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ANALYTICAL CHALLENGE

Solution to collision induced dissociation mass spectrometry challenge

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Our congratulations!

Solution

All of the product ion peaks with *m*/*z* higher than the selected precursor *m*/*z* presented in the "Collision induced dissociation mass spectrometry challenge" [1] originate from ion–neutral reactions between analyte product ions and residual protic solvent molecules present in the mass spectrometer. In general, this is a well-documented phenomenon observed primarily, but not exclusively [2], in ion trap mass spectrometers operating at higher pressure and in which collision-induced dissociation (CID) experiments occur over a longer period of collisional activation compared with quadrupole CID [3, 4]. Depending on the instrument and the analyte, the origin of the solvent can be either residual neutral solvent molecules from electrospray ionization (ESI) [5, 6] or the collision gas [4, 7]. Unlike non-covalent solvent adducts or "clusters" formed during ESI that can be readily desolvated in the mass spectrometer inlet at lower energies than are typically required to break covalent bonds in CID, the ions under discussion represent the products of covalent bond forming ion–molecule reactions. In all the examples presented, the ions first undergo a loss of a neutral molecule (NH_3, H_2O, H_2 or glutamic acid) to produce a reactive product ion that then reacts with water or methanol to give a stable product ion higher in mass than the selected precursor, as detailed in Table 1.

The common structural feature of the reactive product ions that leads to solvent adduction in the examples presented is the ketene functional group (Structure 1).

Ketenes are strong electrophiles with partial positive polarization at the α position, and are susceptible to nucleophilic attack with electrophilic participation. In tandem mass spectrometry (MS/MS), reactive ions with ketene functionality can be formed during collisional activation through ring opening and/or neutral loss reactions that result in a C_β=C_α double bond being inserted adjacent to a carbonyl C_α=O. This is followed by nucleophilic attack by a neutral solvent molecule at the α position and electrophilic participation by the β-carbon or the oxygen [8, 9].

Guanine analogues are perhaps the best studied examples of ketene formation leading to ion–neutral reactions with water in mass spectrometry both as protonated ions and as deprotonated ions before and after dissociation [10–12]. During collisional activation, guanine undergoes tautomerization, ring opening and proton transfer reactions resulting in formation of ketene functionality. The most prominent of these occurs after a ring opening rearrangement accompanied by elimination of ammonia, as shown in Scheme 1. Reaction of this product ion with water leads to the significant peak 1 Da higher than the peak of the selected protonated guanine precursor [10, 11], as shown in Fig. 1 of the challenge [1]. Water adduction is also prominent in the CID of deprotonated guanine in negative ESI-MS/MS, which also results from ketene formation after ring opening [12].

The process by which water is eliminated from dicarboxylic acids was extensively studied by Grossert et al. [5], and involves intramolecular interactions between the ionized and non-ionized carboxyl groups. In the case of deprotonated dodecadedioic acid presented in Fig. 2 of the challenge [1], a +14-dalton peak was observed during

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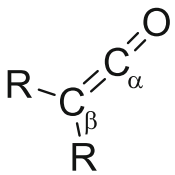
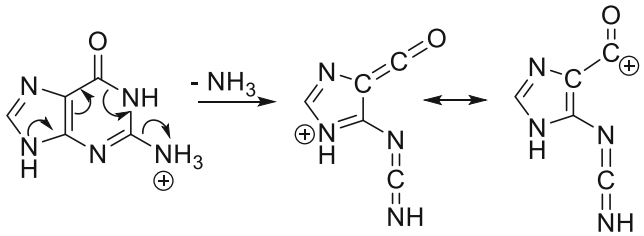
Table 1 Origin of solvent adducts observed in collision-induced dissociation challenge [1]

Analyte	MS/MS precursor (m/z)	Reactive product ion (m/z)	Solvent adduct (m/z)
Guanine	$[M + H]^+$ (152)	$[M + H - NH_3]^+$ (135)	$[M + H - NH_3 + H_2O]^+$ (153)
Dodecandioic acid	$[M + H]^+$ (229)	$[M + H - H_2O]^+$ (211)	$[M + H - H_2O + CH_3OH]^+$ (243)
3-Formylchromone	$[M + H]^+$ (175)	$[M + H - H_2]^+$ (173)	$[M + H - H_2 + H_2O]^+$ (191)
Folic acid	$[M + H]^+$ (442)	$[M + H - Glu]^+$ (295)	$[M + H - Glu + H_2O]^+$ (313)

