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A Study of Formation and Fragmentation of Ionic Complexes of α-Amino Acids and Peptides with Al(III)–Glycerol by Fast Atom Bombardment Mass Spectrometry

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Complexation of aluminum ions {Al(III) in glycerol} with α-amino acids has been studied by FAB mass spectrometry. Twelve α-amino acids and cysteine methyl ester interact with different Al(III)-containing ions and form several cluster ions such as $[M+117]^+$, [117 = {Al(III) + glycerol − 2H}]; $[M+231]^+$, [231 = {Al(III) + glycerol + trifluoroacetic acid − 2H}]; $[M+233]^+$, [233 = {2Al(III) + 2glycerol − 5H}]; $[2M+Al-2H]^+$ and $[2M+117]^+$. Fragmentation of these adduct ions led to the formation of mainly metal-containing product ions. Fragmentation pathways were demonstrated with the help of the dissociation of Leu-d_3, His-d_4, Tyr-d_4, Ser-d_4, Cys-d_4 and Cys methyl ester-d_3 ion complexes in glycerol-d_3. Aliphatic, aromatic and α-amino acids possessing functional side chains show differences in the fragmentation of $[M+117]^+$ ions. Fourteen peptides of different chain lengths (two through six amino acids) also form adducts with Al(III)-glycerol. Dissociation of these complex ions, especially $[M+Al-2H]^+$ ions provides information about side-chain functional groups and some sequence information. The site of the metal-ion interaction is mostly at the amide nitrogens.

The study of metal ion interactions with biologically important molecules in the gas phase is of current interest. Gas phase reactions of ions of alkali metals,[1–7] alkaline earth metals[8,9] and transition metals[9e,10,11] with peptides have been studied. The interaction of metal ions with α-amino acids, the essential constituents of peptides, can also provide information regarding the site of coordination. However, there are few reports of this type of study in the gas phase.

Bouchonnet and co-workers[12] studied the formation and fragmentation of complexes of six aliphatic α-amino acids with Co^+, Ni^+, Cu^+ and $FeCl^+$, using plasma desorption mass spectrometry. Organometallic ions such as $[M+CatCl-H_2CO_2]^+$ and $[M+Cat-H_2CO_2]^+$ which originate from $[M+Cat]^+$ (M = α-amino acid, Cat = transition metal ion), are analogous to $[MH-CO_2H_2]^+$ ions formed from $[M+H]^+$ ions of α-amino acids.[12,13] Recently, Wen *et al.* reported[14] the fragmentation reactions of Cu-cationated α-amino acids, and suggested that there are three pathways for the elimination of (H_2, C, O_2), viz., direct elimination of (H_2CO_2), elimination of (H_2O+CO), and elimination of (CO_2+H_2) from the cationized species. Complexation of amino acids with each of Cu(II), Co(II), Ni(II) and Zn(II), plus diimine ligands such as 2,2′-bipyridyl (bipy), were studied using electrospray ionization by Gatlin and co-workers.[15–18] The dissociation of these {Cu(II)[M − H](bipy)} complexes results in the loss of CO_2, producing ions which, on further fragmentation, show the fission of C_β-C_γ- bonds in aliphatic amino acids.[16] This is in contrast with α-amino acids which have hydroxy, sulfide and disulfide groups transferred to the Cu(bipy). The side-chain functional groups and the $\{C\beta H_2{=}C\alpha HNH_2\}$ fragments compete for coordination to Cu(bpy) in product-ion formation.[17] However, the ε-amino group from lysine, and guanidyl group from arginine do displace the bipyridyl. In the present study, we explore, for the first time, the complexation of α-amino acids with Al(III) in a glycerol-based matrix, and the decomposition of the resulting Al(III)–glycerol–amino acid complexes by fast-atom bombardment (FAB) mass spectrometry.

EXPERIMENTAL

All experiments were carried out using a Kratos (Manchester, UK) Concept 1S double focusing (EB geometry) mass spectrometer. Samples were prepared by dissolving α-amino acids (2–3mg), $AlCl_3$ (~2–3 mg) and trifluoroacetic acid (TFA), and water, 2 μL each in glycerol (~25–30 mg). Peptides (~1mg) were mixed in a glycerol (~10–15mg) matrix containing $AlCl_3$ (1–2mg), TFA (1μL) and water (1μL). Each mixture (1μL) was placed on a stainless-steel probe tip and bombarded with 6–7kV xenon atoms generated with an emission current of 0.1–1mA. The source pressure was maintained at $1-2\times10^{-5}$ torr. The ion beam was then accelerated through 8kV, and the mass spectra were obtained at a resolving power of 1100 (10% valley definition). The spectra (8–10 scans) were averaged and the background was subtracted using a Kratos DART/MACH3 data system. The system was calibrated with 2,4,6-tris(perfluoroheptyl)-1,3,5-triazine.

The Fast-atom bombardment (FAB) spectra recorded for equimolar solutions (all solutions made up to the same molar concentrations) were obtained using identical instrumental conditions, and these samples were prepared by dissolving equal volumes of equimolar solutions of each α-amino acid in constant amounts of glycerol (~25–30 mg), water (2μL) and TFA (2μL) with approximately equal amounts of $AlCl_3$ (~2mg). Linked scan analyses were run by keeping constant B/E for recording product ions and constant B^2/E for recording precursor ions. Helium was used to achieve the attenuation of the main beam by 45–55% so that the collisionally-activated dissociation (CAD) spectra could be recorded. High resolution measurements for the $[His+117]^+$ ion were made at 10 000 (10%

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valley definition) resolving power.

All amino acids (Parish, Vineyard, UT, USA) and peptides (Sigma, St. Louis, MO, USA) were commercially available and were used as received. $AlCl_3$, $GaCl_3$, and glycerol were obtained from Aldrich Co. (Milwaukee, WI, USA). Leu-d_3, His-d_4, Tyr-d_4, Trp-d_4, Ser-d_4, Cys-d_4, Cys methyl ester-d_3 and glycerol-d_3 were prepared by repeating the addition and evaporation of deuterium oxide 2-3 times.

RESULTS AND DISCUSSION

Mass spectra of α-amino acids obtained from $AlCl_3$+glycerol+TFA+water

FAB ionization of a mixture of aluminum chloride in glycerol with a few microliters of TFA generates $[Al(III)]+glycerol-2H]^+$, $[Al(III)+2glycerol-2H)^+$, $[Al(III)+TFA+glycerol-2H]^+$ and $[2Al(III)+2glycerol-5H)]^+$ ions at *m/z* 117, 209, 231 and 233 respectively, together with species derived from these ions by substitution of one hydroxy hydrogen by $(Al(III)+glycerol-3H)$ and with TFA molecules.[19] These interpretations are based in part on deuterium substitution experiments, viz. the shifts in the *m/z* values observed in the spectra of $AlCl_3$+glycerol-d_3+TFA+D_2O mixtures.[19]

