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An electrospray ionization flow tube study of the protonated betaine/ammonia complex

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Abstract

Reactions of protonated betaine, BetH$^+$, with ammonia and deuterated ammonia were studied under thermal conditions with a combination of an electrospray ionization (ESI) source and a flow tube reactor. Reaction with NH_3 in a flow tube pressure range of 0.1–0.45 Torr leads, with a low reaction efficiency of 0.034 ± 0.011, to formation of a collisionally stabilized protonated betaine/ammonia complex, which can have the ion/dipole BetH$^+$.NH_3 structure and/or the ammonium ion stabilized salt–bridge complex structure, Bet.NH_4^+. Reaction with ND_3 leads to a highly efficient H/D exchange of the labile hydrogen of BetH$^+$ presumably via the excited, chemically activated, salt-bridge complex, [Bet.ND_3H^+]*. Ions selected from the ESI source upstream in the flow tube, or generated by the reactions with ammonia midstream and then collisionally stabilized and thermalized to 292 ± 3 K by collisions with He or N_2 carrier gas species at 0.23 ± 0.01 Torr were subjected to multi-collision-induced dissociation (CID) with He or N_2 just before sampling. CID is achieved by raising the potential of the sampling nose cone from 0 to −20 V. The threshold voltage required for multi-collision-induced dissociation of the protonated betaine/ammonia complex to BetH$^+$ and NH_3 is considerably lower than the threshold voltage for dissociation of the protonated betaine homodimer, (Bet)$_2$H$^+$ in accord with the lower binding energy of the former. (Int J Mass Spectrom 195/196 (2000) 11–19) © 2000 Elsevier Science B.V.

Keywords: Salt–bridge complex; ESI; Flow tube; H/D exchange; Collision-induced dissociation

1. Introduction

There has been considerable interest in recent years in the analytical capabilities of mass spectrometry in the field of biomolecules and in the possibility of studying conformations of proteins by gas phase ion chemistry. As part of that effort, techniques have been developed for studying hydrogen–deuterium exchange between protonated peptides and deuterated solvent molecules such as ND_3, D_2O, and CH_3OD. There has been particular interest recently [1–5] in the role of salt–bridge and zwitterion structures in the conformation, reactivity and dissociation mechanisms of gas-phase peptides and proteins. Betaine [$(CH_3)_3N^+CH_2COO^-$], a methylated derivative of glycine, is a naturally occurring zwitterion. It has been pointed out [6] that gas phase complexes of protonated ions with betaine are excellent prototypes for

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In memory of Bob Squires, a good friend and an excellent scientist, who was the first to combine ESI with a flow tube and without whose advice the Hebrew University experimental setup described here would have been impossible.

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quantifying salt–bridge interactions. Dissociation kinetics of protonated dimers of betaine with a series of reference compounds of known gas phase basicity were measured using blackbody infrared radiative dissociation (BIRD) [6].

A salt–bridge complex has been proposed to play a role in the gas-phase H/D exchange of protonated betaine with ND_3 whereas a multiply hydrogen bonded “onium” complex formation was proposed for the exchange of protonated diglycine [1]. We have recently studied [7] the onium complex formation kinetics for protonated diglycine, GLY_2H^+, and NH_3, and the H/D exchange of protonated diglycine with ND_3 [8] using a newly developed electrospray ionization (ESI) flow tube reactor experiment. The latter method, which uses a helium carrier gas, allows us to observe the intermediate complexes between the peptides and the deuterated solvents, which are proposed to have multiply hydrogen bonded onium, or gas phase “salt–bridge” structures. We have the unique capability of observing directly, through collisional stabilization, some of the complexes which have only been indirectly inferred. The present study is devoted to the betaine H^+/NH_3 complex formation, to the H/D exchange between protonated betaine and ND_3 and to collision induced dissociation (CID) of the ammoniated complex of betaine. As noted earlier [7,8], flow tube experiments give experimental data for considerably higher pressure ranges (several tenths of a torr) compared to Fourier transform ion cyclotron resonance (FTICR) experiments [1,6] which are run between $5–8.5 \times 10^{-9}$ Torr [6] and $0.5–1.5 \times 10^{-7}$ Torr [1]. As a result, the kinetics gives information on the competition between carrier gas collisional stabilization of the complex studied, backreaction and/or isotopic exchange.

