



Mass spectrometric study of the dissociation of Group XI metal complexes with fatty acids and glycerolipids: Ag_2H^+ and Cu_2H^+ ion formation in the presence of a double bond

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ABSTRACT

Results of mass spectrometric studies are reported for the collisional dissociation of Group XI (Cu, Ag, Au) metal ion complexes with fatty acids (palmitic, oleic, linoleic and α -linolenic) and glycerolipids. Remarkably, the formation of M_2H^+ ions ($\text{M} = \text{Cu}, \text{Ag}$) is observed as a dissociation product of the ion complexes containing more than one metal cation and only if the lipid in the complex contains a double bond. Ag_2H^+ is formed as the main dissociation channel for all three of the fatty acids containing double bonds that were investigated while Cu_2H^+ is formed with one of the fatty acids and, although abundant, is not the dominant dissociation channel. Also, Cu(I) and Ag(I) ion complexes were observed with glycerolipids (including triacylglycerols and glycerophospholipids) containing either saturated or unsaturated fatty acid substituents. Interestingly, Ag_2H^+ ion is formed in a major fragmentation channel with the lipids that are able to form the complex with two metal cations (triacylglycerols and glycerophosphoglycerols), while lipids containing a fixed positive charge (glycerophosphocholines) complex only with a single metal cation. The formation of Ag_2H^+ ion is a significant dissociation channel from the complex ion $[\text{Ag}_2(\text{L}-\text{H})]^+$ where L = Glycerophospholipid (GP) (18:1/18:1). Cu(I) also forms complexes of two metal cations with glycerophospholipids but these do not produce Cu_2H^+ upon dissociation. Rather organic fragments, not containing Cu(I), are formed, perhaps due to different interactions of these metal cations with lipids resulting from the much smaller ionic radius of Cu(I) compared to Ag(I).

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1. Introduction

Lipids are important molecules that play a large role in biological systems. For example, triacylglycerols (TG) are involved in long-term energy storage and may also be linked to the cytotoxicity and dysfunction of pancreatic islet beta cells [1–3]. Glycerophospholipids (GPs) provide many biochemical functions due to their structural diversity. As well as providing structural support to cells, membrane phospholipids are the source of arachidonic acid, which is required for the formation of lipid mediators [4]. They also provide the precursors for platelet-activating factor molecules, and signaling molecules such as inositol triphosphate and diacylglycerol [4].

TGs and GPs are characterized by a glycerol backbone with linked substituents at each of the three carbons. TGs have three fatty acid substituents, while GPs contains a polar phospho-diester

group that is bound to one of the terminal positions of the glycerol. The hydroxyl moiety on the phosphate group provides the link to a polar head group. The structural diversity of TGs and GPs is attributable to different polar head groups (for GPs) and a wide variety of fatty acyl chains. Studies of structure and functionality of fatty acids are driven by their prominent role in functionality of the lipid family: one of the most abundant classes of molecules in biological systems. A fatty acid consists of a carboxylic group and a long hydrocarbon tail, but it is the nature of the hydrocarbon tail that is crucial in determining the function and properties of the fatty acid since most naturally occurring fatty acids are found predominantly in the esterified form. This includes the number and location of double bonds in the hydrocarbon tail, the stereochemistry of the double bond (*cis/trans*) and the hydrocarbon chain length [5]. Locating double bonds and determining their stereochemistry presents a special analytical challenge.

A substantial amount of information about fatty acids and lipids generally has been gathered from measurements of ion mass and fragmentation (arising from collision induced dissociation) using mass spectrometry together with electrospray ionization (ESI)

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techniques [6,7], but locating double bonds has remained an important challenge. Common mass spectrometric tools, such as low energy collision induced dissociation (CID), generally cannot drive fragmentation of hydrocarbon chains and thus fail to clearly identify the location of double bonds [8,9]. This can be overcome in the higher energy CID regime but because the resulting charge-remote fragmentation of the hydrocarbon chain of the fatty acid is not directed at any particular site the dissociation produces a large number of fragments that can be complex to interpret [10].