FAB ionization of various α-amino acids, added to the mixture of $AlCl_3$, glycerol and TFA, produces many cluster ions in addition to the formation and fragmentation of the protonated molecules. The α-amino acids investigated here may be grouped as Gly, Ala, Val, Leu and Pro, (i.e. aliphatic); Phe, Tyr, Trp and His, (i.e. aromatic); and Glu, Ser, Cys (with functional groups) and Cys methyl–ester hydrochloride. The relative abundances of the ions produced from these twelve α-amino acids, and from the methyl-ester hydrochloride of cysteine, at *m/z* values greater than that of the molecular ions, are listed in Table 1. In addition to adduct ions such as $[M+117]^+$, $[M+231]^+$ and $[M+233]^+$, bimolecular complexes, such as $[2M+Al-2H]^+$ and $[2M+117]^+$ are also formed from Gly, Ala, Val, Leu and His. Identification of these complex ions was aided by comparing with the spectra of Leu-d_3, Tyr-d_4, His-d_4, Ser-d_4, Cys-d_4, and Cys Me ester-d_3 which had been obtained under identical conditions, except that glycerol-d_3 and D_2O were used in place of glycerol and water (Fig. 1). Even though the $[Al+glycerol-2H]^+$ ion at *m/z* 117 (noted as $[117]^+$ passim) exists in the matrix, the origin of the $[M+117]^+$ ion was shown (linked scans at constant B^2/E) to be the $[M+233]^+$ and $[M+231]^+$ ions which were the precursors. High resolution mass measurements for the $[M+117]^+$ of His were consistent with the molecular formula of $C_9H_{15}O_5N_3Al$, with a deviation of only 3.3 ppm (theoretical 272.08270; observed 272.08179).

The data recorded for equimolar solutions of all amino acids under identical conditions, summarized in Fig. 2, reflect the relative proton affinities and interaction affinities for the $[Al(III)+glycerol-2H]^+$ complex for each amino acid. Figure 2 shows that the formation of $[M+H]^+$ and $[M+117]^+$ ions roughly parallel one another. Amino acids with side chains containing functional groups such as -COOH, -OH, and -SH also gave abundant $[M+H]^+$ and $[M+117]^+$ ions. These groups may provide an additional coordination site, which would result in the formation of these relatively abundant ions. However, Pro and cysteine methyl ester hydrochloride form less abundant $[M+117]^+$ ions. Proline formed mainly iminium ions whereas the cysteine ester hydrochloride formed $[M+H]^+$ ions as the base peak. Benzylamine and 1,2-diaminobenzene do not form adduct ions with $[117]^+$ reagent ions. Instead they produce mainly $[M+H]^+$ ions. This can be explained by the high proton affinity of amines. On the other hand, the substitution of alkylamines by a carboxyl group decreases the proton affinity of the amine function by 1.8–3 kcal,[20] which is sufficient to allow aluminum complexation to compete with protonation.

The heavier metal chloride $GaCl_3$ from the same group, forms $[glycerol+GaCl-H]^+$ and $[2glycerol+Ga-2H]^+$ ions with the matrix but does not react in general with amino acids. However, from His, it forms $[M+GaCl_2]^+$ and $[M+GaCl-H]^+$ ions in very low abundances. The mass spectra of Cys and Ser include $[M+GaCl-H]^+$ ions which are more intense in the case of Cys. This contrast between Al and Ga can be explained by an increase of the softness (Hard/Soft Acid Base, Theory) of the metal ions.[21]

Table 1. Relative abundances of ions obtained from α-amino acids with Al(III) and glycerol.

Compd. Name	$[M+H]^+$	$[M+AlO]^+$	$[M+117-H_2O]^+$	$[M+117]^+$	$[M+233]^+$	$[M+231]^+$	Other ions
Gly	100	22	20	77	16	24	12 $[2M+117]^+$ 18 $[M+349]^+$
Ala	100	6	7	25	12	16	9 $[2M+117]^+$
Val	86	8	10	48	8	14	17 $[2M+117]^+$ 7 $[M+209]^+$
Leu	78	11	12	55	8	12	20 $[2M+117]^+$ 7 $[2M+Al-2H]^+$ 7 $[M+209]^+$
Pro	100	7	6	22	7	13	5 $[M+209]^+$ 11$[2M+Al+TFA-2H]^-$
Phe	71	15	7	51	12	6	10 $[M+347]^+$
Tyr	100	6	—	24	5	10	9 $[M+209]^+$
Trp	76	8	3	52	—	—	10 $[M+209]^+$
His	100	9	3	44	9	4	15 $[M+AlF-H]^+$ 14 $[2M+Al-2H]^+$ 8 $[2M+143-2H]^+$
Glu	100	—	14	87	41	—	
Ser	100	9	6	87	15	8	—
Cys	100	7	—	47	6	13	14 $[M+209]^+$
Cys Me	100	6	—	10	3	13	—

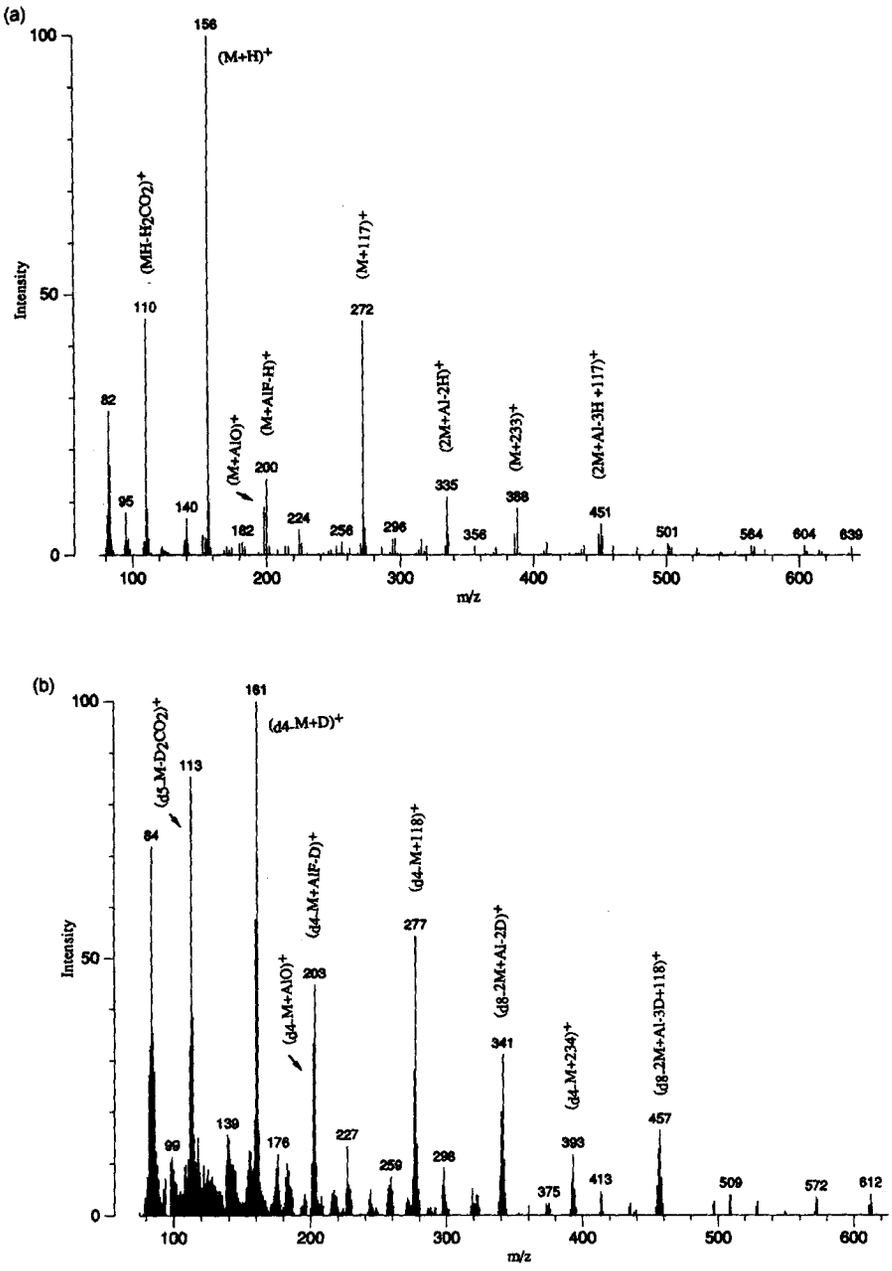


Figure 1. (a) FAB mass spectrum of His obtained using $AlCl_3$+glycerol+trifluoroacetic acid+H_2O. (b) FAB mass spectrum of His-d_4 obtained using $AlCl_3$+glycerol-d_3+trifluoroacetic acid+D_2O.

