all lie within 1.4 kcal mol^{-1} of the most stable structure. As well, with the same caveat as noted above, the relative calculated entropy changes with respect to the monomer and protonated monomer of alanine methyl ester range from −34.6 cal mol^{-1} K^{-1} (DiAla5) to −38.7 cal mol^{-1} K^{-1} (DiAla6). The most entropically favorable isomer, DiAla7, with an associated entropy change of −32.6 cal mol^{-1} K^{-1} is 5.3 kcal mol^{-1} higher in energy than DiAla1 at the B3LYP/6-31 + G(d,p) level of theory and 5.5 kcal mol^{-1} higher in energy at the B3LYP/6-311 + G(2d,2p)//B3LYP/6-31 + G(d,p)) level of theory. A comparison of these energetics with the experimentally observed data would suggest that this conformer with no secondary intermolecular interaction (intra–intra) is unlikely to be formed in the high-pressure ion source in any appreciable quantity. It is of interest to note that a comparison of the enthalpy change values calculated for all of the isomers of the proton-bound dimers of the methylesters of glycine and alanine shows the influence of the steric hindrance in the presence of low energy structures. That is to say, that the introduction of the methyl group on the carbon α to the amine function leads to an increase in the energy difference between analogous conformers. For example, in the case of glycine, four conformers were found to lie within 0.5 kcal mol^{-1} of the lowest energy structure, whereas in the case of alanine, only one other structure is found which satisfies this condition.

An analogous trend was found for the proton-bound dimer of methyl ester of valine, for which the same conformational investigation was performed but, due to the increased molecular size and increased computational resources required, fewer conformers were investigated in detail. Of these, two were found to be almost isoenergetic and the more stable of these two is presented in Fig. 5 as DVal1. For the reasons discussed below, structures lacking the secondary interaction were more extensively explored and two of these are also given in Fig. 5 as DVal 2 and DVal 3. The differences of ∼4 and ∼7 kcal mol^{-1} in the enthalpy changes for formation of DVal 2 and DVal 3 relative to DVal1 points once again to the important contributions of secondary interactions to the stability of these poly-functional dimer species.

Structures for the proton-bound dimers of the methylesters of glycine and alanine presenting a 'salt-bridged' R_1–COO^-–$^+HNH_2$–R_2 type of interaction were also optimized and are reported as DiGly-SB and DiAla-SB in Figs. 3 and 4, respectively. Both of them represent similar structures. The strength of the intermolecular electrostatic interaction is reflected in the short distance between the oxygen of the carboxylate function and the intermolecular proton (1.48 Å for DiGly-SB; 1.50 Å for DiAla-SB) and a consequent lengthening of the intermolecular proton–nitrogen bond (1.10 Å for DiGly-SB and DiAla-SB relative to 1.04 Å in the protonated monomers) in comparison to the other structures, DiGly1–DG6 and DiAla1–DiAla7, given in Figs. 3 and 4, respectively. The two carbon–oxygen bonds of the carboxylate group have the same length, 1.25 Å, which is 0.04 Å longer than the typical carbon–oxygen double bond of the ester group, both in DiGly-SB and DiAla-SB structures. These 'salt-bridged' structures are more stable by 4.9 (DiGly-SB) and 5.5 kcal mol^{-1} (DiAla-SB) with respect to the most stable non rearranged isomers, DiGly1 and DiAla1. These isomers would necessarily be the result of the migration of a methyl group from the ester oxygen to the nitrogen of the amino group to lead to a very strong electrostatic, intermolecular interaction between the carboxylate anion and ammonium cation ion moieties. The barrier for such a migration of the methyl group is expected to be high. For example, a barrier of 96 kcal mol^{-1} has been calculated for the GlyMe–Na^+ complex [18] and in conjunction with the present work a barrier of 76 kcal mol^{-1} has been determined for this transfer within $GlyMeH^+$. While these barriers are clearly too large to permit a unimolecular transfer of this type within the protonated monomers, it is conceivable that a "methyl cation transport" type of mechanism might make such a transfer feasible within protonated dimer or higher order clusters. The data obtained in the present work for the protonated dimers would suggest however that these transfers do not occur to any appreciable extent within the temperature range examined.

It is of some interest to compare the most stable structures obtained for the proton-bound dimers of these amino acid methyl esters with those obtained for the proton-bound dimers of the amino acids themselves. As noted above the most stable structure found for the proton-bound dimers of the amino acid methyl esters involves a primary hydrogen bond interaction between a proton of the ammonium ion of the protonated moiety and the amine nitrogen of the neutral moiety. This is supplemented by a secondary intermolecular hydrogen bond interaction between a second ammonium ion proton and the carbonyl oxygen of the neutral moiety and, finally, a third, weaker intramolecular hydrogen bond interaction between the remaining ammonium ion proton and the carbonyl oxygen of the protonated ester moiety. This bonding motif is similar to that found by Williams and co-workers in their calculations for proton-bound dimers of glycine and alanine [29]. These authors presented structures