In the present study we combine CID with the electrospray ionization flow tube reactor. Bohme and co-workers have recently developed [9,10] a selected ion flow tube (SIFT)-CID experiment. In this application multi-collision-induced dissociation with the carrier gas is achieved by raising the potential of the sampling nose cone. This CID technique was found to be useful for the determination of bond connectivities and for distinguishing isomeric ions. We are attempting to apply it to the small noncovalently bound biomolecule/ammonia complexes studied here. The success of using CID in conjunction with ion/molecule reactions, so that the products of these reactions can be analyzed, has been demonstrated recently [11] for H/D exchange reactions of protonated diglycine.

The study of ion/molecule reactions of protonated biomolecules by means of a flow tube technique necessitates the introduction of these intact ions into the tube. Bob Squires developed the combination of ESI with the flowing afterglow method in a pioneering study [12]. We constructed a similar simple ESI source for our flow tube reactor and applied it to the study of reactions of GLY_2H^+ [7,8]. This ESI flow tube reactor has been employed in the present study of the zwitterion complex of protonated betaine, $BetH^+$ with ammonia.

2. Experiment

We constructed a SIFT apparatus some years ago, which is been described in detail elsewhere [13]. This apparatus has been modified to work with an ESI source connected directly to the flow tube, as in the work of Poutsma et al. [12] and not through the injector quadrupole; reactant ions were thus not mass selected. The system has been described in detail [7,8]. Briefly, the SIFT consists of a flow reactor that is 123 cm in length and has an inner diameter (i.d.) of 74 mm. A neutral reagent is introduced into the flow tube through either one of two ring inlets. Tylan mass flow controllers define the flow rate of the neutral reactant into the flow tube. The quadrupole mass analyzer is housed in a differentially pumped chamber that is separated from the flow tube by a nose cone (NC) skimmer with a 1.0 mm sampling orifice. In the conventional operation of the apparatus, a small NC voltage of −1.25 V is used for focusing ions into the analysis quadrupole. Helium or nitrogen buffer gas enters the flow tube at the upstream end near an electron impact ion source through another Tylan flow controller. It is pumped through the tube by a Roots blower with flow velocities of up to about 9000 cm s^{-1} with typical pressures of a few tenths of a torr.

The present experiments were run at pressures ranging from 0.1 to 0.45 Torr and at reaction times of several milliseconds.

The electrospray ion source was designed following the design of the Bob Squires group [12]. A capillary tube serves as the interface between the electrospray and the helium or nitrogen flow reactor. Stainless-steel tubes 15 cm in length and 0.05 cm i.d. are employed. The entire assembly is inserted into the flow tube at a distance of ~96 cm from the sampling orifice, 135° to the direction of the carrier gas flow, through an O-ring-type vacuum fitting. A capillary tube of 0.05 cm i.d. introduces an air leak into the flow tube with a pressure of 0.07 Torr and a flow rate of 1.3 L/min (STP); these numbers have to be added to the carrier gas pressure and flow rate when calculating rate constants. Ions are electrosprayed ~10 mm through ambient air into the grounded capillary tube from a stainless steal syringe needle biased at 4700 V dc. Dilute solutions of the analyte of interest in a polar solvent are delivered to the electrospray needle at flow rates of 3.3 μL min^{-1} from a 5000 μL syringe mounted on a model 100 KD Scientific Syringe Pump.

Second-order rate coefficients are obtained by monitoring the intensity of the primary ion decay as a function of the neutral gas B concentration introduced downstream. Product ion distributions are obtained by plotting the percentage of each product ion as a function of the gas B flow rate. Isotopic exchange is thus followed as a function of the concentration of ND_3 at a constant reaction time. Product ion distributions are corrected via the measured mass discrimination factors of the detector quadrupole mass filter and for ^{13}C contributions. Extrapolating product ion percentage curves to zero flow rate yields the percentage distribution of the primary products.