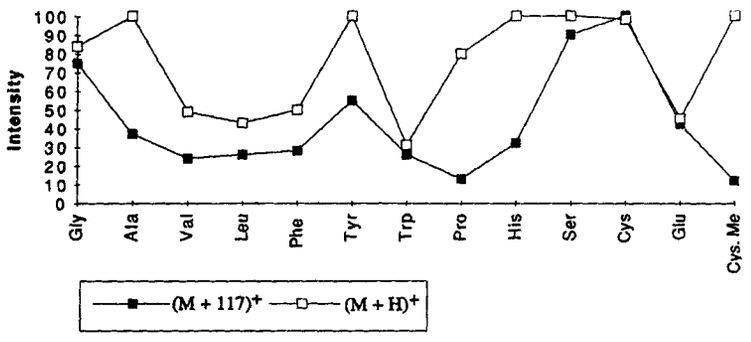


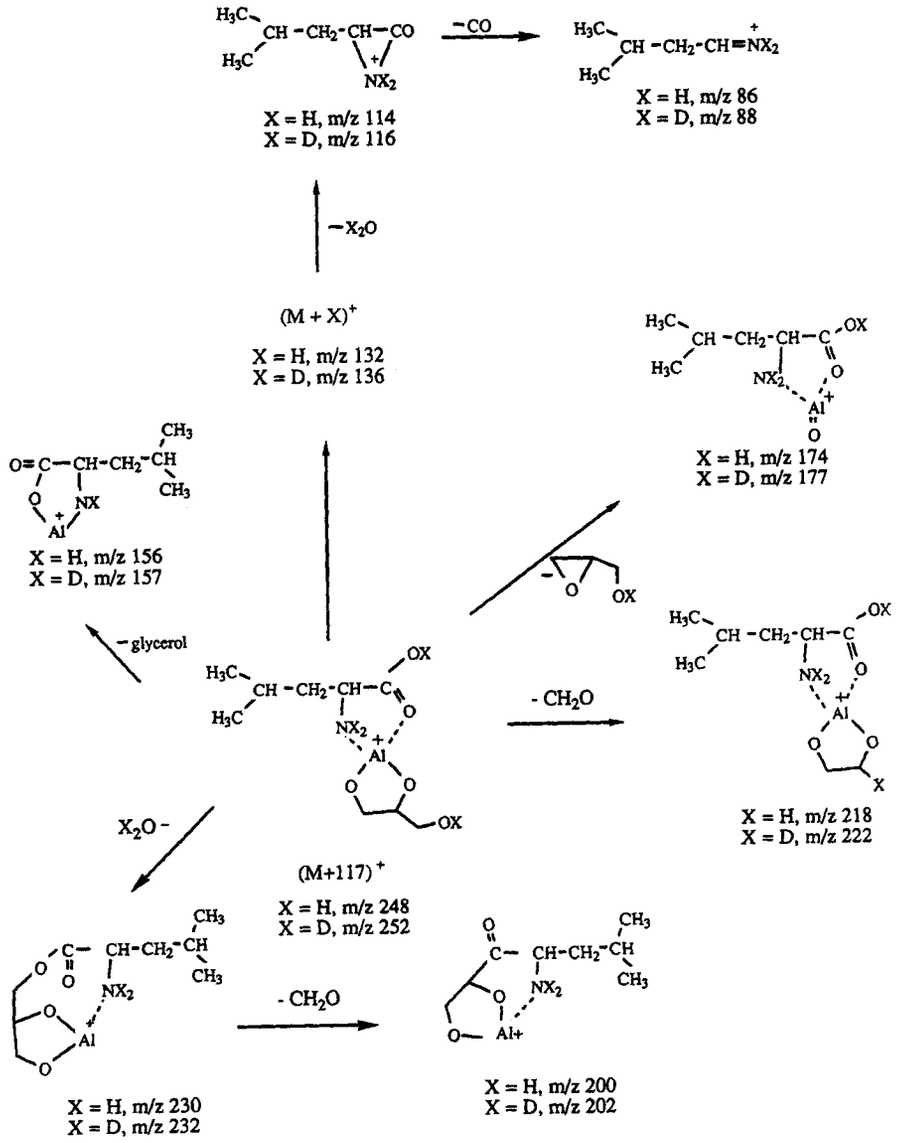
Figure 2. Relative abundances of the $[M+117]^+$ and $[M+H]^+$ ions for equimolar amounts of the common amino acids.

Collisionally-activated dissociation of $[M+117]^+=[M+Al(III)+glycerol-2H]^+$ adduct ions

Aliphatic amino acids. Collisionally-activated dissociation (CAD) of $P^+=[M+117]^+$ ions of Gly, Ala, Val, Leu, and Pro produces $[P-H_2O]^+$ ions. Gly, Ala and Leu also give abundant $[P-30]^+$ and $[P-74]^+$ ions, whereas Pro produced $[P-H_2O]^+$ and $[P-30]^+$ ions with almost equal abundances. The CAD spectrum of the Leu-d_3 complex shows the retention of all deuterium atoms in the $[P-30]^+$ product ion. Therefore, the $[P-30]^+$ ion is probably due to the elimination of a CH_2O group from the glycerol moiety. Loss of one D atom in the formation of the $[P-75]^+$ ions, establishes the loss of epoxypropanol (CH_2OCHCH_2OD) from the glycerol skeleton. In a similar way, glycerol elimination from the perdeuterated P^+ ion shows the loss of 95u (glycerol-d_3). The glycerol-d_3 loss binds Al(III) to the carboxylic acid and amino groups of the amino acid in a substitution process involving exchangeable protons (Scheme 1). During D_2O exchange experiments, the CAD of P^+ ions of Leu also shows the formation of ions at *m/z* 86, 114, 117, i.e. increases in mass by 2, 2 and 1 unit respectively. The CAD of $P^=$ ions of Gly and Ala also shows ligand losses and the formation of $[Al(III)+glycerol-2H-CH_2O]^+$ ions.

The CAD spectrum of the $P^+=[M+117]^+$ ion of Val shows the formation of the $[M+H]^+$ ion and subsequent elimination of H_2CO_2 from that ion. Other amino acids such as Gly, Ala, Leu and Pro also give $[M+H]^+$ ions followed by loss of HCOOH to yield iminium ions. Proline formed a particularly stable iminium ion. However, no direct loss of a H_2CO_2 group from P^+ nor any loss of CO from $[P-H_2O]^+$ ions is observed for aliphatic amino acids. Earlier studies had, however, reported elimination of (H_2CO_2) from metal complexes of α-amino acids.[12,14] Ion intensities formed from $P^+=[M+117]^+$ ions in the CAD experiments are listed in Table 2.

