Multiply protonated betaine clusters are stable in the gas phase†‡

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Multiply protonated clusters of betaine are formed *via* electrospray ionisation and fragment *via* competitive betaine neutral loss and charge separation; theoretical calculations suggest the $[M_n + 2H]^{2+}$ ions consist of a $[M_2 + 2H]^{2+}$ core based on the hydrogen bonded carboxylic acid dimer.

Multiply charged ions play important roles in chemistry and biochemistry, and there has been considerable interest in studying their gas phase chemistry.[1] When multiply charged ions are desolvated, their gas phase properties come to the fore. Thus multiply charged protein ions can unfold in the gas phase,[2] while the interplay between attractive forces and Coulomb repulsion can result in the presence of a barrier, and can lead to charge separation/reduction reactions competing with simple solvent evaporation as the size of the cluster decreases in gas phase solvated metal cations[1b] and dianions.[3] For example, charge reduction *via* ion evaporation (eqn (1)) competes with solvent evaporation (eqn (2)) in the hydrated, triply charged lanthanide cluster ions, $[Ln(H_2O)_n]^{3+}$ (where Ln = La, Tb and Lu) when the clusters reach the critical size of n = 17–28.[4] Similarly, ion evaporation (eqn (3)) becomes an important fragmentation channel in competition with water loss (eqn (4)) for the hydrated sulfate anion, $[SO_4(H_2O)_6]^{2-}$.[3] In contrast, very few studies have examined whether clusters containing two singly charged ions are stable in the gas phase. Apart from amino acid and peptide clusters, which can exhibit multiple hydrogen bonding,[5] and metal salt clusters,[6] Kass appears to have been the first to address the stability of simple organic systems solely capable of being stabilized by a few hydrogen bonds.[7] Using theoretical calculations, he examined several clusters between two mono anions. While the dissociation of these clusters is always exothermic, in many instances hydrogen bonding can make the clusters kinetically stable with respect to dissociation. For example, even the least stable cluster between chloride and deprotonated malonic acid has a barrier of 0.8 kJ mol^{-1} to dissociation. During a recent study on the intercluster reactions of the $[M_2 + H]^+$ and $[M_2 + Na]^+$ clusters of the zwitterion betaine ($M = (CH_3)_3NCH_2CO_2$),[8] we noticed the formation of $[M_n + 2H]^{2+}$ and $[M_n + 3H]^{3+}$ clusters. Here we use electrospray ionisation (ESI), high resolution mass measurements and collision induced dissociation (CID) tandem mass spectrometry in a hybrid linear ion trap FT-ICR mass spectrometer[9] to examine the minimum size clusters, n, for which these clusters are still stable in the gas phase. In addition, we examine the structure and stability of the $[M_2 + 2H]^{2+}$ of betaine using DFT and PM3 calculations, and potential isomers of the $[M_4 + 2H]^{2+}$ using PM3 calculations.[10]

$$[Ln(H_2O)_n]^{3+} \rightarrow [Ln(OH)(H_2O)_a]^{2+} + [H(H_2O)_b]^+ + cH_2O \quad (1)$$

$$\rightarrow [Ln(H_2O)_{n-x}]^{3+} + xH_2O \quad (2)$$

$$[SO_4(H_2O)_6]^{2-} \rightarrow [HSO_4(H_2O)_2]^- + [HO(H_2O)_3]^- \quad (3)$$

$$\rightarrow [SO_4(H_2O)_5]^{2-} + H_2O \quad (4)$$

ESI/MS of a solution of betaine[9] yields a series of protonated and sodiated clusters of types $[M_n + H]^+$ and $[M_n + Na]^+$ as well as the multiply charged clusters $[M_n + 2H]^{2+}$, $[M_n + 3H]^{3+}$ and $[M_n + 4H]^{4+}$, as highlighted in Fig. 1. These clusters are unique to betaine, not being observed for its isomer, *N*,*N*-dimethylglycine methyl ester. The multiply charged ions are readily identified by unique m/z values when they have odd numbers of n for $[M_n + 2H]^{2+}$ and $[M_n + 4H]^{4+}$, and where $n \neq$ multiple of 3 for $[M_n + 3H]^{3+}$. In cases where the multiply charged clusters