MS/MS tandem mass spectrometry



Structure 1 Ketene functional group



Scheme 1 Dissociation of protonated guanine forming a reactive ketene

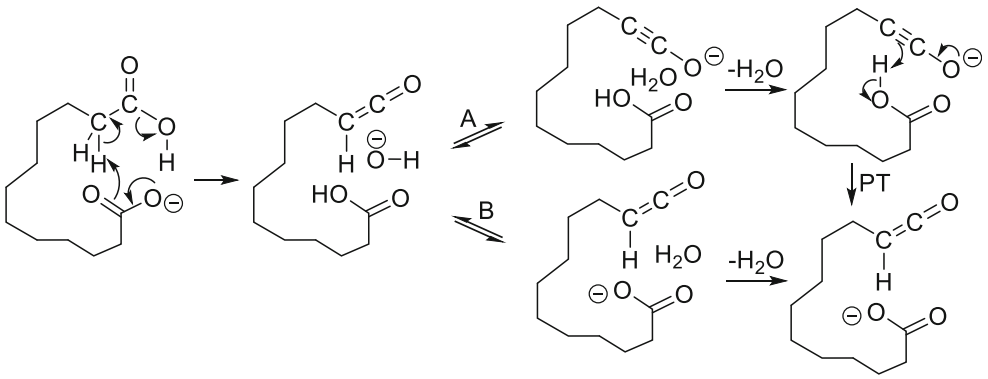
CID only when methanol was used as a solvent in ESI [5]. This can be attributed to formation of a reactive product of water loss during CID and subsequent ion–molecule reaction with residual methanol present in the ion trap. Grossert et al. focussed primarily on the formation of ynolate anions (these can be considered equivalent to ketene anions [13]), and ketene functionality is shown as part of an ion–neutral complex that acts as a precursor to the ynolate ion (Scheme 2, reaction A) [5]. Furthermore, ynolate anions do not undergo direct hydration, but instead rapidly rearrange to form ketenes that readily undergo hydration in solution [13, 14]. In MS/MS of deprotonated dodecanedioic acid, this could occur after formation of the proposed ynolate anion, as shown in reaction A in Scheme 2, or directly during dissociation of the ion–neutral complex, as shown in reaction B in Scheme 2. Intramolecular proton transfer and charge stabilization reactions are expected to be involved, and the potential for multiple product ion structures after methanol addition to the reactive product was also highlighted by Grossert et al. [5].

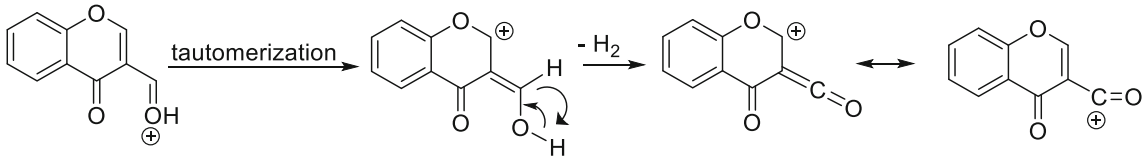
The simplest example of ketene formation presented was for 3-formylchromone, which shows another relatively uncommon loss for even-electron ions in CID: H_2 [7]. The product of H_2 loss reacts with water in CID to form the species corresponding to the +16-dalton peak at m/z 191 detected in CID of protonated 3-formylchromone, as shown in Fig. 3 of the challenge [1]. As shown in Scheme 3, elimination of H_2 from a 3-formylchromone tautomer yields a ketene that can react with water to give a carboxylic acid.

The most broadly applicable example presented was for sequential CID of folic acid, shown in Fig. 4 of the challenge [1], which reacts with water after elimination of a glutamic acid moiety in MS/MS [3]. In general, this reactivity can be expected for conjugated imines or esters where elimination of a good leaving group that can accommodate a charge occurs adjacent to a conjugated carbonyl group, as shown in Scheme 4 for folic acid.

