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Positive and negative ion electrospray mass spectrometric studies of some amino acids and glutathione, and their interactions with alkali metal ions and methylmercury(II)

Allan J. Canty[a,*], Ray Colton[b], Antonella D'Agostino[b], John C. Traeger[b]

[a]*Chemistry Department, University of Tasmania, Hobart, Tas. 7001, Australia*
[b]*School of Chemistry, La Trobe University, Bundoora, Vic. 3083, Australia*

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Abstract

Positive and negative ion electrospray mass spectrometry (ESMS) has been used to investigate solutions of some simple amino acids (glycine, histidine, methionine and cysteine) and the small peptide glutathione. Negative ion ESMS detected the simple anions [AA-H$^-$] in each case (AA = amino acid) and often the dimeric species [(AA)$_2$-H]$^-$ were also observed. In the positive ion mode ESMS allowed the observation of the protonated cluster ions [H(AA)$_x$]$^+$ (x = 1–4). Some interactions between alkali metal ions and the amino acids were observed, but in general the peaks in the ES mass spectra were weak and the alkali metal complexation could not compete with protonation. In contrast, methylmercury(II) reacted strongly with the amino acids and glutathione. In addition to species of 1:1 stoichiometry, all the amino acids gave peaks in their ES mass spectra corresponding to the ions [(MeHg)$_2$(AA-H)]$^+$, while glutathione gave a clear indication of the formation of a 3:1 species.

Keywords: Electrospray mass spectrometry; Metal ion complexes; Mercury complexes; Alkyl complexes; Amino acid complexes

1. Introduction

Electrospray mass spectrometry (ESMS) is a new technique which allows the very gentle transfer of ionic species from solution to the gas phase, where they can then be examined by conventional mass spectrometric methods. The technique was largely developed by Yamashita and Fenn [1,2] and it has had enormous impact in biochemistry since it allows large molecules, such as proteins, to be readily observed by mass spectrometry. The protein in solution is multiply protonated by the mobile phase and a series of peaks corresponding to the intact protein with varying degrees of protonation is observed, from which the molecular weight of the protein can be deduced [3–6]. The application of ESMS to inorganic, organometallic and simple organic systems has so far been relatively neglected, and although many proteins and large molecules have been examined by ESMS, the simple amino acids have also been neglected. The first part of this paper therefore deals with the positive and negative ion ES mass spectra of several amino acids and the small peptide glutathione.

We are interested in the interaction of metal species, especially methylmercury(II), with biological substrates. The interaction of methylmercury(II) with sulfur containing amino acids, peptides, and larger biomolecules is a key process in the biological behaviour of mercury. Major contributions to understanding of these processes in the solid state have involved X-ray crystallographic studies [7–10]. For example, an X-ray structural study of the 2:1 complex of penicillamine (Structure 1) has been reported [10], and phenylmercury(II) penicillamine and cysteine analogues of this complex have been isolated [11].

The estimation of the stoichiometries and stability constants for methylmercury(II)/amino acid complexes in solution has involved a variety of techniques, including potentiometric [12,13] and NMR methods [14–17], but direct observation of species in solution has been dif-

*Corresponding author.

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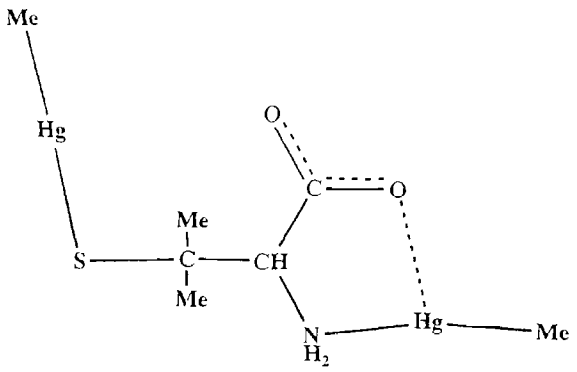