Complexes of fatty acids as well as more complex lipids with metal cations have previously been subject to mass spectrometric investigation. Sodium [11], lithium [11] and iron [12] ion complexes of fatty acid esters as well as di-lithiated complexes of deprotonated fatty acids [13,14] exhibit charge-remote fragmentation. Similar results are observed with alkaline earth metal complexes of deprotonated fatty acids [15]. Dissociation of TGs and GPs as sodium and lithium adducts have been explored and are employed routinely in analysis while the fragmentation of a number of transition metal complexes have also been reported [16–19].

Some of us have recently reported a method termed ozone induced dissociation (OzID) for the identification of double bond position in complex lipids via gas phase reactions of mass-selected lipid ions with ozone in an ion-trap mass spectrometer [20]. Preliminary observations suggest that the charge carrier (i.e., $[M+H]^+$, $[M+Li]^+$ or $[M+Na]^+$) can influence the reactivity of the unsaturated lipid ion towards ozone. We envisage that metal cations that attach to a double bond might be effective in catalyzing the oxidation and thus might enhance the efficacy of the OzID protocol. In order to facilitate a chemical reaction at the double bond, it would be necessary for the metal cation to have a strong interaction with the double bond. Since alkaline and alkaline earth metal ions have been shown previously not to interact strongly with double bonds in complexes with fatty acids [11,13,14], we chose a larger cation for our exploratory studies. Here we report, as a first step in possible future studies of metal assisted oxidation of double bonds in biological molecules, the results of our studies of complexes of Group XI metal cations with four fatty acids, palmitic, oleic, linoleic and α -linolenic, with increasing number (0–3) of double bonds (9-ocatdecanoic acid, 9,12-ocatdecadienoic acid, 9,12,15-ocatdecatrienoic acid). Copper, silver and gold provide more opportunities for binding with their larger d-orbitals, while the study of a group in the periodic table, rather than a random collection of metal cations, provides more insight into periodic trends in reactivity that might occur. The measurements also were extended to several different lipids, some containing unsaturated fatty acids.

2. Experimental

All experiments were performed using a modified MDS SCIEX QTrap 2000 instrument. Solutions of interest were subject to electrospray ionisation using a TurboSpray source at a typical electrospray voltage of 5 kV applied. The instrument was operated both in the triple-quadrupole (Q-q-Q) and the Q-q-LIT (linear ion trap) mode; the linear ion trap provided higher sensitivity and mass resolution when required. Four different fatty acids (palmitic, oleic, linoleic and α -linolenic), all commercially available from Sigma (99.9% purity), were used in our experiments; all were dissolved in methanol. These fatty acids have different levels of unsaturation, from no double bonds in palmitic acid to 3 double bonds in linolenic acid. Solutions of copper(I) chloride (Sigma, 99.95%), copper(II) nitrate (Sigma, 99.9%), silver(I) nitrate (Sigma, 99.9%) and gold(III) chloride (Sigma, 99.95%) in methanol were used as sources of Group XI metal cations. Different lipids 1,3-dipalmitoyl-2-oleoylglycerol (TG(16:0/18:1/16:0)), 1,2-distearoyl-sn-glycero-3-phosphocholine (PC(18:0/18:0)),

palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (PC(16:0/18:1)), 1,2-distearoyl-sn-glycero-3-phosphoserine (PS(18:0/18:0)) and 1,2-oleoyl-sn-glycero-3-phospho-*rac*-(1-glycerol) (PG(18:1/18:1)) were all purchased from Sigma, 98% purity. Lipid and metal solutions were typically 10–50 μ M, mixed in 1:1 ratio with methanol as the solvent. Lipid complexes containing 1 and 2 metal ions were analyzed in the positive mode of the ESI-MS.