CID can be applied to products of ion/molecule reactions carried out in the flow tube or to ions injected into the flow tube through the ESI source. The sample ions are dissociated by increasing the voltage on the NC, following the work by Baranov and Bohme [10]. The extent of collisional dissociation of the sampled ions is controlled by the magnitude of the NC voltage (U_{nc}) which can be varied in our experiments from 0 to −20 V. Upon entering the detection region of the apparatus, ions experience sequentially a front-lens voltage ($U_{fl} = 0$ to -100 V), a quadrupole field (rf/dc), and a channeltron voltage (from −2000 to −2500 V). In the present series of experiments, the voltage on the central element of the front lens was held constant at $U_{fl} = -65$ V. The other two elements of the front lens were held at slightly negative voltages with respect to the nose cone. Conversion from laboratory ion energy to the centre-of-mass energy frame is possible through multiplication of the nose cone voltage by a mass factor [10]. In the case of helium as a carrier gas, CID can take place in collisions with the helium as well as with oxygen and nitrogen from the air entering through the ESI source. This leads to at least two different mass correction factors. Pure nitrogen was employed as the carrier gas in some of the CID experiments, in order to avoid this confusion.

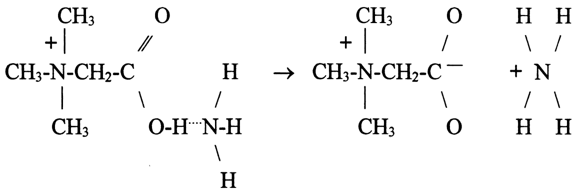
Betaine (Bet) was a sample from Aldrich with a stated minimum purity of 98%. A 3×10^{-5} M solution of Bet in 1% formic acid, 50% methanol, and 49% water was employed to electrospray the protonated Bet. ND_3 was from Cambridge Isotope Laboratories with a stated isotopic purity of ≥99 at. % D.

3. Results and discussion

Two major ions were observed upon introduction of the betaine solution into the flow tube through the ESI source: protonated betaine, $BetH^+ - (CH_3)_3N^+CH_2COOH$, and the proton bound dimer of betaine, $(Bet)_2H^+$, at *m/z* 118 and 235, respectively. The protonated monomer was observed to be reactive with neutral ammonia while the dimer was not. Ammonia cannot switch betaine out of the protonated dimer in view of the much higher proton affinity of betaine, PA (Bet) = 246.7 kcal/mol [6], compared to that of ammonia, PA (NH_3) = 204 kcal/mol [14]. However, ammonia forms a complex with the protonated betaine monomer, at *m/z* 135,

$$\mathrm{BetH^+ + NH_3 \xrightarrow{M} Bet.NH_4^+} \qquad (1)$$

Calculations by Campbell et al. [1] at the PM3 level have indicated that NH_3 forms a relatively strong hydrogen bond to betaine (12.6 kcal/mol) and that furthermore, proton transfer to NH_3, forming a salt–bridge, is exothermic by 18 kcal/mol. As a result, reaction (1) may be considered to proceed in two steps: 1. Formation of a hydrogen bonded ion dipole complex between protonated betaine and NH_3 followed by, 2. Proton transfer within the complex and formation of the betaine zwitterion stabilized by NH_4^+ (Scheme 1).



Scheme 1.

Results of a typical flow tube experiment are presented in Fig. 1. The reactant ion decay is linear on a semilogarithmic plot of the ion count rate as a function of the neutral flow rate. Effective second-order rate coefficients have been deduced from such plots and the experimental rate constant for reaction (1) is: k_{exp} (1) = (7.1 ± 2.3) × 10^{-11} $\mathrm{cm^3}$/molecule s at a flow tube pressure range of 0.1–0.45 Torr. The reaction efficiency can be calculated from the ion/dipole collision rate constant calculated by using the parametrized expression of Su and Chesnavich [15], k_{coll} (1) = 2.08 × 10^{-9} $\mathrm{cm^3}$/molecule s, and is: $r_{\mathrm{eff}}(1) = 0.034 \pm 0.011$. The results are summarized in Table 1, where they are compared with our previous data [7,8] for protonated diglycine, GLY_2H^+. The low reaction efficiency for production of the collisionally stabilized betaine.NH_4^+ complex is an indication for a high degree of backreaction of the "chemically activated" excited collision complex, (betaine.NH_4^+)* which is formed initially in the protonated betaine/ammonia collision. Reaction (1) is considered to proceed in two consecutive steps:

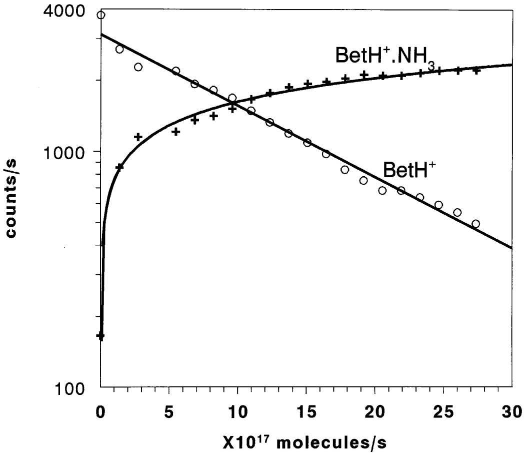


Fig. 1. Variation of reactant and product ion count rates with ammonia flow rate for the reaction of protonated betaine with NH_3 [reaction (1)].

Table 1
Ion/molecule reaction rate constants (in units of 10^{-9} $\mathrm{cm^3}$/molecule s)

Reaction pair	k_{exp}	k_{c}	r_{eff}
GLY_2H^+/ NH_3	k' = 0.3–1.3 k'' = 0.004–0.024	2.04	0.15–0.64 0.002–0.01
GLY_2H^+/ ND_3	0.63 ± 0.2	1.94	0.32 ± 0.1
Betaine/NH_3	0.071 ± 0.023	2.08	0.034 ± 0.011
Betaine/ND_3	1.5 ± 0.16	1.92	0.78 ± 0.08

$$\mathrm{BetH^+ + NH_3 \underset{k_1}{\overset{k_1}{\rightleftharpoons}} (BetNH_4^+)^*} \qquad (1a)$$

$$\mathrm{(Bet.NH_4^+)^* + M \xrightarrow{k_2} Bet.NH_4^+ + M} \qquad (1b)$$

Application of the steady state approximation to (Bet.NH_4^+)* yields an effective binary rate coefficient, k_{eff}, such that [7]

$$1/k_{\mathrm{eff}} = 1/k_1 + (k_{-1}/k_1k_2)1/[\mathrm{M}] \qquad (2)$$

Fig. 2 presents a plot of $1/k_{\mathrm{eff}}$ as a function of 1/[M], where k_{eff} is the experimental rate constant for a set of experiments with He as carrier gas. The forward rate

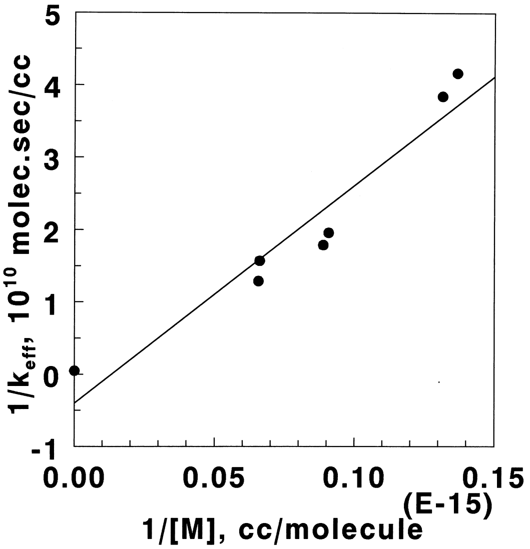


Fig. 2. Reciprocal effective binary rate coefficient as a function of reciprocal third body concentration for the reaction of protonated betaine with NH_3; M is a mixture of He, O_2, and N_2. The point at $1/[M] = 0$ is calculated as $1/k_{\mathrm{eff}} = 1/k_1$.

constant of the first step, k_1 has been calculated above to be $k_1 = k_{\mathrm{coll}}(1) = 2.08 \times 10^{-9}$ cm^3/molecule s. The value of $k_2 = \beta\, k_{c2}$ can be estimated as before [7] from the collisional rate constant of the excited complex, k_{c2} with third body particles, M, which in our case are He, N_2, and O_2, and $\beta \leq 1$, the stabilization efficiency. If $k_2 \approx 1 \times 10^{-10}$ cm^3/molecule s, then substituting k_1 and k_2 into the slope of Fig. 2 leads to the rate constant for the unimolecular back reaction, $k_{-1} \approx 6 \times 10^7$ s^{-1}. Analogous plots to that of Fig. 1 obtained earlier for diglycine [7] demonstrated two slopes, with fast and slow decay rates, respectively (see Table 1). This was ascribed to the unimolecular backreactions of interconverting chemically activated isomers with different well depths, for example, the ion–dipole complex and the hydrogen-bonded $GLY_2NH_4^+$ complex. A similar behavior was expected for the ion–dipole–salt–bridge complex pair in the case of betaine. This is not the case (see Fig. 1 and Table 1). An alternative interpretation of the diglycine data was the possible role of two (or more) GLY_2–NH_4^+ complexes, hydrogen bonded at different positions or two complexes of GLY_2H^+ protonated at different sites whose protonation site does not vary during the complexation and backreaction [7]. These alternative isomeric structures are possible for diglycine but not for betaine and can explain the different complexation kinetics of the two systems.