ficult. Potentiometric titrations of amino acids and glutathione in the presence and absence of methylmercury(II) compounds have indicated the formation of 1:1 complexes in all cases, but often the computational models for solution behaviour provide best agreement with experimental results when polynuclear species are included. For example, with the sulfhydryl amino acid cysteine, the complexes $MeHgSCH_2CH(NH_2HgMe)CO_2$, $[MeHgSCH_2CH(NH_2HgMe)CO_2H]^+$ and $[(MeHg)_2SCH_2CH(NH_3)CO_2]^+$ are suggested for the solution speciation, in addition to species of 1:1 stoichiometry [13].

Although NMR has been used to detect binding of methylmercury(II) to hemoglobin [17] and to glutathione in human erythrocytes [18], the NMR method of determining the composition of the complexes formed in solution is also indirect. The systems are extremely labile on the NMR time scale so exchange competition between the amino acid and a mercury thio derivative, which gives rise to exchange average signals, has been used to study complexation of amino acids with methylmercury(II). The variation of the positions of these average signals provides evidence for both the number of methylmercury(II) groups attached to the amino acid and also the site of coordination. Thus for cysteine, the first methylmercury(II) is attached to sulfur and the coordination site of a second MeHg(II) group is pH dependent between sulfur and nitrogen [15]. However, although spectroscopic detection of the mode of binding of one organomercury(II) group to a range of sulfur containing amino acids and glutathione is well documented, interactions involving more than one organomercury(II) group are less well understood.

We have recently demonstrated that ESMS can readily observe simple cationic methylmercury(II) complexes as their intact ions [19], so it seemed likely that the technique could provide useful information on the speciation in solution of methylmercury(II) complexes of amino acids. The second part of this paper therefore describes an ESMS investigation of the interaction of small biomolecules with alkali metal cations and methylmercury(II). The amino acids used in this study were glycine (Gly), histidine (His), L-methionine (Met), L-cysteine (Cys), together with the small peptide glutathione (Glu). The general symbol AA will be used for all of these biomolecules.

2. Experimental

The methylmercury(II) complex of *N*-methylpyrazole (Mepz), $[MeHg(Mepz)]NO_3$, was prepared as described [20] and used as a source of methylmercury(II). The pyrazole ligand is a very weak donor and readily displaced, and the ionic formulation (non-volatile) and high crystallinity of this compound reduces the hazards associated with manipulations of highly toxic methylmercury(II) compounds.

Electrospray mass spectra were obtained with a VG Bio-Q triple quadrupole mass spectrometer (VG BioTech, Altrincham, Ches., UK). Positive ion mass spectra were acquired using a water/methanol/acetic acid (50:50:1%) mobile phase and the procedures described previously [19]. A mobile phase of 50:50 water/isopropanol was used for negative ion ES mass spectrometry as this medium has recently been shown to best suppress corona discharge in the ion source, with resultant enhanced negative ion ES response [21]. Unless stated otherwise ES mass spectra were recorded with a low voltage (40 V) on the first skimmer electrode (B1) to minimise fragmentation. Increasing the voltage on B1 enhances the formation of product ions by collisional activation within the ion source.

3. Results and discussion

All peaks in the ES mass spectra are identified by the most abundant *m/z* value in the isotopic mass distribution. In all cases the experimental and calculated isotopic mass distributions were in excellent agreement.

Negative ion ES mass spectra of the biomolecules were examined using water/isopropanol (50:50) as the mobile phase. In all cases the intact monoanion was the base peak in the mass spectrum but there was usually another peak of reasonable intensity which can be assigned to the ion $[(AA)_2\text{-}H]^-$. These species may be regarded as an amino acid anion hydrogen bonded to another amino acid molecule or, equivalently, two amino acid anions hydrogen bonded to a proton. These weakly bonded cluster ions were not observed at higher collision energies. Fig. 1(a) shows the negative ion ES mass spectrum of L-histidine as a typical example.