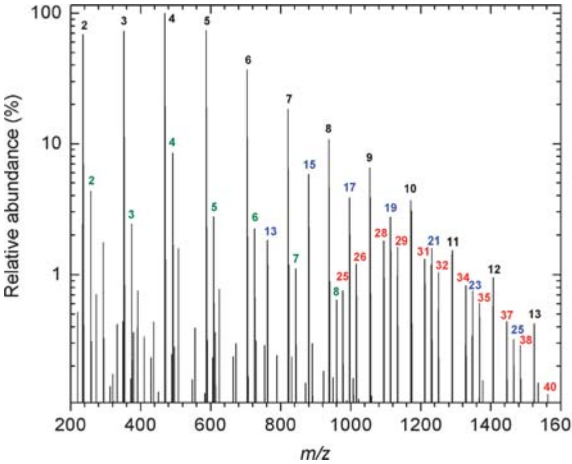

Fig. 1 LTQ-FT ICR ESI high resolution MS of a solution of betaine.[9] The spectrum contains series of singly charged clusters protonated $[M_n + H]^+$ (n = black) and sodiated $[M_n + Na]^+$ (n = green), doubly charged clusters $[M_n + 2H]^{2+}$ (n = blue) and triply charged clusters $[M_n + 3H]^{3+}$ (n = red).

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† Electronic supplementary information (ESI) available: Fig. S1 (exact mass measurement of $[M_{13} + 2H]^{2+}$ cluster), Fig. S2 (B3LYP/6-31+G(d) calculated Cartesian coordinates and energies (Hartrees) for species relevant to the $[M_2 + 2H]^{2+}$), Fig. S3 and S4 (PM3 calculated Cartesian coordinates and energies (Hartrees) for species relevant to the $[M_2 + 2H]^{2+}$ and $[M_4 + 2H]^{2+}$), Table S1 (Predicted m/z values for $[M_n + xH]^{x+}$ clusters of betaine). See DOI: 10.1039/b808124g

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have the same m/z value as singly charged clusters, we take advantage of high resolution mass measurements, since $[M + H]^+$ and $[M_2 + 2H]^{2+}$ can be distinguished based on the spacing of the isotopes.

The smallest doubly charged cluster observed is $[M_{13} + 2H]^{2+}$. Its stoichiometry was confirmed by accurate mass measurements (Supplementary Fig. S1).† The combination of high resolution mass measurements and collision induced dissociation provides further evidence for the formation and stability of these multiply charged betaine clusters, as shown in Fig. 2. Thus there is no evidence in the high resolution mass spectrum that $[M_{21} + 2H]^{2+}$ is contaminated with $[M_{42} + 4H]^{4+}$ (inset of Fig. 2a). MS/MS on $[M_{21} + 2H]^{2+}$ gives a combination of charge separation (eqn (5)) and betaine evaporation (eqn (6)). A range of charge separation products are observed, with products $x = 8$–10 dominating. The smallest doubly charged cluster formed *via* betaine evaporation is $[M_{15} + 2H]^{2+}$.[11] In contrast, both high resolution (inset of Fig. 2b) and MS/MS on $[M_{11} + H]^+$ reveal the presence of the multiply charged betaine clusters $[M_{22} + 2H]^{2+}$, $[M_{33} + 3H]^{3+}$ and $[M_{44} + 4H]^{4+}$. The MS/MS spectrum shown in Fig. 2b is rich and contains a series of singly, doubly and triply charged ions. The presence of $[M_n + 2H]^{2+}$ and $[M_n + 3H]^{3+}$ where $n > 22$ and $n > 33$ must arise from charge separation reactions of $[M_{33} + 3H]^{3+}$ (eqn (7)) and $[M_{44} + 4H]^{4+}$ (eqn (8)). Once again, $[M_{15} + 2H]^{2+}$ is the smallest doubly charged cluster formed. A very minor amount of $[M_{32} + 3H]^{3+}$ is observed, but it is not clear if this is due to loss of a betaine neutral or a charge separation reaction of $[M_{44} + 4H]^{4+}$.