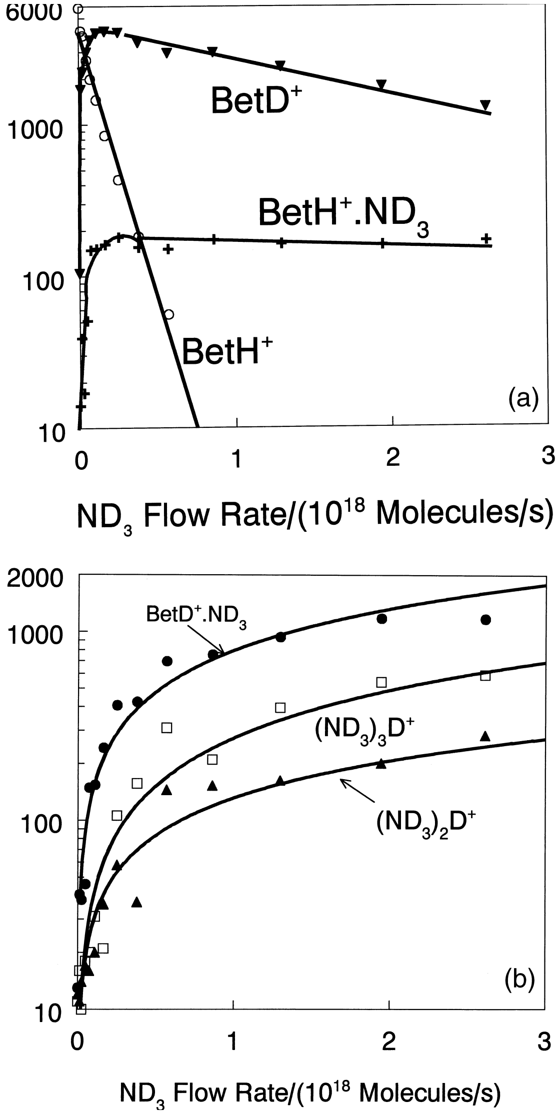


Fig. 3. (a) Variation of reactant ($BetH^+$) and primary ($BetD^+$ and $BetH^+.ND_3$) product ion count rates with ND_3 flow rate for the reaction of protonated betaine with deuterated ammonia [reaction (3a)]. (b) Variation of the secondary [$BetD^+.ND_3$ and $(ND_3)_2D^+$] and tertiary [$(ND_3)_3D^+$] product ion count rates with ND_3 flow rate.

The salt–bridge mechanism has been suggested [1] to be responsible for H/D exchange between protonated betaine and ND_3. Direct observation of the complexes is not achievable in the low pressure FTICR experiments [1]. Since we are able to directly observe the collisionally stabilized complex at our flow tube pressures, we subjected the protonated betaine to H/D exchange with deuterated ammonia in the flow tube and the incorporation of deuterium was monitored as a function of ND_3 flow rate at a constant reaction time. Typical results are plotted in Fig. 3 for a flow tube pressure of 0.4 Torr and a reaction time of 3.5 ms. There is only one labile exchangeable hydrogen in betaine. Fig. 3(a) gives the ion count rates as a function of ND_3 flow rate for the protonated betaine reactant, $BetH^+$, and for the primary reaction products—deuterated betaine, $BetD^+$, and the ND_3 salt–bridge complex of protonated betaine, $BetH^+.ND_3$ (or $Bet.ND_3H^+$). Fig. 3(b) presents the count rates as a function of ND_3 flow rate for the secondary and tertiary reaction products. The ND_3 complex of deuterated betaine, $BetD^+.ND_3$ (or rather its salt bridge stabilized isomer, $Bet.ND_4^+$) is formed by the reaction of deuterated betaine and ND_3 and consecutive reactions of this complex lead to deuteron bound deuterated ammonia clusters,