Positive ion ES mass spectra of the biomolecules in aqueous methanol were examined using water/methanol/acetic acid (50:50:1%) as the mobile phase. In each case the intact ion of the singly protonated species gave rise to the base peak in the mass spectrum. At relatively low B1 voltages (40–50 V), all of the bio-

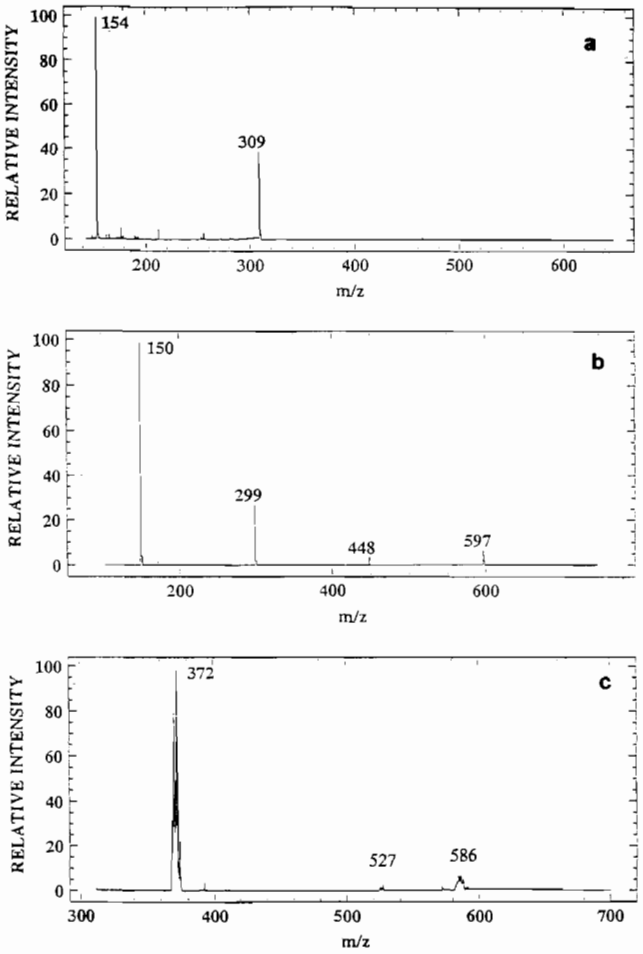

Fig. 1. (a) Negative ion ES mass spectrum of L-histidine. (b) Positive ion ES mass spectrum of L-methionine. (c) Positive ion ES mass spectrum of a solution containing L-histidine and $[MeHg(Mepz)]^+$.

molecules gave rise to cluster ions containing several amino acid molecules hydrogen bonded to a proton. However, peaks due to these ions were relatively weak and disappeared at higher collision energies. Fig. 1(b) shows the positive ion ES mass spectrum for L-methionine with peaks assigned to ions consisting of one (*m/z* 150), two (*m/z* 299), three (*m/z* 448) and four (*m/z* 597) methionine molecules attached to a proton. Positive ion ESMS data for all the biomolecules are summarised in Table 1.

There are several systems known in which alkali metal cations, as well as protons, may be added to generate species which may then be observed by ESMS. Examples include polyethylene glycols [22], polydentate oxygen donor ligands [23] and neutral transition metal complexes of Schiff bases [24]. Addition of NaCl and KCl solution to the biomolecules was therefore investigated and the data are summarised in Table 2. In general, the alkali metal adducts gave rise to peaks which were weak relative to the protonated cations. For some biomolecules, alkali metal adducts were not observed.