Aromatic amino acids. The CAD of $P^+=[M+117]^+$ complexes derived from aromatic amino acids, such as Phe, Tyr, Trp and His, resulted in fragment ions such as $[P-H_2O]^+$, $[P-74]^=$ and $[P-92]^+$ (Table 2). However, there is no direct loss of a CH_2O group from the P^+ ion. Its loss is observed from $[P-H_2O]^+$ ions; this process is dominant for His complexes, and this observation may be related to the



Scheme 1. Mechanism for collisionally-activated dissociation of $P^+=[M+117]^+$ ion for M=Leu. When D_2O was used (X=D) instead of H_2O (X=H), $P^+=[deutero\text{-}M+118]^+$.

additional coordination sites available to the metal ion. The CAD of P^+ ions of Phe forms $[MH - H_2CO_2]^+$ ions. The His complex also undergoes elimination of a H_2CO_2 group from $[P - 74]^+$ ions to give ions at *m/z* 152. Fragmentation of the $[M+Al-2H]^+$ ion, i.e. $[P - 92]^+$, leads to the formation of an aziridine, by the removal of an AlO neutral, while decarboxylation of the same ion may give rise to an ion at *m/z* 136. The fragmentation of P^+ ions of His is confirmed with perdeuteration experiments (Scheme 2). The Trp complex produces abundant $[P - H]^+$ ions, unlike other α-amino acids complexes of this type, and also undergoes C_α–C_β fission to produce ions due both to the loss of a CH_2-indole group and to ions corresponding to $[CH_2\text{-indole}]^+$ at *m/z* 130. CAD of the P^+ complexes of Phe and Tyr also yields tropylium and hydroxy-tropylium (or related ions) respectively, due to C_α–C_β cleavage. Tyr shows an additional interesting fragmentation, the formation of an ion at *m/z* 209, assigned to the adduct of [Al(III)+glycerol − 2H]$^+$ with the (OC_6H_4) group after the elimination of 89 mass units from the analyte. This species also eliminates water. The chelation of metal ions through the aromatic groups[22] for tyrosine may explain the formation of ions at *m/z* 209 and 191, in addition to chelation with -NH_2 and -COOH groups which leads to the formation of other ions at *m/z* 107, 117, 136, 206, 224, 250 and 280. However, the comparison of CAD spectra of complexes of Tyr and Tyr-d_4 suggests that the ion at *m/z* 209 corresponds to a group of isomeric ions with a different number of acidic protons including π-complex formation (Table 2).

Amino acids with COOH, SH or OH side chains. Aluminum (III)-glycerol, $P^+=[M+117]^+$ complex ions of Cys, Ser and Glu, show some CAD fragments, e.g., $[P - H_2O]^+$, $[M+AlO]^+$ pr $[P - 74]^+$ and $[M+Al-2H]^+$ or $[P - 92]^+$ ions in common with other amino acids. The spectrum of the Ser complex shows the formation of a $[P - 30]^+$ ion which does not shift in *m/z* value for the complex of Ser-d_4, suggesting the elimination of a CH_2O group. Ions corresponding to $[P - H_2O/D_2O]^+$, $[P-H_2O/D_2O - CH_2O]^+$, which then eliminate NH_2/ND_2, are also observed from the P^+ complex ion of Ser and P^+ ion of Ser-d_4.

CAD experiments on the P^+ ion of Cys gave fragment ions due to the loss of ammonia directly from P^+, as well as from the $[P - H_2O]^+$ ion. The reverse process of water elimination from $[P - NH_3]^+$ ions was also observed. The formation of $[P - NH_3]^+$ ions with the assistance of an OH group of [Al(III)+glycerol − 2H]$^+$ ions is excluded, because this is not a common process, so a nucleophilic displacement by the S atom is involved (Scheme 3). Fragment ions due to elimination of $HSCH_3/DSCH_2D$, (SH/CH_2OH) or (SD/CH_2OD) and CH_2O groups from P^+ ion, and due to the removal of CO from $[M+Al-2H]^+$, are also observed. Formation of characteristic fragment ions for Cys

Table 2. Collisionally-activated dissociation spectra of $P^+=[M+\{Al(III)+(glycerol-2H)\}]^+=[M+117]^+$ complexes.

Amino acid	P^+ ion *m/z*	Fragments {*m/z*, (int. %)}
Gly	192	175 (23), 174(100), 162 (32), 144 (16), 118 (30), 117 (16).
Ala	206	189 (21), 188 (100), 177 (8), 176 (42), 164 (4), 159 (11), 158 (15), 132 (27), 117(15), 87 (7), 61 (12).
Val	234	216 (12), 204 (4), 186 (2), 160 (2), 142 (2), 118 (100), 72 (4).
Leu	248	247 (13), 230 (100), 218 (36), 200 (19), 174 (24), 156 (5), 132 (3), 114 (8), 86 (13).
Leu-d_3	252	233 (34), 232 (100), 222 (49), 202 (25), 177 (24), 176 (19), 118 (10), 116 (10), 88 (8).
Pro	232	214 (91), 202 (100), 184 (16), 158 (39), 140 (4), 116 (5), 114 (5), 70 (30).
Phe	282	281 (59), 264 (100), 252 (5), 234 (50), 208 (56), 190 (40), 120 (22), 117 (10), 91 (8)
Tyr	298	280 (41), 250 (41), 224 (41), 209 (100), 206 (27), 191 (14), 136 (6), 135 (3), 117 (15), 107 (8).
Tyr-$d_{3,4}$	303	285 (32), 284 (43), 283 (100), 252 (22), 228 (40), 213 (29), 212 (90), 211 (60), 210 (26), 195 (12), 194 (13), 193 (15), 192 (23), 191 (23), 108 (3).
Trp	321	320 (100), 303 (17), 293 (16), 273 (23), 247 (19), 191 (3), 130 (3).
His	272	271 (12), 256 (8), 254 (8), 226 (16), 225 (19), 224 (100), 198 (50), 180 (19), 152 (20), 137 (10), 136 (6).
His-d_4	277	259 (4), 258 (8), 234 (14), 233 (16), 228 (38), 227 (100), 203 (20), 202 (70), 201 (31), 83 (23), 182 (22), 155 (23), 154 (13), 139 (22), 138 (13).
Glu	264	246 (100), 228 (28), 218 (67), 216 (33), 200 (31), 190 (33), 172 (25), 144 (79), 117 (5), 100 (17).
Ser	222	205(11), 204 (17), 192 (100), 186 (7), 179 (9), 176 (22), 174 (15), 158 (14), 148 (11), 117 (9), 102 (14), 61 (8).
Ser-d_4	227	207 (17), 197 (17), 179 (27), 177 (18), 159 (18), 152 (10), 118 (8), 104 (21), 103 (8).
Cys	238	237 (21), 221 (86), 220 (39), 203 (52), 192 (31), 191 (23), 190 (100), 174 (29), 164 (64), 146 (48), 135 (11), 118 (35), 117 (24), 102 (8), 87 (8), 86 (12), 76 (10), 61 (9).
Cys-d_4	243	225 (26), 224 (31), 223 (100), 203 (22), 195 (8), 193 (42), 168 (7), 148 (4), 120 (3).
Cys Me	252	251 (93), 235 (95), 223 (69), 221 (36), 204 (64), 197 (66), 179 (50), 178 (100), 160 (31), 118 (24), 102 (8), 87 (8).
Cys Me-d_3	256	239 (9), 238 (24), 237 (10), 206 (36), 181 (100), 161 (16), 120 (17).