analogous to DiGly3 and DiAla3, which are only slightly higher in energy than DiGly1 and DiAla1, as the suggested global minima. In contrast, our calculations of the glycine proton-bound dimer found a global minimum that was not, in fact, analogous to any of the structures found for the amino acid esters in the present work. The glycine proton-bound dimer found in that study exhibited a N–$H^+\cdots$O hydrogen bond as the primary intermolecular hydrogen bond between an ammonium ion proton and the carbonyl oxygen of the neutral moiety. This primary interaction was then supplemented by two additional intramolecular hydrogen bonds. One of these was between a second ammonium ion proton and the carbonyl oxygen in the protonated moiety while the second was between the carboxyl hydrogen and the amine nitrogen of the neutral moiety. Thus, the presence of the acidic carboxylic acid hydrogens in the underivatized amino acids changes the proton-bound dimer structure dramatically relative to that found in the esters. Significantly, a zwitterionic structure derived from the most stable protonated glycine dimer structure by an actual intermolecular proton transfer within the neutral moiety was found to be almost identical in energy to the analogue of DiGly 1.

As noted above, the agreement between the experimental thermochemical data for the formation of the proton-bound dimer of valine methyl ester and computational results for formation of the most stable calculated structure is not good. This lack of agreement could suggest that the most stable dimer structure is not being formed under our experimental conditions and, if this is the case, it would likely be the result of an entropically more favorable structure being formed in the temperature regime investigated. A logical reason for this would seem to be the possibility that the most compact calculated structure, DiVal1, could lead to a considerable degree of restriction of internal rotation of the bulky *i*-propyl groups in this form of the proton-bound dimer. This would give rise to a species which is very much lower in entropy than one in which fewer intermolecular hydrogen bonds exist, leaving the *i*-propyl groups much less sterically encumbered. DiVal2 and DiVal 3 are two possible structures for which this would be true. In this regard, it is of interest to note that, at an average temperature for the valine methyl ester results of ∼173 °C, the free energy calculated using the computational data for DiVal1 is less favorable than that obtained from the experimental ΔH and ΔS values. Unfortunately, the inability of the computational method employed to adequately determine entropies of species exhibiting significant restriction of internal rotation prohibits a more rigorous analysis of the valine methyl ester data.

5. Conclusions

The clustering reactions of the three simplest amino acid methylesters were investigated by means of pulsed high pressure mass spectrometry experiments and DFT calculations. The formation of the proton-bound homodimers was characterized and it has been shown that the most stable isomers should be formed in the case of glycine and alanine whereas an entropically favorable structure would favored in the case of valine. The DFT calculations show that the most stable species for each of the proton-bound dimers is one in which three hydrogen bonds are formed involving the three ammonium ion hydrogens of the protonated amino acid ester moiety. These include intermolecular hydrogen bonds to the amine nitrogen and the carbonyl oxygen of the neutral molecule and an intramolecular hydrogen bond to the carbonyl oxygen of the protonated species. Other possible low energy isomers for the proton-bound dimers were investigated and the five lowest energy isomers were all found to involve the primary N–$H^+\cdots$N intermolecular hydrogen bond interaction. A system of nomenclature has been developed to denote the types of secondary hydrogen bond interactions found in these isomers. The agreement between experimental and computational data for the association reaction energetics was found to be excellent for the glycine and alanine systems while the agreement for the valine system was less than satisfactory. The experimental binding energy was found to be less than that predicted for the most stable DiVal species and this has been interprets in terms of formation of a less strongly bound, but more entropically favorable DiVal species in which no secondary intermolecular hydrogen bonds are present. This species might be expected to dominate due to the gain of internal rotations which would otherwise be restricted, at high entropic cost in the case of DiVal, in a species exhibiting secondary intermolecular hydrogen bonding.

Acknowledgements

Financial support of this work by the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged. The authors also wish to acknowledge the extremely valuable assistance of Professor Michael Chong in the preparation and purification of the amino acid methyl esters from their hydrochloride salts.

References

[1] J.P. Simons, Phys. Chem. Chem. Phys. 6 (2004) E7.
[2] (a) M. Yamashita, J.B. Fenn, J. Phys. Chem. 88 (1984) 4671;
(b) M. Yamashita, J.B. Fenn, J. Phys. Chem. 88 (1984) 4451.
[3] F. Hillenkamp, M. Karas, R. Beavis, B. Chait, Anal. Chem. 63 (1991) A1193.
[4] E.R. Williams, V.H. Wysocki, Int. J. Mass Spectrom. 219 (2002) VII.
[5] E.N. Kitova, W.J. Wang, D.R. Bundle, J.S. Klassen, J. Am. Chem. Soc. 124 (2002) 13980.
[6] E.N. Kitova, D.R. Bundle, J.S. Klassen, J. Am. Chem. Soc. 124 (2002) 5902.
[7] R. Daneshfar, E.N. Kitova, J.S. Klassen, J. Am. Chem. Soc. 126 (2004) 4786.
[8] M.J. Meot-Ner, Am. Chem. Soc. 96 (1974) 3168.
[9] M.J. Meot-Ner, Am. Chem. Soc. 106 (1984) 278.
[10] M. Meot-Ner, Acc. Chem. Res. 17 (1984) 186.
[11] M. Meot-Ner, J. Am. Chem. Soc. 110 (1988) 3071.
[12] M. Meot-Ner, J. Am. Chem. Soc. 110 (1988) 3075.
[13] F. Rogalewicz, Y. Hoppilliard, G. Ohanessian, Int. J. Mass Spectrom. 227 (2003) 439.
[14] F. Rogalewicz, G. Louazel, Y. Hoppilliard, G. Ohanessian, Int. J. Mass Spectrom. 228 (2003) 779.
[15] F. Rogalewicz, Y. Hoppilliard, G. Ohanessian, Int. J. Mass Spectrom. 206 (2001) 45.
[16] Y. Hoppilliard, F. Rogalewicz, G. Ohanessian, Int. J. Mass Spectrom. 204 (2001) 267.