Table 1
Negative and positive ion electrospray mass spectra for amino acid species

Amino acid	Negative ions (*m/z*)	Positive ions (*m/z*)
Glycine (Gly)	$[Gly\text{-}H]^-$ (74) $[(Gly)_2\text{-}H]^-$ (149)	$[HGly]^+$ (76) $[H(Gly)_2]^+$ (151) $[H(Gly)_3]^+$ (226)
Histidine (His)	$[His\text{-}H]^-$ (154) $[(His)_2\text{-}H]^-$ (309)	$[HHis]^+$ (156) $[H(His)_2]^+$ (311) $[H(His)_3]^+$ (466)
L-Methionine (Met)	$[Met\text{-}H]^-$ (148) $[(Met)_2\text{-}H]^-$ (297)	$[HMet]^+$ (150) $[H(Met)_2]^+$ (299) $[H(Met)_3]^+$ (448) $[H(Met)_4]^+$ (597)
L-Cysteine (Cys)	$[Cys\text{-}H]^-$ (120)	$[HCys]^+$ (122) $[H(Cys)_2]^+$ (243) $[H(Cys)_3]^+$ (364)
Glutathione (Glu)	$[Glu\text{-}H]^-$ (306)	$[HGlu]^+$ (308) $[H(Glu)_2]^+$ (615)

In contrast, positive ion ES mass spectra of the amino acids in the presence of $[MeHg(Mepz)]^+$ indicated replacement of the labile *N*-methylpyrazole ligand and strong coordination of the mercury fragment to the amino acid. In all cases, the peak due to the intact ion $[MeHg(AA)]^+$ was the base peak, although other weaker peaks due to $[MeHg(AA)_2]^+$ and $[(MeHg)_2(AA\text{-}H)]^+$ were also observed. Fig. 1(c) shows the positive ion ES mass spectrum of L-histidine in the presence of $[MeHg(Mepz)]^+$. The base peak at *m/z* 372 is due to $[MeHg(His)]^+$, whilst those at *m/z* 527 and 586 are assigned to $[MeHg(His)_2]^+$ and $[(MeHg)_2(His\text{-}H)]^+$, respectively. However, it should be noted that in ES mass spectra the relative peak heights are not necessarily a measure of relative concentrations. Although the peaks at *m/z* 527 and 586 are small relative to that at *m/z* 372, they do in fact have a reasonable signal to noise ratio to the base line. Data for ions observed following other reactions of $[MeHg(Mepz)]^+$ are summarized in Table 2.

ESMS cannot distinguish between isomers so the stoichiometries of methylmercury(II):biomolecule species are tabulated in a manner that does not indicate the sites of $MeHg^+$ coordination. The strongest peaks for MeHg(II) compounds correspond to 1:1 species, and the binding of mercury to the biomolecules in these is expected to be as reported from structural studies of model complexes, e.g. binding to cysteine as $[MeHgSCH_2CH(NH_3)CO_2H]^+$. Polynuclear species are detectable for all of the biomolecules studied, but the relevant peaks are of low intensity relative to those of the 1:1 adducts except for the glutathione species which gives rise to a relatively intense peak for the 2:1 (*m/z* 738) adduct and a moderate peak for the 3:1

Table 2
Positive ions observed in ES mass spectra after addition of metal ions to amino acids

Amino acid	Alkali metal adducts (m/z)	$MeHg^+$ adducts (m/z)
Gly		$[MeHg(Gly)]^+$ (292); $[MeHg(Gly)_2]^+$ (367); $[(MeHg)_2(Gly\text{-}H)]^+$ (506)
His	$[Na(His)]^+$ (178)	$[MeHg(His)]^+$ (372); $[MeHg(His)_2]^+$ (527); $[(MeHg)_2(His\text{-}H)]^+$ (586)
Met	$[Na(Met)]^+$ (172) $[K(Met)]^+$ (188)	$[MeHg(Met)]^+$ (366); $[MeHg(Met)_2]^+$ (515); $[(MeHg)_2(Met\text{-}H)]^+$ (580)
Cys	$[Na(Cys)]^+$ (144) $[K(Cys)]^+$ (160)	$[MeHg(Cys)]^+$ (338); $[MeHg(Cys)_2]^+$ (459); $[(MeHg)_2(Cys\text{-}H)]^+$ (552)
Glu		$[MeHg(Glu)]^+$ (524); $[(MeHg)_2(Glu\text{-}H)]^+$ (738); $[(MeHg)_3(Glu\text{-}2H)]^+$ (952)