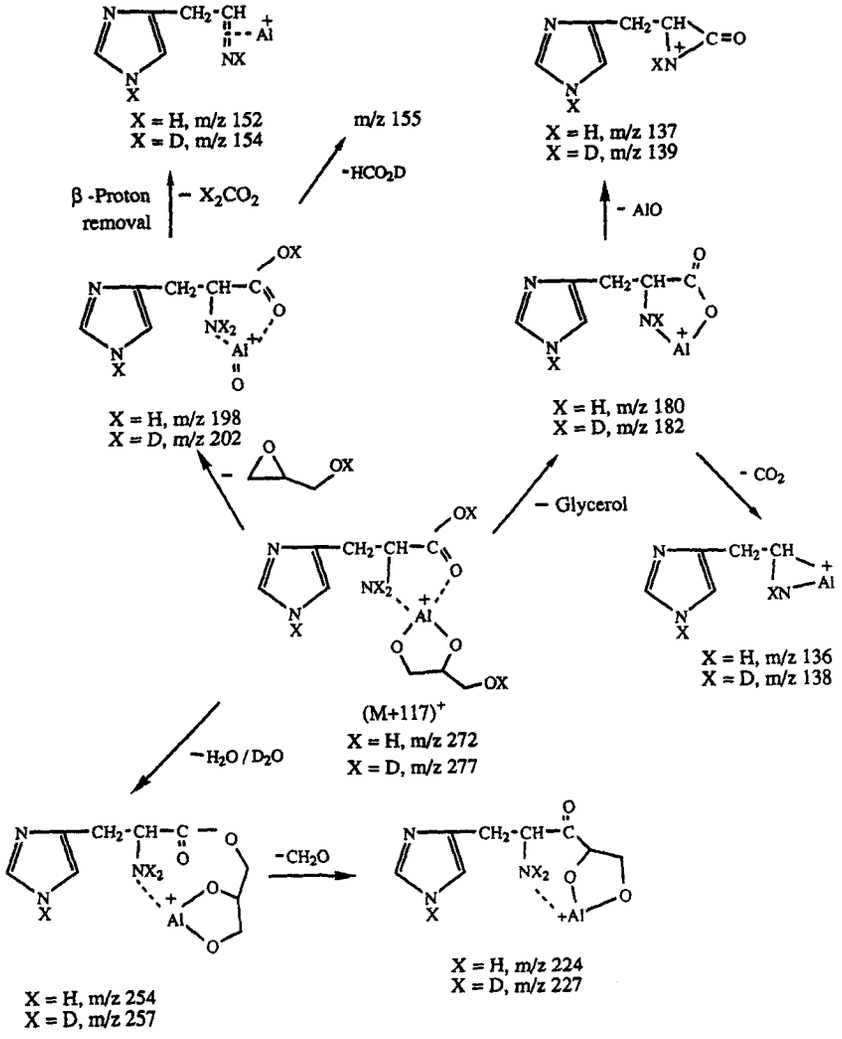
was confirmed by the fragmentation of the P^+ ion of Cys-d_4 (Scheme 3).

When the Glu complex dissociated it gave a $[P-H_2CO_2]^+$ ion which is characteristic of the presence of the side-chain acid group. This was followed by the loss of 74 mass units, in addition to the formation of $[M+Al-2H]^+$ and $[M+AlO]^+$ ions. The P^+ adduct of Cys Me ester also shows the loss of the $HSCH_2H$ group, which is a characteristic of the cysteine moiety, as well as formation of $[P-H]^+$, $[P-OH/OD]^+$, and $[P-HCOOCH_3/DCOOCH_3]^+$ ions due to the presence of the ester functionality. The remaining fragmentations are similar to those observed for P^+ complex ions of aliphatic amino acids. We identified the fragments by comparison with the perdeuterated ion dissociations.

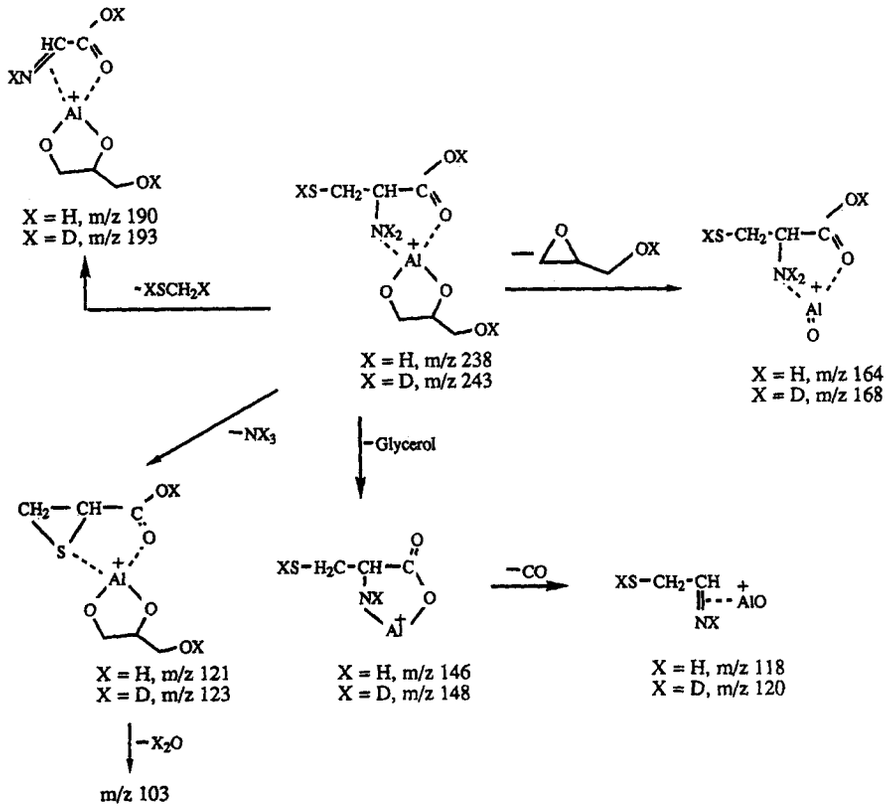
Cys, Gly and Ser complexes produce ions due to loss of (H_2CO_2), by the sequential elimination of H_2O and CO, or by direct loss of H_2CO_2 (formic acid), from the P^+ ion. The elimination of CH_2O and of CO from $[P-H_2O]^+$ may be competitive processes for amino acids possessing additional coordination sites. The formation of the $[P-H_2O-CO]^+$ ion may be more favored in Cys, Glu and Ser than the elimination of CH_2O from $[P-H_2O]^+$, which is favored for aliphatic amino acids. However, direct removal of a CH_2O group from the P^+ complex is a facile process for both Ser and Pro.