$$\begin{array}{c} \mathrm{BetH^+ + ND_3} \underset{k_{-1D}}{\overset{k_{1D}}{\rightleftharpoons}} \mathrm{[BetH^+.ND_3]^*} \rightleftharpoons \mathrm{[Bet.ND_3H^+]^*} \rightleftharpoons \mathrm{[BetD^+.ND_2H]^*} \xrightarrow{k_{3D}} \mathrm{BetD^+ + ND_2H} \\ \downarrow k_{2D}(\mathrm{M}) \\ \mathrm{BetH^+.ND_3,\ Bet.ND_3H^+,\ BetD^+.ND_2H} \end{array} \tag{3a}$$

$$\begin{tikzcd} \mathrm{BetD^+ + ND_3} \arrow[r, rightleftharpoons] & \mathrm{[BetD^+ND_3]^*} \arrow[r, "+\mathrm{ND_3}"] \arrow[d, "\mathrm{M}"] & \mathrm{(ND_3)_2D^+ + Bet} \arrow[d, "+\mathrm{ND_3}"] \\ & \mathrm{BetD^+.ND_3} & \mathrm{(ND_3)_3D^+} \end{tikzcd} \tag{3b}$$

Second order effective rate coefficients were deduced from the linear part of the semilogarithmic decay of the $BetH^+$ [Fig. 3(a)] reacting via reaction (3a), k_{exp} (3a) $= (1.5 \pm 0.16) \times 10^{-9}$ cm^3/molecule s. The collision rate for $BetH^+$ with ND_3 has been calculated with the parametrized expression of Su and Chesnavich [15], $k_{1D} = 1.92 \times 10^{-9}$ cm^3/molecule sec. The reaction efficiency obtained is thus $r_{eff}(3a) \approx (1.5 \pm 0.16)/1.92 = 0.78 \pm 0.08$. These results are included in Table 1. The fractional abundances of the two primary products of reaction (3a), $BetD^+$ (isotope exchange) and $BetH^+.ND_3$ (or its isomers having the same mass to charge—*m/z*, ratios) are plotted as a function of the ND_3 flow rate in Fig. 4. Extrapolation of such plots to zero flow rate gives branching ratios of $BetD^+ = 96 \pm 1\%$ and $BetH^+.ND_3 = 4 \pm 1\%$. Provided the kinetic isotope effect is small, $k_{3D}/k_{-1D} = 3/1$, due to the statistical factor in the breakup of the $[Bet.ND_3H^+]^*$ salt–bridge complex in reaction (3a). We can estimate on this basis a reaction efficiency of $r_{eff}(3a) = 0.76$ in very good agreement with the estimate given above. According to this, 24.2% of the collisions lead to backreaction, 72.7% lead to isotope exchange and 3% lead to collisional stabilization of the salt–bridge complex. There is internal consistency, within experimental errors, between the results for the efficiencies of the reactions of $BetH^+$ with NH_3 and ND_3, respectively. The present data give reliable values for the thermal rate constants involved; they also yield for the first time information concerning the complexes formed in the process of H/D exchange.

The final set of results relates to CID of the salt–bridge complex. In a preliminary experiment, ions formed in the electrospray ion source were subjected to CID. In this experiment conditions were such that there was an excess of the betaine homodimer, $(Bet)_2H^+$ over the monomer, $BetH^+$. Under

CID the dimer is observed to dissociate (Fig. 5) at relatively high nose cone voltages in excess of ≈14V when a mixture of He and air is used as the carrier gas. By using N_2 as the carrier gas we could demonstrate that the threshold dissociation voltage observed is due to collisions with nitrogen. The dissociation threshold voltage for collisions with helium is outside of our presently available range of nose cone voltages (however, collisional dissociation with oxygen from the air introduced through ESI is possible). Production of the $BetH^+.NH_3$ complex is observed upon introduction of ammonia at a relatively high flow rate into the tube and CID of the ion mixture (Fig. 6) results in formation of $BetH^+$ at a considerably lower nose cone voltage of ≈5 V with a second rise at the higher voltages observed previously (Fig. 5). The low energy process is ascribed to CID of the $BetH^+.NH_3$ complex in the region between the nose cone and the exit from the flow tube,