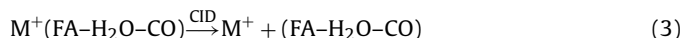
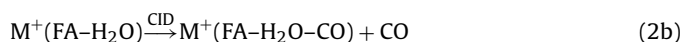
3. Results and discussion

Experiments were performed with a series of metal cations belonging to Group XI of the periodic table. Copper has two common oxidation states Cu(I) and Cu(II), both of which were part of this study. Only single oxidation states of silver and gold, Ag(I) and Au(I), were selected because they are the most common. Au(I) was derived via electrospray of a solution containing an Au(III) salt.

Two distinct classes of metallated ion complexes were observed in the course of our experiments: complexes of a single metal cation with one or more fatty acids and complexes of two or more metal cations with fatty acids. In the single-metal complexes of Cu(II) one of the fatty acids is deprotonated so that the net charge of the complex becomes 1+. Depending on the number of metal cations within the complex, one or more of the fatty acid molecules become deprotonated in order to reduce the net positive charge of the complex to 1+. No multiply charged complexes of Group XI metal cations with fatty acids observed under our experimental operating conditions.

3.1. Complexes of single Group XI metal cations with fatty acids

The complexes of single Group XI metal cations with fatty acids observed in our experiments appear to be mostly electrostatic in nature, with up to 3 acids attached to a metal cation. When subjected to CID, the complexes of Ag(I) and Au(I) dissociate to produce the metal cation (reaction (1)), with minor charge-remote fragmentation of the fatty acid chain (Fig. 1). Alternatively, sequential losses of water and carbon monoxide (reactions (2a) and (2b)) can precede the loss of the metal cation (reaction (3)).



Such a small presence of the charge-remote fragmentation channel is indicative of mostly electrostatic interactions with little electron withdrawal and little activation of the bonds adjacent to the metal cation-binding site.

Unlike the relatively simple behaviour shown by Ag(I) and Au(I) ions, both Cu(I) and Cu(II) almost exclusively produce charge remote fragmentation products. Copper or copper containing cations have not been identified conclusively as dissociation products in either case in our experiments due to overlap with numerous products resulting from charge-remote fragmentation, but we cannot rule out their presence in small amounts.

3.2. Complexes of multiple Group XI metal cation with fatty acids

Complexes of deprotonated fatty acids with more than one metal cation were observed with the three fatty acids containing one or more double bonds (oleic, linoleic and linolenic) but were absent with the only saturated acid in our study (palmitic acid).

The complexes, $M_n(FA-H)_{n-1}(FA)_{1,2}^+$ ($n=2, 3$), are likely created by interactions of M^+ cations with deprotonated and neutral carboxylic groups of the fatty acids. In each complex there is one

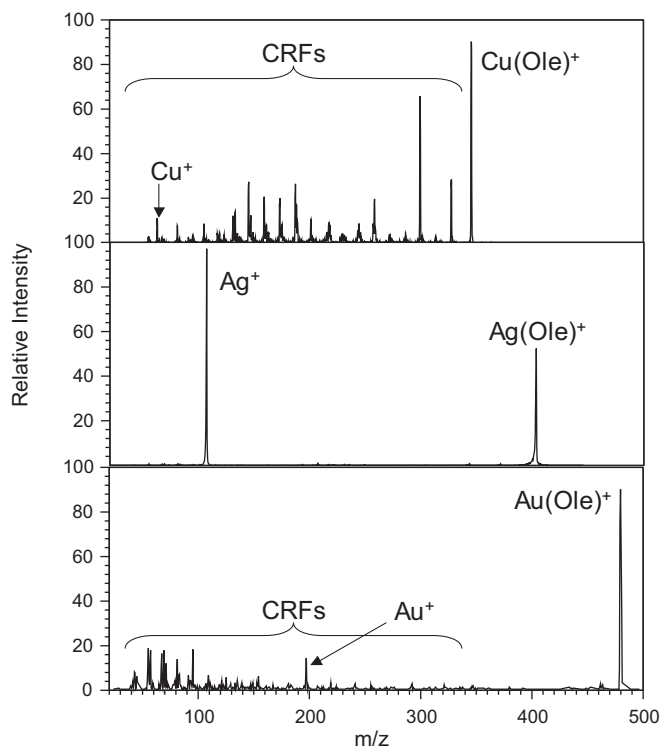
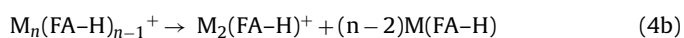
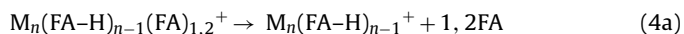