$$[M_n + 2H]^{2+} \rightarrow [M_{n-x} + H]^+ + [M_x + H]^+ \qquad (5)$$

$$\rightarrow [M_{n-y} + 2H]^{2+} + yM \qquad (6)$$

$$[M_n + 3H]^{3+} \rightarrow [M_{n-x} + 2H]^{2+} + [M_x + H]^+ \qquad (7)$$

$$[M_n + 4H]^{4+} \rightarrow [M_{n-x} + 3H]^{3+} + [M_x + H]^+ \qquad (8)$$

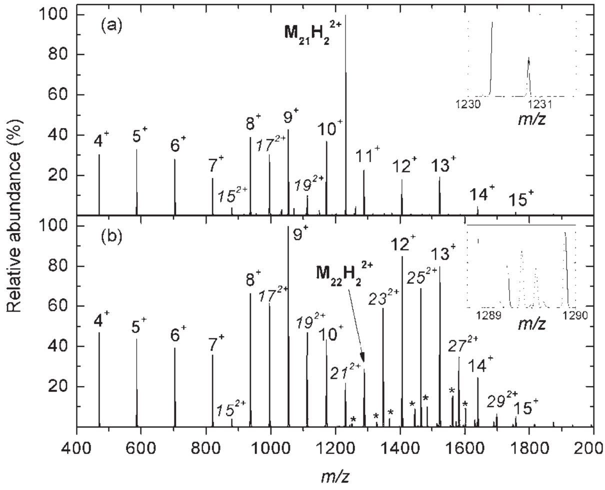

Fig. 2 CID/MS/MS of betaine clusters: (a) $[M_{21} + 2H]^{2+}$ (inset is high resolution of the precursor ion); (b) overlapping $[M_{11} + H]^+$, $[M_{22} + 2H]^{2+}$, $[M_{33} + 3H]^{3+}$ and $[M_{44} + 4H]^{4+}$ (inset is high resolution of the precursor ion). In order to simplify the spectra, the fragments $[M_n + H]^+$ and $[M_m + 2H]^{2+}$ are referred to as n^+ and m^{2+}. Triply charged fragments are indicated by a *.

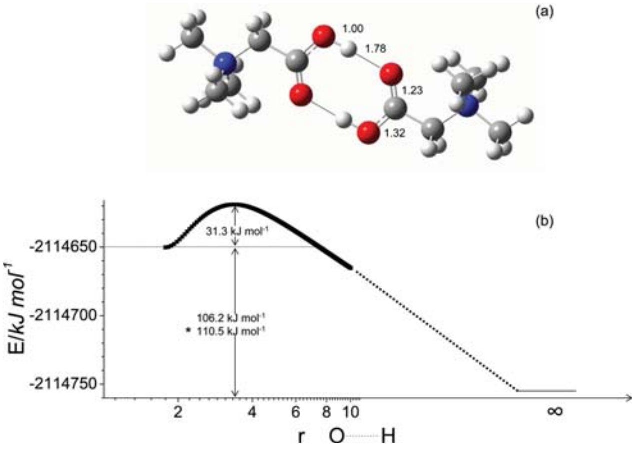

Fig. 3 DFT calculations (B3LYP/6-31+G(d)) associated with the $[M_2 + H]^{2+}$ closed centrosymmetric dimer structure of betaine (C atoms in grey, O atoms in red and N atoms in blue): (a) fully optimized structure (C_s symmetry, distance between both N atoms is 8.85 Å); (b) energy plot as a function of O···H bond length. The value of DE indicated by * is corrected to include the zero-point vibrational energy. The dashed line from 10 Å is an extrapolation.

How are these clusters structurally organized in order for them to be stable in the gas phase? Attractive forces such as hydrogen bonding[5a,7] and dipole–dipole interactions[11,12] must counterbalance the repulsive Coulombic interactions of the two (or more) cations.[13] Intrigued by the possibility that the core of the doubly charged clusters might adopt the closed centrosymmetric dimer structure typically observed in the self-association of carboxylic acids,[14,15] we have calculated the structure of this dimer and its energy of dissociation using both the PM3 semi-empirical method and DFT calculations. At the B3LYP/6-31+G(d) level of theory, the minimum shown in Fig. 3a is found. A similar dimer structure is found at the PM3 level of theory (supplementary Fig. S2†). The dissociation energy (DE) of this dimer into two protonated betaines (eqn (5), where $n = 2$ and $x = 1$) is predicted to be exothermic by 110.5 kJ mol^{-1} at the B3LYP/6-31+G(d) level of theory and 121.7 kJ mol^{-1} using the PM3 method. We also estimated the barrier associated with dissociating the dimer by systematically increasing the two O···H bonds and examining the change in energy at the B3LYP/6-31+G(d) level of theory (Fig. 3b). These calculations reveal that the dimer dication is kinetically stable with respect to dissociation to two protonated monomers, with a barrier of approximately 31.3 kJ mol^{-1}.