[17] F. Rogalewicz, Y. Hoppilliard, G. Ohanessian, Int. J. Mass Spectrom. 201 (2000) 307.
[18] J.M. Talley, B.A. Cerda, G. Ohanessian, C. Wesdemiotis, Chem. Eur. J. 8 (2002) 1377.
[19] M.M. Kish, G. Ohanessian, C. Wesdemiotis, Int. J. Mass Spectrom. 227 (2003) 509.
[20] S. Hoyau, J.P. Pelicier, F. Rogalewicz, Y. Hoppilliard, G. Ohanessian, Eur. J. Mass Spectrom. 7 (2001) 303.
[21] F. Rogalewicz, G. Ohanessian, N. Gresh, J. Comput. Chem. 21 (2000) 963.
[22] S. Hoyau, G. Ohanessian, J. Chem.—A Eur. 4 (1998) 1561.
[23] S. Hoyau, G. Ohanessian, J. Am. Chem. Soc. 119 (1997) 2016.
[24] A.S. Lemoff, M.F. Bush, E.R. Williams, J. Am. Chem. Soc. 125 (2003) 13576.
[25] R.A. Jockusch, A.S. Lemoff, E.R. Williams, J. Phys. Chem. A 105 (2001) 10929.
[26] R.A. Jockusch, E.R. Williams, J. Phys. Chem. A 102 (1998) 4543.
[27] W.D. Price, R.A. Jockusch, E.R. Williams, J. Am. Chem. Soc. 120 (1998) 3474.
[28] W.D. Price, R.A. Jockusch, E.R. Williams, J. Am. Chem. Soc. 119 (1997) 11988.
[29] W.D. Price, P.D. Schnier, E.R. Williams, J. Phys. Chem. B 101 (1997) 664.
[30] T.B. McMahon, Int. J. Mass Spectrom. 200 (2000) 187.
[31] M. Meot-Ner, Int. J. Mass Spectrom. 227 (2003) 525.
[32] B. Bogdanov, H.J.S. Lee, T.B. McMahon, Int. J. Mass Spectrom. 210 (2001) 387.
[33] B. Bogdanov, T.B. McMahon, Int. J. Mass Spectrom. 219 (2002) 593.
[34] B. Bogdanov, M. Peschke, D.S. Tonner, J.E. Szulejko, T.B. McMahon, Int. J. Mass Spectrom. 187 (1999) 707.
[35] K. Norrman, T.B. McMahon, J. Am. Chem. Soc. 118 (1996) 2449.
[36] M. Meot-Ner, E.P. Hunter, F.H. Field, J. Am. Chem. Soc. 101 (3) (1979) 686.
[37] J.E. Szulejko, T.B. McMahon, Int. J. Mass Spectrom. Ion Process. 109 (1991) 279.
[38] P.J. Stevens, F.J. Devlin, C.F. Cheblowski, M.J. Frisch, J. Phys. Chem. A 98 (1994) 11623.
[39] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, V.G. Zakrzewski, J.A. Montgomery Jr., R.E. Stratmann, J.C. Burant, S. Dapprich, J.M. Millam, A.D. Daniels, K.N. Kudin, N.C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, A.G.A. Petersson, Y.P.Y. Ayala, Q. Cui, K. Morokuma, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J. Cioslowski, J.V. Ortiz, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, J.L. Andres, C. Gonzalez, M. Head-Gordon, E.S. Replogle, J.A. Pople, Revision A.6 ed., Gaussian, Inc., Pittsburgh PA, 1998.
[40] R. Yamdagni, P. Kebarle, J. Am. Chem. Soc. 95 (1973) 3504.
[41] S.A. Raspopov, T.B. McMahon, J. Mass Spectrom. 40 (2005) 1536.
[42] J.E. Del Bene, M.J. Frisch, J.A. Pople, J. Phys. Chem. 89 (1985) 3669.
[43] J.E. Del Bene, M.J.T. Jordan, J. Mol. Struct. 573 (2001) 11.
[44] J.A. Platts, K.E. Laidig, J. Phys. Chem. 99 (1995) 6487.
[45] E.F. Strittmatter, E.R. Williams, J. Phys. Chem. A 104 (2000) 6069.
[46] M. Meot-Ner, L.W. Sieck, J. Am. Chem. Soc. 113 (1991) 4448.