Fig. 1. MS² spectra of Group XI metal cation complexes with oleic acid. The spectra represent an average of spectra taken at collision voltages from 0 to 60 V in 5 V increments and so provide a more complete picture than a single collision energy spectrum would. CRFs indicate charge remote fragmentation products which are not identified individually for clarity. Individual MS² spectra at 30 and 60 V are provided in the Supplement (Figs. S1 and S2).

metal cation that is not bound to a deprotonated carboxylic group and this results in an overall charge of 1+. The extent of the complexation is expected to be highly dependant on the concentration of the fatty acid and the metal cation in solution, as well as the ratio between the two. The dissociation of complexes with more than one metal cation results in M₂(FA-H)⁺ cations (reactions (4a) and (4b)), mostly by loss of neutral fatty acids or in the sequential loss of M(FA-H) complexes.



M(FA-H) is sometimes lost as sequential loss of CO₂ and M(FA-H-CO₂) from M_n(FA-H)_{n-1}⁺, but this is a minor dissociation channel. It is interesting to note that Cu(II) also forms complexes with more than one metal cation present. Complexes of the type Cu₂(FA-H)₃(FA)_{0,1}⁺ were observed in which the higher oxidation state of the copper is offset by further deprotonation of the fatty acids. These complexes undergo loss of one or two neutral fatty acids, forming Cu₂(FA-2H)(FA-H)⁺. This ion dissociates further by loss of neutral (FA-2H) to produce Cu₂(FA-H)⁺ in which the two copper cations have been reduced from their 2+ oxidation state to a 1+ state.

As far as we can tell, M₂(FA-H)⁺ is the final product of the dissociation of the fatty acid complexes containing more than one metal cation, other possible products being below the detection limit. This ion is somewhat peculiar in that two transition metal cations are attached to a relatively small molecule, albeit with multiple sites for cation attachment. The fact that this type of complex is observed in our experiments only with unsaturated fatty acids suggests that the double bond plays an important role in strengthening the binding of the second metal cation. This is different from what is typically

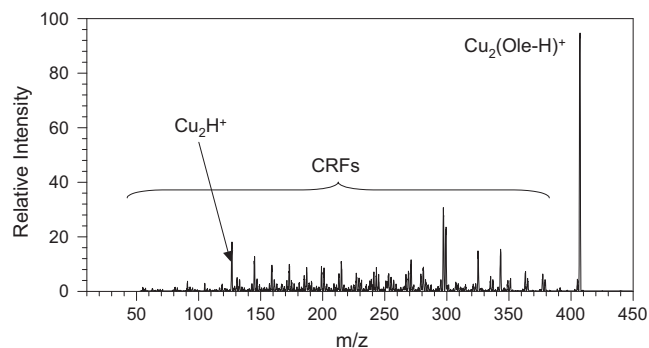


Fig. 2. MS² spectrum of the copper (I) complex Cu₂(Ole-H)⁺, with oleic acid, showing formation of Cu₂H⁺. The spectrum represents an average of spectra taken at collision voltages from 0 to 60 V in 5 V increments and so provides a more complete picture than a single collision energy spectrum would. CRFs indicates charge remote fragmentation products which are not identified individually for clarity.

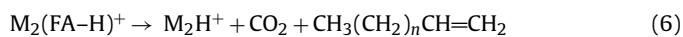
implied in anion-bridged complexes of some smaller metal cations such as lithium [13]. In such cases the metal cations are usually proposed to be each bound in a proton-like fashion to one of the carboxylic oxygens.