In order to address how "solvation" of the betaine dimer dication by other betaine molecules might change the structure and energetics of dissociation, we have optimized a range of different structures of the $[M_4 + 2H]^{2+}$ cluster using the PM3 method. Three structures, fully optimized without symmetry constraints, are shown in Fig. 4. The most stable structure maintains the hydrogen bonded carboxylic acid dimer motif, with each end of the dimer having its trimethylammonium group solvated by the carboxylate of one betaine molecule (Fig. 4a).[16] Solvation changes the DE into two $[M_2 + H]^+$ clusters to 80.7 kJ mol^{-1}, around 40 kJ mol^{-1} less than that for the parent $[M_2 + 2H]^{2+}$ dimer.[17] Other structures with the hydrogen bonded carboxylic acid dimer motif in which only one trimethylammonium group is doubly solvated are less

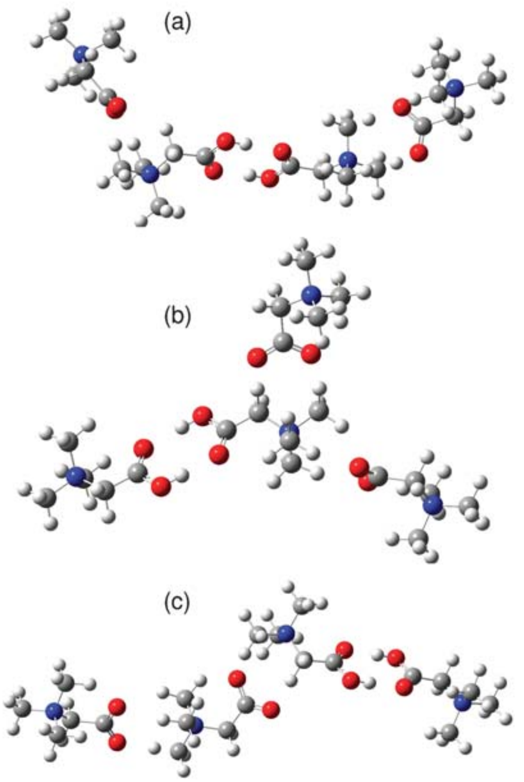

Fig. 4 PM3 calculated structures of $[M_4 + 2H]^{2+}$ clusters of betaine, relative energies (RE) and DE to two $[M_2 + H]^+$ clusters given in kJ mol^{-1}: (a) dimer core solvated by a betaine at each end (RE = 0 kJ mol^{-1}; DE = −80.7 kJ mol^{-1}); (b) dimer core solvated by two betaines at one trimethylammonium (RE = 38.2 kJ mol^{-1}; DE = −118.9 kJ mol^{-1}); (c) dimer core sequentially solvated at one trimethylammonium (RE = 31.1 kJ mol^{-1}; DE = −111.8 kJ mol^{-1}).

stable (Fig. 4b and c). We also attempted to calculate a structure of a $[M_4 + 2H]^{2+}$ cluster which does not contain the hydrogen bonded carboxylic acid dimer motif, but rather consists of two $[M_2 + H]^+$ clusters. This structure did not optimize, undergoing charge separation, with both $[M_2 + H]^+$ clusters being separated by large distances (>47 Å after >100 steps). These results suggest that kinetically stable $[M_n + 2H]^{2+}$ clusters may adopt a core structure consisting of a hydrogen bonded carboxylic acid dimer motif that is "solvated" by betaine molecules.

The fact that we have demonstrated simple multiply protonated betaine clusters are stable in the gas phase raises the possibility of observing other fundamentally important multiply charged clusters related to the ESI process.[18]

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10 All structure optimizations were performed using the Gaussian 03 molecular modelling package (Gaussian 03, Revision B.04, M. J. Frisch *et al.*, see full citation in Supplementary material†). Structures of the carboxylic acid dimers were optimized at the B3LYP level of theory with the standard 6-31+G(d) basis set. All optimized structures were subjected to vibrational frequency analysis to establish that they corresponded to minima (*i.e.* no imaginary vibrational frequencies). The final energies used to calculate the dimer DE involve correcting the electronic energies with the unscaled zero-point vibrational energies. For calculations carried out using the PM3 method, the final electronic energies were used to calculate the relative stabilities and reaction energetics.

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