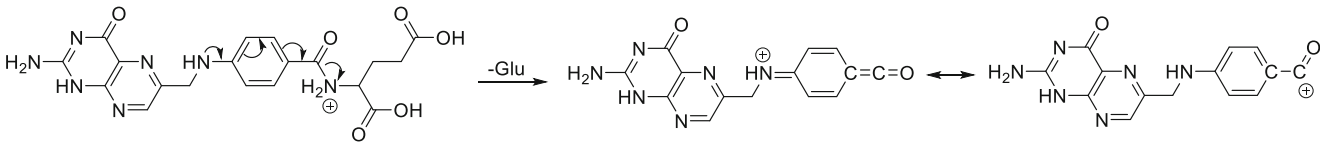
The hydration of neutral ketene has been extensively studied experimentally and theoretically, and two pathways of similar energy exist, leading to H_2O addition across the $C_\beta=C_\alpha$ bond to form a carboxylic acid, as shown in

Scheme 2 Pathwayys of water loss from deprotonated dodecanedioic acid. PT = proton transfer





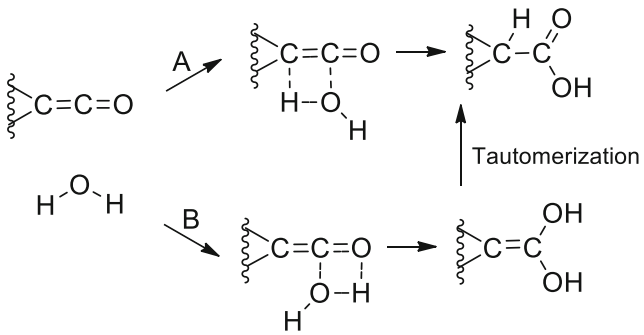
Scheme 3 Dissociation of protonated 3-formylchromone forming a reactive ketene



Scheme 4 Dissociation of protonated folic acid forming a reactive ketene

reaction A in Scheme 5, or across the C_{α}=O bond to form an enediol, as shown in reaction B in Scheme 5 [8, 9]. In solution, the less stable enediol product, which is formed through a slightly lower energy transition state, easily undergoes proton transfer to the more stable carboxylic acid through a solvent bridge involving two or more additional water molecules [8]. In the gas phase, vacuum in a mass spectrometer, where ion–neutral reactions are more likely to occur with a single water molecule, direct formation of a stable carboxylic acid by the mechanisms shown in reaction A in Scheme 5 could be considered more feasible. This could, however, be followed by proton transfer from the β-carbon to form an more stable carboxylic acid, which could be expected in cases such as 3-formylchromone, where chromone-3-carboxylic acid is detected as the final product of water adduction [7]. Because there often exist multiple feasible pathways for ketene formation (Scheme 2) and addition of solvent molecules to ketene (Scheme 5), in most cases further computational studies would be required to fully elucidate these complex mechanisms.

The reactions shown in Scheme 5 are for neutral ketene, and are presented as charge-remote processes. Thus, they apply equally to both protonated and deprotonated ions where ketene is formed during collisional activation, and indeed



Scheme 5 Addition of water to neutral ketene

many ions that react with water during CID do so as both protonated and deprotonated ions. However, the presence of charge and its localization on the reactive ketene centre clearly also plays an important role in the reactivity of ketene-containing ions in CID. In the case of cationic ketenes, a partial positive charge on the α-carbon further promotes nucleophilic attack at this centre. In the case of anionic ketenes, localization of the negative charge at the ketene moiety reduces the electrophilic character of the ketene, so neutral ketene can be considered the most reactive moiety. A simple model for assessing the potential for positive polarization of the α-carbon of a ketene is to examine the possible resonance structures for localization on the α-carbon, as shown for positive ions in Schemes 1, 3 and 4.

In conclusion, the reactivity of ions with residual solvent in CID is related to formation of specific, highly electrophilic functionality during CID. Examples from the literature currently include ketenes, as described here, as well as aryl cations, positively charged P, Si or Sn centres [3], halogen anions [4] and hydroxyl anions [15]. Solvent adducts can create problems with interpretation of MS/MS data or quantitation in targeted scan modes such as selected reaction monitoring [16], but with an understanding of their origin and properties, solvated product ions can also be highly diagnostic for the de novo identification of unknown compounds in non-target analysis [17]. An unfortunate trend has arisen in MS(/MS) spectral databases of cropping spectral data to within only a few daltons of the selected precursor *m*/*z*, which prevents the detection of many solvent adducts (+14, +16, +18, +32). In these cases, it is likely that potentially valuable information for the identification of unknown compounds is being discarded. Further work is needed to fully explore the full range of compounds and substructures that can react with residual solvents in the mass spectrometer, to fully elucidate the mechanistic details of these reactions and to exploit this phenomenon for identification of unknown compounds by CID.

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