Collisionally-activated dissociation of $[M+231]^+=[M+Al(III)+TEA+glycerol-2H]^+$ adduct ions

The $A^+=[M+231]^+$ complexes of Gly and Ala yield $P^+=[M+117]^+$ and $[A-72]^+$ ions, respectively, as the major processes of fragmentation (Table 3). Other ions from Gly are due to loss of 28 mass units, while Ala complexes lose 30u (CH_2O), 114u (TFA), 102u and also form $[M+H]^+$ ions. Even though Val, Leu, and Pro complexes show loss of 72u, they also give ions due to the loss of a trifluoroacetic acid moiety and of glycerol. Further fragmentation of the A^+ ion is consistent with the fragmentation of individual $[M+117]^+$ ions. Removal of 72u from the unlabelled A^+ ion can account for the loss of (2HF and HCH_2OH) from Leu and loss of 74u from the labelled A^+ ion of Leu-d_3, (2HF and DCH_2OD), which occurs within the reaction time window of the field-free region. The CAD of A^+ ions of Tyr and Phe also yields $P^+=[M+117]^+$ and $[A-72]^+$ ions, while formation of other ions is similar to the fragmentation of the corresponding $[M+117]^+$ ions. The A^+ ions of Cys



Scheme 2. Mechanism for collisionally-activated dissociation of $P^+=[M+117]^+$ ion for M=His. When D_2O was used (X=D) instead of H_2O (X=H), $P^+=[deutero\text{-}M+118]^+$.



Scheme 3. Mechanism for collisionally-activated dissociation of $P^+=[M+117]^+$ ion for M=Cys. When D_2O was used (X=D) instead of H_2O (X=H), $P^+=[\text{deutero-M}+118]^+$.

also give $[A-72]^+$ ions, and the CAD spectrum of A^+ of the Cys-d_4 adduction showed similar shifts in the *m/z* values as were observed in the Leu-d_3 complex. This common behaviour again excludes the involvement of the -SH functional group of Cys in the elimination of 72u from unlabelled A^+ ions and of 74u from labelled A^+ ions. The A^+ ion of Cys Me ester loses neutral TFA as the major pathway. The preferential loss of neutral TFA over glycerol from the A^+ ions reflects the strength of binding of bidentate ligands vs. monodentate ligands with aluminum.

Collisionally-activated dissociation of $[M+233]^+=[M+2Al(III)+2glycerol-5H]^+$ adduct ions

This complex is formed from the amino acid (M) via chelation of two Al(III) ions bonded to the oxygens of two glycerol molecules which have eliminated five protons. The

Table 3. Collisionally-activated dissociation spectra of $A^+=[M+\{Al+(glycerol+TFA-2H)\}]^+=[M+231]^+$ complexes.

Amino acid	A^+ ion (*m/z*)	Fragments {*m/z*, (int. %)}
Gly	306	290 (5), 278 (16), 234 (17), 192 (100), 174 (10), 137 (5), 118 (3).
Ala	320	290 (5), 248 (100), 246 (11), 219 (18), 218 (22), 206 (29), 192 (11), 189 (15), 147 (4), 90 (19).
Val	348	347 (23), 277 (6), 276 (4), 259 (8), 235 (57), 234 (100), 216 (16), 205 (6), 191 (8), 160 (12), 118 (58), 72 (22).
Leu	362	361 (60), 346 (17), 334 (13), 320 (10), 290 (56), 272 (8), 248 (100), 230 (10), 174 (4), 132 (7), 86 (10).
Leu-d_3	367	348 (7), 293 (19), 292 (28), 252 (100), 251 (59), 232 (5).
Pro	346	274 (18), 255 (9), 232 (100), 214 (11), 202 (15), 178 (7), 158 (16), 116 (83), 70 (11).
Phe	396	395 (33), 380 (12), 368 (9), 324 (100), 312 (9), 306 (19), 282 (61), 250 (19), 203 (7), 166 (13), 147 (4), 120 (12), 105 (3).
Tyr	412	340 (14), 322 (12), 301 (25), 298 (100), 209 (17).
Cys	352	351 (67), 336 (13), 318 (31), 306 (45), 280 (100), 262 (28), 250 (17), 238 (36), 224 (22), 206 (18), 178 (9), 147 (6).
Cys-d_4	358	341 (17), 340 (23), 339 (29), 309 (48), 308 (24), 284 (99), 283 (100), 282 (11), 264 (31), 209 (5).
Cys Me	366	365 (47), 349 (12), 347 (21), 295 (6), 274 (5), 252 (100), 204 (6), 180 (11), 178 (12), 137 (7), 136 (8).
Cys Me-d_3	371	296 (3), 276 (3), 257 (30), 256 (100), 255 (62), 182 (9), 181 (12), 139 (5).

$C^+=[M+233]^+$ complexes of Phe, His and Ser/Ser-d_4 gave $[C-H]^+$, $[C-H_2O]^+$, $[C-CH_2O]^+$ and P^+ ions on CAD. On further fragmentation, the $[C-H_2O]^+$ ions lost (CH_2O). The $[C-CH_2O]^+$ ions lost H_2O to produce $[C-48]^+$ ions. From the Ser system, loss of (CH_2O) from C^+ ions is followed by the loss of 74u to give ions of *m/z* 234. The perdeuteration experiments confirmed the loss of 74u from C^+ ions. However, the C^+ ions of Phe and His lost 74u directly. On further fragmentation, these $[C-74]^+$ ions, lost 74u (AlO_2CH_3) to produce ions at *m/z* 250 and 240 from Phe and His respectively. The C^+ ions of Ser yielded ions of *m/z* 162 corresponding to an adduct of the $AlOCH_3$ species with an analyte [M − H] ion. This interpretation was also confirmed by the deuteration experiments. These differences clearly indicate the involvement of side-chain groups in the fragmentation of these $C^+=[M+233]^+$ adduct ions.

Collisionally-activated dissociation of $[2M+117]^+$ and $[2M+Al(III)-2H]^+$ adduct ions

Under CAD conditions, the bimolecular complexes $[2M+117]^+$, for M=Gly, Ala, Val and Leu, produce mainly $[M+117]^+$ ions. Losses of H˙ and of neutral glycerol are also observed for M=Val and Leu. Further fragmentation patterns of these ions are similar to those of the corresponding $P^+=[M+117]^+$ ions. The $[2M+Al-2H]^+$ ions for M=Val also lose H_2O followed by elimination of CO_2, to produce ions of *m/z* 197. An ion of *m/z* 72, corresponding to $[C_3H_7CH=NH_2]^+$ is also observed. The formation of ions of *m/z* 160 (i.e. by loss of i-$C_3H_7CHCONH$) and *m/z* 159 (i.e. by loss of i-$C_3H_7CHCONH_2$) from the $[2M+Al-2H]^+$ complex for M=Val reflect the different ways of binding Al(III) with amino acids. Binding of both amino acids through the amino groups may also explain the elimination of water and CO_2 and the formation of *m/z* 160, while binding through the amino group of one amino acid and the carboxylate group of the other could explain the formation of *m/z* 159.

The $[2M+Al-2H]^+$ complex for M=His also loses H˙, H_2O and H_2CO_2. The removal of an entire side-chain involves cleavage of the C_α–C_β bond with the migration of one exchangeable proton from the amino group of the precursor ion. These processes yield ions of *m/z* 334, 317, 289, and 254 respectively, and are confirmed by appropriate mass shifts in the deuteration experiments. The formation of the ion of *m/z* 198 is also a facile process corresponding to loss of the (NHCOCH-R) group, where R represents the histidine side-chain.

The $[M+209]^+$ ion (209=117+glycerol) for M=Cys shows loss of a cysteine moiety and results in an ion of *m/z* 209. The $[2M-H+AlOCOCF_3]^+$ complex for M=Pro loses 28u, neutral TFA and CF_2CO_2 (by migration of F to metal), and also forms $[MH-CO_2H_2]^+$ ions.