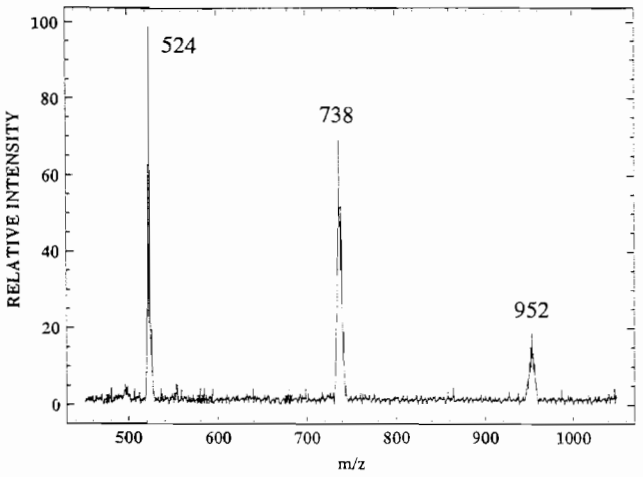

Fig. 2. Positive ion ES mass spectrum of a solution containing glutathione and $[MeHg(Mepz)]^+$.

adduct (m/z 952), as shown in Fig. 2. Polynuclear MeHg(II):glutathione species have been previously postulated in NMR studies [15], but this work provides the first direct observation of such species.

4. General discussion

Positive and negative ion ES mass spectra of the amino acids can be observed readily because they are all weak acids so both anionic and cationic species, as well as the neutral amino acids, are all present simultaneously in the same solution.

The ES mass spectra of the solutions of the amino acids with added methylmercury(II) provide substantial new information on the speciation in such solutions. Methylmercury(II)/amino acid complexes of 1:2 stoichiometry have been postulated to exist in solution from potentiometric measurements, but these suggestions have been based upon the best fits between experimental data and computational models which make assumptions on the identities and concentrations of various 1:1 species at various pHs. This work provides more direct evidence for their existence. However in all cases, including the simplest amino acid glycine, the ES mass spectra also showed the presence of 2:1 methylmercury(II) amino acid complexes. In the case of glycine, the second methylmercury(II) group can only be coordinated to the carboxylate group. Carboxylate anions coordinated to mercury have been observed previously in ES mass spectra [25]. However, in all the potentiometric and NMR studies of methylmercury(II) amino acid complexes only complexation to sulfhydryl and amine groups has been considered, so these ESMS results introduce new considerations into the binding of MeHg(II) to amino acids. Mercury coordination does not occur at all the potential sites for some of the amino acids. For example, with cysteine only two methylmercury(II) groups coordinate to give $[(MeHg)_2(Cys\text{-}H)]^+$, and not three to form $[(MeHg)_3(Cys\text{-}2H)]^+$ under the pH conditions of the mobile phase. However, three methylmercury(II) groups are clearly coordinated to glutathione, but no indication of this complex was obtained from the potentiometric measurements.

It should also be noted that for each methylmercury(II) complex tabulated, there may well be the corresponding deprotonated species also present in solution, which cannot be detected by ESMS. Thus for example, $[MeHg(His)_2]^+$ is detected by ESMS, but the neutral MeHg(His)(His-H), which may also be present in amounts depending on the pH of the solution, will not be observed. Thus it is apparent that the speciation in these solutions is extremely complex.

The results reported here indicate that electrospray mass spectrometry is a suitable method to probe the interaction of organomercury(II) species and other metallic cations with amino acids and peptides.

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