$$BetH^+.NH_3 + M(N_2, O_2) \rightarrow BetH^+ + NH_3 + M(N_2, O_2) \quad (4)$$

whereas the high energy process is due to

$$(Bet)_2H^+ + M(N_2, O_2) \rightarrow BetH^+ + Bet + M(N_2, O_2) \quad (5)$$

It is known that the binding energy of the betaine homodimer (1.45 eV) is considerably higher [6] than that of the ammonia/betaine mixed dimer, since ammonia has a considerably lower gas phase basicity than betaine. The binding energy for dissociation of the ion/molecule complex according to reaction (4) is $0.5 \text{ eV} \leq D \leq 1.2 \text{ eV}$ [1,6]. It is thus not surprising that the CID dissociation threshold of the homodimer is considerably higher than that of the ammonia heterodimer (Figs. 5 and 6). An unscaled center-of-mass energy frame has been introduced [10] by multiplying the nose cone voltage by the mass factor $m_M/(M_{ion} + m_M)$, where m_M is the mass of the collision gas and M_{ion} is the mass of the parent ion undergoing CID. DT is defined as the dissociation threshold in this frame and while the SIFT-CID technique was found [10] to have only limited application in the determination of absolute or relative bond energies, the DTs deduced here for reactions (4) and (5) are 0.85 and 1.5 eV in fairly good agreement with the expected values. The betaine/ammonia heterodimer dissociates to NH_4^+ at a considerably higher threshold voltage (Fig. 6) than that of reaction (4), as is expected for the dissociation of the ammonia salt–bridge complex,

$$Bet.NH_4^+ + M \rightarrow NH_4^+ + Bet + M \quad (6)$$

Partial formation of NH_4^+ from CID of protonated ammonia homodimer ions present in the mixture cannot be excluded and the threshold voltage observed may be due to this latter reaction.

It has been demonstrated [6] that the time frame and the maximum collision energy have a profound effect on the product ratio for the dissociation of heterodimers of protonated betaine. There are energy/entropy tradeoffs and it is quite possible that high energy (kV), short time frame CID experiments could lead to preferential proton retention by ammonia although this is clearly not the case in the present experiment. Is the NH_4^+ stabilized betaine salt–bridge complex more stable than the ion/dipole $BetH^+.NH_3$ complex remains an open question. As noted earlier, calculations at the PM3 level indicate that this is the case [1]. On the other hand, the BIRD experiments [6] have indicated that when betaine is more basic than the reference base by ~2 kcal/mol or more, the ion/molecule conformation of the dimer is more favored. These experimental results have suggested [6] that the most stable structure of the ammonia/betaine dimer is the ion/molecule complex due to the significantly lower gas phase basicity of ammonia. The present CID results are in agreement with that conclusion in view of the retention of the proton by betaine observed in these experiments, albeit for relatively low energy CID. We are in the process of

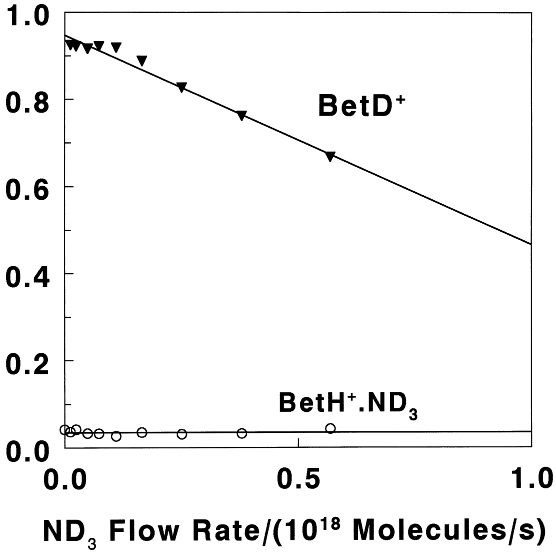


Fig. 4. Product ion distribution as a function of ND_3 flow rate for the reaction of protonated betaine with deuterated ammonia. The branching ratios for the primary product ions is shown. $BetD^+$ decays with increasing flow rate because of its consecutive reactions [see reaction (3b)], whereas collisionally stabilized $BetH^+.ND_3$ is unreactive.

calculating the protonated betaine/ammonia potential energy profile by density functional theory (DFT) methods, to obtain further insight and more quantitative information on the relative stabilities of the isomeric complexes [16].