Table 4. List of peptides investigated (molecular masses in brackets).

Ala-Tyr (252)	1					
Ala-Ser (176)	2					
Gly-Gly-Gly (189)		3				
Glu-Val-Phe (393)		4				
Leu-Gly-Phe (335)		5				
Ala-Gly-Ser-Glu (362)			6			
Val-Gly-Asp-Glu (418)			7			
Gly-Gly-Gly-Gly (246)			8			
Gly-Gly-Gly-Gly-Gly (303)			9			
Tyr-Gly-Gly-Phe-Leu (555)			10			
Gly-Ala-Ala-Ala-Ala (359)			11			
Val-Glu-Ser-Ser-Lys (548)			12			
Val-Glu-Pro-Ileu-Pro-Tyr (716)					13	
Gly-Gly-Gly-Gly-Gly-Gly (360)					14	

Mass spectra of peptides obtained from $AlCl_3$+glycerol+TFA+water

The mass spectra of fourteen peptides, ranging in length from two to six amino acids (Table 4), were studied by FAB mass spectrometry in the mixture of $AlCl_3$ in glycerol, TFA and water as matrix. All of these peptides (M) formed complex ions with Al(III) ions corresponding to $[M+117]^+$, $[M+Al-2H]^+$, $[M+Al-3H+117]^+$, $[M+233]^+$ and $[2M+Al-2H]^+$. In addition, the formation and fragmentation of $[M+H]^+$ ions yielded fragment ions, which provide conventional sequence information. The relative intensities of ions observed with *m/z* values which are greater than that of the molecular ion are given in Table 5. Dipeptides Ala-Tyr (**1**) and Ala-Ser (**2**), and tripeptides $(Gly)_3$ (**3**), Glu-Val-Phe (**4**) and Leu-Gly-Phe (**5**) form $[M+117]^+$ and $[M+233]^+$ ions. In addition, **4** and **5** give $[M+Al-2H]^+$ and $[2M+Al-2H]^+$ ions (Fig. 3).

Tetrapeptides such as Ala-Gly-Ser-Glu (**6**), Val-Gly-Asp-Glu (**7**) and $(Gly)_4$ (**8**) and pentapeptides $(Gly)_5$ (**9**), Tyr-Gly-Gly-Phe-Leu (**10**) and Gly-Ala-Ala-Ala-Ala (**11**) give mainly $[M+Al-2H]^+$ and $[M+Al-3H+117]^+$ ions. As the peptide chain length increases the abundances of $[M+Al-2H]^+$ and $[M+Al-3H+117]^+$ ions are enhanced in **9** and **10**. However, **12** still gives both $[M+H]^+$ ions and

Table 5. Relative abundances of cluster ions from peptides 1–14 obtained using Al(III) with glycerol.

Compd.	$[M+H]^+$	$[M+117]^+$	$[M+Al-2H]^+$	$[M+Al-3H+117]^+$	$[M+233]^+$	Other ions
1	18	11	—	—	7	
2	23	12	—	—	21	
3	66	32	5	—	25	
4	10	7	21	6	—	6 $[2M+Al-2H]^+$
5	18	14	5	5	—	7 $[2M+Al-2H]^+$ 4 $[2M+Al-3H+117]^+$
6	6	—	8	5	—	—
7	5	3	5	7	—	2 $[M+Al-4H+(117)_2]^+$
8	43	54	5	—	12	—
9	11	7	26	11	4	—
10	—	—	14	16	—	—
11	10	—	5	4	—	—
12	4	2	12	5	—	—
13	3	—	2	2	—	—
14	3	5	2	—	—	—

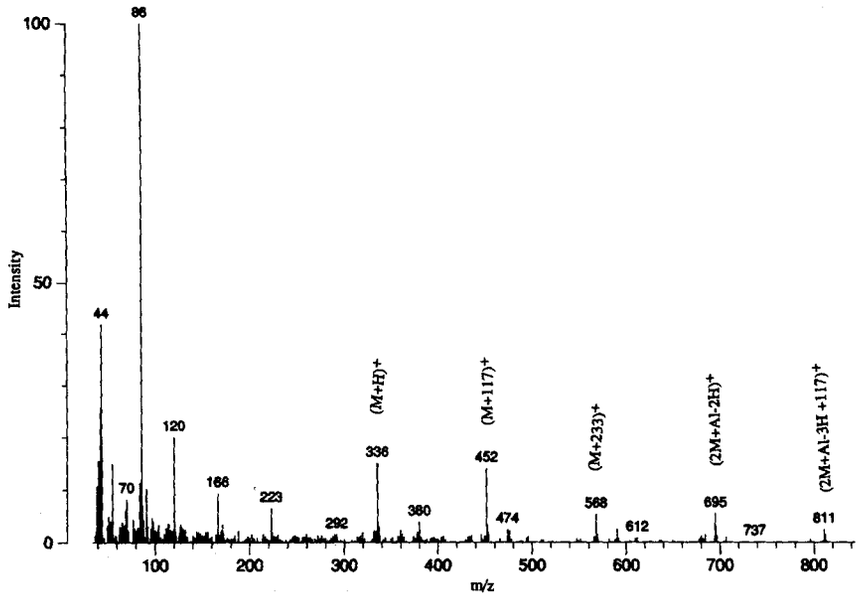


Figure 3. FAB mass spectrum of Leu-Gly-Phe (**5**) obtained using $AlCl_3$+glycerol+trifluoroacetic acid+H_2O.

significant $[M+Al-2H]^+$ ions. The hexapeptides **13** and **14** form less abundant $[M+Al-2H]^+$ ions.

Collisionally-activated dissociation of complex ions of peptides M

$[M+117]^+$ ions. The CAD of the $P^+=[M+117]^+$ ion of **1** gave $[P-H_2O]^+$ followed by the loss of CH_2O to produce *m/z* 321. Loss of 74u (epoxypropanol) from the P^+ ion gave $[M+AlO]^+$ ions. Ions due to the loss of glycerol are not significant. The CAD spectrum of the P^+ ion from **1** also shows the elimination of 86u from the $[P-H_2O]^+$ ion. The other dipeptide **2** produced $[P-CH_2O]^+$ ions as the major product due to the presence of Ser, while $[P-H_2O]^+$, $[P-H_2O-CH_2O]^+$ and $[P-74]^+$ ions were also observed. Ions due to the loss of 86u from $[P-H_2O]^+$ ions was also observed for compound **2**, presumably from the alanine residue, which is common to these two dipeptides.

The P^+ complex ion of **3** (Gly_3) gave $[P-H_2O]^+$, $[P-H_2O-CH_2O]^+$ (100%), $[P-74]^+$ and $[P-glycerol]^+$ ions, which is a similar pattern to the dissociation of $[M+117]^+$ ions when M represents glycine. The $[M+117]^+$ ions of **8** yielded $[P-NH_2]^+$, and $[P-H_2O]^+$ ions which subsequently lost CO_2 to give ions at *m/z* 301. The formation of $[M+Al-2H]^+$ ions was also observed. However, we observed that the $P^+=[M+117]^+$ ions of **9** give $[M+Al-2H]^+$ ions as the base peak. Ions due to the losses of 74u (epoxypropanol), from the glycerol moiety and 113u gave ions at *m/z* 346 and 307 respectively from P^+. The P^+ complex of **5** gave fragment ions, $[P-H_2O]^+$ which then lost CH_2O to produce *m/z* 404, and other abundant ions due to $[P-NH_2]^+$, $[P-H_2O]^+$, $[M+AlO]^+$, $[M+Al-2H]^+$ and $[M+AlO-HCOOH]^+$. The spectrum also shows the formation of an ion of *m/z* 86 i.e. $[C_3H_7CH_2CH-NH_2]^+$ from the Leu moiety. From the P^+ ion, ions of *m/z* 287, due to loss of 91u neutral from the Phe moiety, and an adduct ion of $[Phe+117]^+$, are also observed. From these observations we conclude that the metal ion is either bonded to multiple sites to form a heterogeneous population of P^+ ions, and/or undergoes rapid site-exchange before the fragmentation which has been reported for alkali metal chelated peptides.[1-7] In contrast the P^+ ion of peptide **4** produced mainly $[P-H_2O]^+$, and $[P-glycerol]^+$ ions followed by water loss.