Dissociation of M₂(FA-H)⁺ cation produces two main channels, one is formation of a M₂H⁺ ion (reaction (5)) and the other is charge-remote fragmentation of the fatty acid chain.



The ratio of these two channels depends on the identity of M⁺. Copper shows M₂H⁺ formation only with oleic acid and even then there is still a significant presence of charge remote fragmentation (see Fig. 2). Fragmentation of silver complexes is dominated by M₂H⁺ formation with charge remote fragmentation products contributing only a minor channel (see Fig. 3). Gold exhibits a complete absence of M₂H⁺ formation. The identity of the M₂H⁺ ion is confirmed by the mass changes observed in the MS² spectra upon dissociation of different peaks in the M₂(FA-H)⁺ isotopic pattern (see Fig. 4).

Possible products in reaction (5) are the dehydrogenated fatty acid, FA-2H (one extra double bond) or CO₂ and an alkene hydrocarbon chain as shown in reaction (6).



In the dehydrogenated fatty acid the new double bond is likely to be conjugated with an existing double bond, providing extra stability for the product. However, this would require transfer of a second hydrogen atom from the location of the new double bond to the carboxylic group (one being transferred to form M₂H⁺), possibly posing a significant kinetic barrier to this process. On the other hand, CO₂ and alkene formation requires only one hydrogen atom to be transferred, that from the β-carbon in the fatty acid. Although the latter products might be less thermodynamically stable than the dehydrogenated fatty acid, they may be kinetically more favourable.

3.3. Complexes of single Group XI metal cations with glycerolipids

Single Cu(I) and Ag(I) cations form adducts with all the glycerolipids that were studied. Triacylglycerol (TG) complexes fragment in a similar manner, mainly by subsequent losses of two saturated fatty acid chains. This is followed by the loss of glycerol moiety (C₃H₅O) and formation of R'COOHM⁺ ion (R' = unsaturated). A minor, loss of neutral RCOO⁻M⁺, is observed with both metal cations. While both saturated and unsaturated fatty acid chains are represented with Ag(I), only unsaturated fatty acid salts are formed

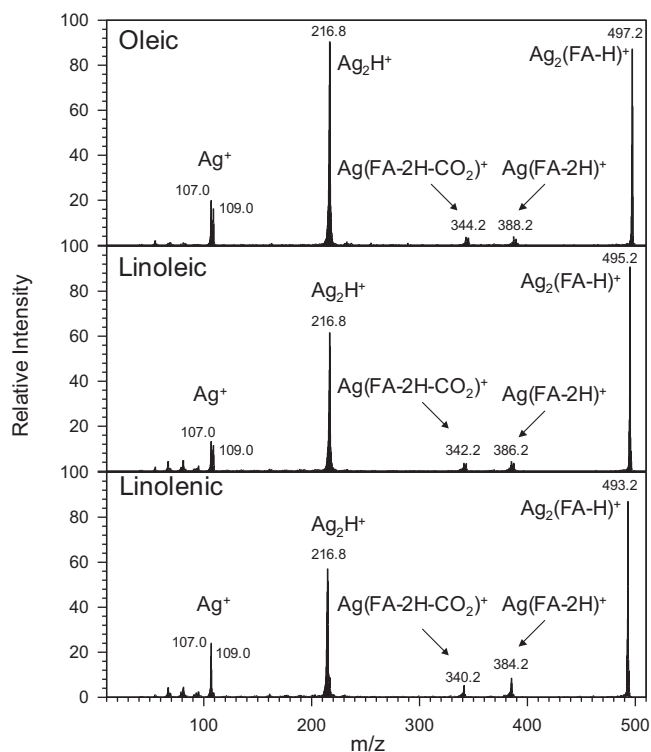


Fig. 3. MS² spectra of silver (I) complexes, Ag₂(FA-H)⁺, with three deprotonated unsaturated fatty acids, oleic (top), linoleic (middle) and α-linolenic (bottom). All three exhibit a high proportion of Ag₂H⁺ formation. The spectra represent an average of spectra taken at collision voltages from 0 to 60 V in 5 V increments and so provide a more complete picture than would a single collision energy spectrum.