4. Conclusions

The ability to study under thermal conditions the kinetics of formation of the proton bound complex of betaine and ammonia and of its role in the isotope exchange between protonated betaine and deuterated ammonia has been demonstrated in an electrospray flow tube experiment. The combination of the flow tube experiment with CID studies of the ion complex demonstrates the proton retention by betaine. The breakup into protonated betaine and neutral ammonia can be due to direct cleavage of the $BetH^+.NH_3$ ion/dipole complex, if this is the more stable isomer as indicated by BIRD experiments [6]. Alternatively, the salt–bridge complex, $Bet.NH_4^+$ could be the more stable structure [1]; if this is the case, low energy CID of the type used here could lead to proton transfer followed by dissociation of the ion/dipole complex.

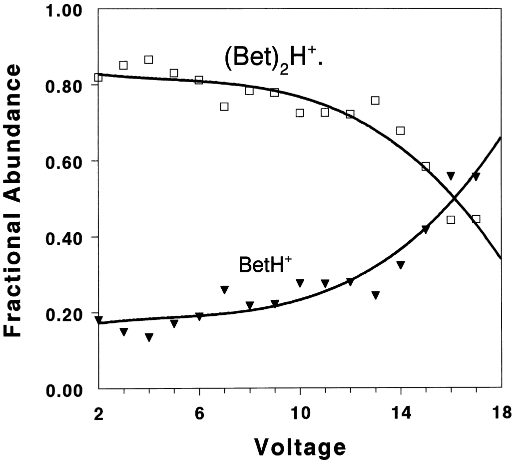


Fig. 5. Multicollision CID results for $(Bet)_2H^+$ on the laboratory energy scale. The measurements were performed at 292 ± 3 K and at a carrier/collision gas total pressure of 0.23 ± 0.01 Torr out of which 22% is air and 78% is He. The protonated homodimer of betaine and the protonated monomer are produced in the electrospray ion source and injected into the flow tube. The ion fractional abundances are plotted versus the sampling nose cone voltage.

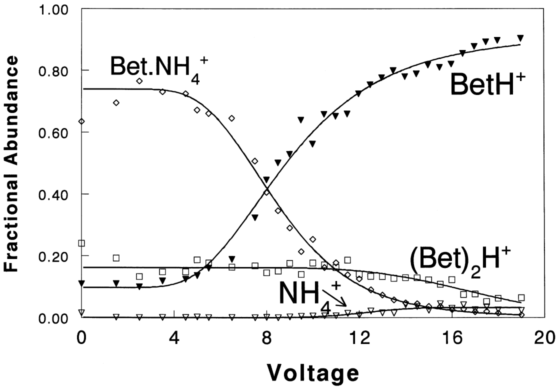


Fig. 6. Multicollision CID results for the betaine/ammonia salt–bridge complex, $BetH^+.NH_3/Bet.NH_4^+$, and for the $(Bet)_2H^+$ homodimer. The measurements were performed at 293 ± 3 K and at a carrier/collision gas total pressure of 0.23 ± 0.01 Torr out of which 22% is air and 78% is He. The betaine/ammonia complex was formed by reaction (1) in the flow tube. The ion fractional abundances are plotted vs. the sampling nose cone voltage. $Bet.NH_4^+$ (diamond); $(Bet)_2H^+$ (square); $BetH^+$ (closed inverted triangle); NH_4^+ (open inverted triangle).

Further flow tube experiments with other protonated betaine complexes are planned as are higher level DFT calculations. These should clarify some of the unresolved questions concerning the relative stability of the ion/dipole and salt–bridge complexes. We have studied two systems by the electrospray ionization—flow tube technique, diglycine [7,8] and betaine (present results), which have been studied previously by other methods. We have demonstrated the capabilities of our new technique to observe complexes of small protonated biomolecules with interesting solvent molecules, to study quantitatively their formation kinetics under truly thermal conditions, to study their hydrogen/deuterium exchange kinetics and to characterize them by collision induced dissociation. We now plan to apply this method to other more interesting biomolecules.

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