$[M+Al-2H]^+$ ions. The fragmentation of this complex for peptide **4** shows loss of H_2O to form the base peak. The loss of 46 mass units may be due to the presence of Glu, which can eliminate H_2CO_2 and/or CO followed by expulsion of H_2O, which yields an ion of *m/z* 372. Elimination of the Phe residue (147u) from *m/z* 372 could account for an ion observed at *m/z* 225. Ions due to the elimination of a neutral Val fragments {71u, $(HN=CH-C_3H_7\}$ and of 145u (probably from Glu) are also observed to produce ions at *m/z* 347 and 273 respectively. All these fragmentation pathways suggest that the $[M+Al-2H]^+$ ions have a cyclic structure in which the Al(III) is directly attached to the NH group of the N-terminus and to the oxygen of the C-terminus carboxylate group.

The complex of **6** gives primarily loss of H_2O. Other abundant ions, created by consecutive losses of H_2O and CH_2O, are characteristic for the presence of Ser. The formation of ions due to consecutive losses of 28u, 46u and 64u indicates the presence of amino acids with carboxyl groups in the side-chain.

The CAD of $[M+Al-2H]^+$ ions for peptide **7** produce a loss of H_2O which suggests one of the three possible processes. Thus, facile elimination of water may reflect anhydride formation from two carboxyl groups of adjacent side chains (Asp and Glu), or cyclic amide formation from free side-chain carboxyl groups and the N-terminus NH_2, or the aluminum might have attached to the peptide backbone. No further fragment ions are observed.

The $[M+Al-2H]^+$ ions for peptide **11** lose H_2O, suggesting that the NH_2 and COOH groups may be free. Subsequent losses of 29u and 73u can lead to the formation of ions at *m/z* 337 and 293 respectively from $[M+Al(III)-2H-H_2O]^+$ ions. The loss of a neutral Ala molecule (89u) from the C-terminus leads to an abundant ion at *m/z* 295. The formation of an ion at *m/z* 284 can be readily interpreted in terms of the attachment of the aluminium ion to amide nitrogens, with migration of a hydrogen atom to the carbonyl of the second Ala. The $[M+Al-2H]^+$ complex for peptide **9** gave fragment ions at

m/z 87, 147, 177, 235, 273 and 299 with significant abundances. However, *m/z* 310 (H_2O loss) formed the base peak. CAD of the $[M+Al-2H]^+$ complex ion of peptide **10** showed loss of 115u ($C_4H_9CHCOOH$), due to the fission of the C_α–C_β bond of Leu from the parent ion to give *m/z* 465. Other significant product ions at *m/z* 463 and 295 are also present in the spectrum.

$[M+Al-3H+117]^+$ ions. The formation of such complex ions is observed in significant abundance from peptides where the formation of the $[M+117]^+$ ion itself is less favored. In such complexes, one aluminum binds to the sites where deprotonation occurs easily and the other aluminum ion may chelate with the peptide chain. CAD of the complex for peptide **6** gave mainly H_2O loss followed by CH_2O loss (*m/z* 455), while elimination of 44 u from *m/z* 455 was also observed. Loss of 74u is due to the elimination of epoxypropanol (glycerol $-H_2O$). The corresponding complex ion of **7** gives fragment ions due to losses of H_2O and of glycerol. The $[M+Al-3H+117]^+$ complex for peptide **9** loses 74u from the glycerol moiety.

The $[M+Al-3H+117]^+$ complex ion for peptide **10** did not lose water, as do similar peptide complexes, but rather lost 74u, 107u, 115u and 116u, as well as forming an ion at *m/z* 116. The loss of a hydroxybenzyl group from Tyr was favored over the cleavage of benzyl from Phe in **10**. Due to cleavage of the C_α–C_β bond of the C-terminal acid, the loss of 115u (probably from Leu) is observed. The CAD of this complex ion also resulted in the formation of $[M+Al-2H]^+$ by the loss of 116u {Al(III)+glycerol $-3H$}, which is followed by the elimination of 115u through similar fission of the C_α–C_β bond of the C-terminus acid to yield an ion at *m/z* 465. The CAD of the $[M+Al-3H+117]^+$ complex ion for peptide **11** yielded a loss of 74u as the dominant process in the spectrum. Loss of a neutral Ala molecule (89u) was also observed and other product ions at *m/z* 409 and 485 were also significant.

The $[M+Al-3H+117]^+$ complex for peptide **12** produced significant ions at *m/z* 671 (loss of H_2O), 579 (loss of H_2O and glycerol), followed by elimination of 116u which produced an ion at *m/z* 463. The formation of an ion at *m/z* 577 can be explained by assuming that the peptide is cyclic in structure before the elimination of 112u from Lys. Formation of a less abundant ion at *m/z* 597, due to the elimination of glycerol, was also observed.

$[M+233]^+ = [M+2Al(III)+2glycerol-5H]^+$ ions. The CAD of the $[M+233]^+$ complexes for peptides **3**, **4** and **5** produces ions due to loss of glycerol. This loss is followed by consecutive losses of CH_2O, H_2O, and CH_2O. From the complex for peptide **3**, loss of 116u (glycerol+Al $-3H$), i.e. the formation of the $[M+117]^+$ ion was observed, followed by removal of CH_2O.

CONCLUSIONS

All α-amino acids used in this study interact with different Al(III) containing ions. Complexes of Al(III) and amino acids are generated initially by complexation to amino and carboxylate groups in the analyte. The fragmentation of these adduct ions shows the removal of ligands (74u and 92u), attached to the aluminum ion, which results in direct binding of Al(III) to amino and carboxylate groups by the substitution of exchangeable protons. The well-documented elimination of epoxypropanol (74u) from glycerol is observed. The formation of $[M+Al-2H]^+$ ions is another indication of direct bonding of aluminum ions to deprotonated sites in α-amino acids. However, earlier reports[12, 14] proposed the insertion of the metal ion into C-COOH bonds by the migration of a hydrogen atom from the β-position to the metal ion. The functional groups in the side-chains of the amino acids assist in the coordination of the metal ion, and lead to differences in the fragmentation processes. This study may be helpful in understanding the reactions of Al(III) with electron-rich substrates in biological systems.

Peptides of different chain lengths also formed complex ions with Al(III) ions. As the number of α-amino acids in the chain increased, the formation of $[M+Al-2H]^+$ and $[M+Al-H+117]^+$ ions was favored for peptides **9** and **10** due to the availability of more amide nitrogens. Dissociation of these complex ions gave rise to fragment ions which are significant for identifying side-chain functional groups.

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