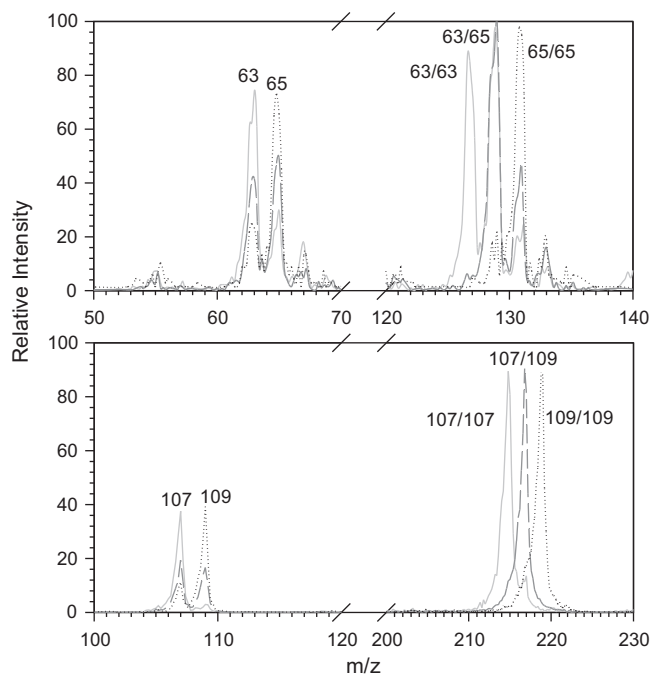


Fig. 4. Comparison of cation isotope patterns in the MS² spectra of silver(I) (bottom) and copper(II) (top) complexes with oleic acid. The solid gray line indicates spectra taken for Cu⁶³Cu⁶³ and Ag¹⁰⁷Ag¹⁰⁷ complexes, the dashed gray line indicates spectra taken for Cu⁶³Cu⁶⁵ and Ag¹⁰⁷Ag¹⁰⁹ complexes and the dotted black line indicates spectra taken for Cu⁶⁵Cu⁶⁵ and Ag¹⁰⁹Ag¹⁰⁹ complexes. Ag₂H⁺ and Cu₂H⁺ can be identified unambiguously from these spectra.

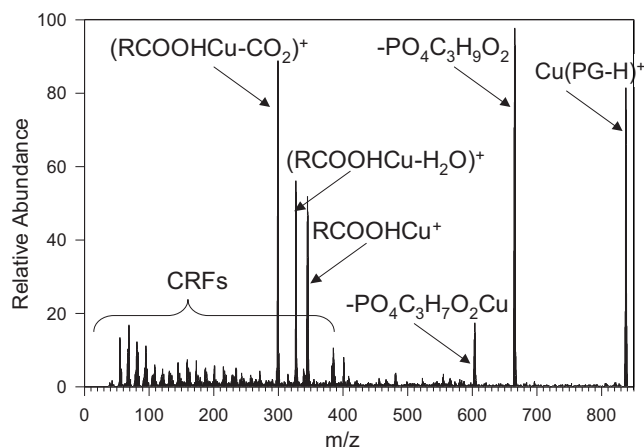


Fig. 5. A composite MS² spectrum of single copper(I) complex with PG(18:1/18:1). The spectrum represent an average of spectra taken at collision voltages from 0 to 130 V in 5 V increments to provide a more complete picture than a single collision energy spectrum would.

with Cu(I). These fragment further to produce various charge-remote fragments with sequences that are difficult to determine.

Complexation of silver to phospholipids was also studied including PC(18:0/18:0), PC(16:0/18:1), PS(18:0/18:0) and PG(18:1/18:1). All of these phospholipids complex with one silver cation and dissociate in a similar manner. The main channel is the loss of the headgroup, such as serine or choline, with the loss of the silver salt of the head group being the minor product. Further dissociation of [Ag⁺L-(head)] results in the neutral loss of one of the fatty acid substituents as a ketene rather than a carboxylic acid. The resulting product ion fragments readily to produce abundant RCOOHAg⁺ ion. Cu(I) provided similar results to Ag(I), but the intensity of the RCOOHCu⁺ ion was lower and the intensity of the various charge-remote fragments was higher with the latter presumably accompanied by the loss of neutral CuH (see Fig. 5). Loss of neutral CuH is also an indicator that (RCOOHCu-CO₂)⁺ ion likely contains Cu⁺ inserted into a C–H bond, rather than being a hydrocarbon complex of Cu⁺.

3.4. Complexes of two Group XI metal cations with lipids

Formation of lipid complexes with two metal cations was observed but only under very restricted conditions. The lipid had to possess at least one unsaturated fatty acid chain, an acidic headgroup to facilitate proton substitution with a metal and no other positively charged groups could be present in the molecule (such as choline headgroup). Of the lipids used PG(18:1/18:1) was the only one to fulfill all these requirements. The dissociation of the [Ag₂(PG-H)]⁺ shows two distinct channels (see Fig. 6). The first is identical to the one described for the dissociation of the [AgPG]⁺ complex, loss of a headgroup, this time as a silver salt (PO₄C₃H₆O₂Ag), followed by loss of the ketene form of a fatty acid substituent. The second dissociation channel is more interesting, with the cleavage occurring within the phosphate group (loss of PO₃C₃H₇O₂). The remaining fragment retains both silver cations and this facilitates formation of RCOOAg₂⁺ in the next step, leading to predominant formation of Ag₂H⁺.

The dissociation of the equivalent Cu(I) complex, [Cu₂(PG-H)]⁺, was observed to be different under similar operating conditions. The main dissociation channel is loss of the fatty acid chain, followed by the loss of a glycerol moiety (C₃H₈O₂). Further fragmentation leads to the formation of a fatty acid complex with two copper cations, RCOOCu₂⁺. Contrary to what was observed with the

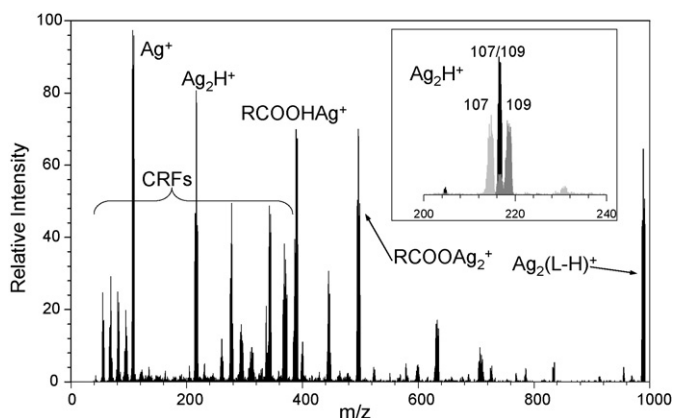


Fig. 6. A composite MS² spectrum of double silver(I) complex with PG(18:1/18:1) including all three silver isotopic combinations. Ag₂H⁺ peak is blown up, showing different isotopic combinations. The spectrum represent an average of spectra taken at collision voltages from 0 to 130 V in 5 V increments, for all three combinations of silver isotopes. This provides a more complete picture than a single collision energy spectrum would, while allowing for easy identification of silver-containing ions.

fatty acids, the RCOOCu₂⁺ ion created by the in-source dissociation of the [Cu₂(PG-H)]⁺ ion did not produce Cu₂H⁺, dissociating instead by the loss of CuH neutral followed by the loss of CO₂ and charge-remote fragmentation of the fatty acid chain. This indicates that the RCOOCu₂⁺ ions produced by the dissociation of [Cu₂(PG-H)]⁺ have different structures than those created from solutions of Cu(I) and fatty acids. The most likely modification that leads to preferential CuH loss is a consequence of either a hydrogen shift to one of the copper cations or of the insertion of copper into a C–H bond.

3.5. Trends in M₂H⁺ ion formation from Group XI metal cation complexes with fatty acids

The formation of Ag₂H⁺ ion from various silver complexes has been studied by Khairallah and O'Hair [21]. These authors found that multiple CID experiments on silver-amino acid clusters of the type [(M–H+Ag)_n+Ag] lead to the formation of Ag_n⁺ and Ag_{n-1}H⁺ clusters (*n*=3, 5 and 7). Their investigation showed that the counter ion, (M–H)⁻, in the cluster must have favorable redox properties to facilitate the reduction of Ag⁺ under CID conditions. This requirement is met and Ag₂H⁺ is formed upon CID in our study with the deprotonated fatty acid substituent of the lipid (RCOO⁻). However, we find that the copper cation Cu(I) with a redox potential of *E*=0.52 V is not as easily reduced upon CID as is Ag(I) with a redox potential of *E*=0.80 V. This makes formation of Cu₂H⁺ less favourable and puts it in competition with other channels such as CuH formation or charge-remote fragmentation. In the case of the [Cu₂(PG-H)]⁺ cluster, for which no Cu₂H⁺ formation is observed from the RCOOCu₂⁺ cluster, structural changes appear to be induced in the CID process that make reduction unfavourable. Of the three Group XI metals, gold, even though it has the highest redox potential (1.69 V), is handicapped by a much higher ionization energy (9.2 eV compared to ~7.7 for Cu and Ag). This likely makes formation of Au₂H⁺ prohibitive compared to other channels, such as charge-remote fragmentation.

4. Conclusions

The most interesting feature of the dissociations of Group XI metal ion complexes with the lipids investigated here is the appearance of the M₂H⁺ ion (with M=Cu, Ag). This ion is tied to the presence of the double bond in the fatty acid chain and is especially

selective with the Ag(I) ion. It is the main dissociation product with all three of the unsaturated fatty acid complexes in our study. Formation of M₂H⁺ ion in glycerolipids is observed with silver only and is influenced by the presence of other functional groups in the glycerolipid. There appears to be a strong preference towards formation of singly charged complexes, requiring deprotonation in order to attach a second metal cation. In two PC glycerolipids (16:0/18:1 and 18:0/18:0) we propose that the formation of the complex with two Ag(I) cations is prevented by the presence of the positively charged choline headgroup, while TG glycerolipid lacks a deprotonation site. Of the glycerolipids studied only PG (18:1/18:1) formed the complex with two Ag(I) cation and dissociated form Ag₂H⁺. Direct comparison in our experiments of the influence of unsaturation on the formation of the glycerolipid complex with two metal cations was only possible with the two PC glycerolipids, one saturated and one unsaturated, neither of which formed the complex. Despite this, we propose that there is enough similarity between saturated PS and unsaturated PG glycerolipids to infer that, similar to fatty acid observations, complex with two metal cations is facilitated by the presence of the fatty acid double bond.

Formation of the M₂H⁺ ion suggests that the larger size of the transition metal cation allows for concomitant coordination to the double bond and the site of the carboxylic group. Since formation of the M₂H⁺ ion appears to be dependant on the ionization energy of the respective metal, it is possible that all three Group XI metal cations exhibit similar binding (to both carboxylic and double bond sites) and that formation of the M₂H⁺ ion is governed by thermodynamic and not structural criteria. It remains to be seen whether the interaction of an atomic metal cation with the double bond in a fatty acid can be utilized to direct and catalyze functionalization of the double bond by gas-phase ion/molecule reactions, with a strong oxidant such as ozone, for example.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ijms.2010.09.031](https://doi.org/10.1016/j.ijms.2010.09.031).

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