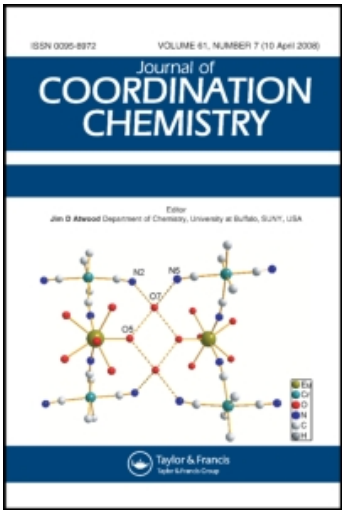

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FACILE DETECTION OF ORGANOMETALLIC DERIVATIVES OF PEPTIDES USING ELECTROSPRAY MASS SPECTROMETRY

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NOTE

FACILE DETECTION OF ORGANOMETALLIC DERIVATIVES OF PEPTIDES USING ELECTROSPRAY MASS SPECTROMETRY

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Electrospray mass spectrometry (ESMS) is shown to provide a facile and versatile method for the rapid *in situ* characterisation of adducts between the organometallic electrophile $[(\eta^5\text{-}C_6H_7)Fe(CO)_3]^+$ (Fed^+) and a range of peptides.

KEYWORDS: electrospray mass spectroscopy, organometallics, peptides

INTRODUCTION

We have recently demonstrated[1] that dienyl cations of the type $[(\eta^5\text{-}RC_6H_6)Fe(CO)_3]^+$ (**1**; a, R = H; b, R = 2-MeO) provide a new class of reagents for the selective labelling of peptides and proteins. Cysteine and histidine residues, in particular, are highly preferred targets. In several cases the modified peptides could be isolated as solids, but, in general, confirmation of peptide labelling was obtained from *in situ* ^1H and ^{13}C NMR studies, including 2D NMR spectra. More recently, others[2] have suggested that FTIR spectroscopy may also be used as a probe for such peptide labelling. The latter technique, however, is restricted to peptide binding by organometallic reagents containing IR-sensitive carbonyl ligands.

We now report that the recently-developed technique of electrospray mass spectrometry[3] (ESMS) provides a particularly facile and versatile method for the rapid characterisation of adducts between organometallic species and peptides. ESMS has already found extensive application in the structural analysis of large biopolymers, especially proteins.[4] In contrast, only a few studies have described the ES mass spectra of coordination and organometallic complexes.[5–7] However, we have found[7] ESMS to be the method of choice for the identification of π-hydrocarbon organometallic salts, including dienyl cations of the type **1** . Particu-

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larly significant for the present work was our observation[7] that relatively labile adducts of the type **2** between cations **1** and nucleophilic reagents, Nuc (*e.g.*, imidazoles, tertiary phosphines) exhibited strong molecular ions in their ES mass spectra. These fragile adduct species could not be readily identified by FAB mass spectrometry, due to fragmentation loss of the Nuc substituent. The unique features of ESMS, namely the ability to identify biopolymers and the very soft nature of the ionization process, suggested that it may provide a useful method for the rapid *in situ* identification of adduct formation between peptides and organometallic reagents.

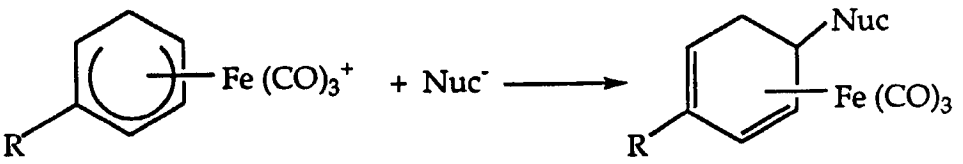
RESULTS AND DISCUSSION

The results summarised in Table 1 and Figure 1, below, confirm this prediction. In each case a dilute solution of the appropriate amino acid or peptide was made up in water, the pH adjusted to the value indicated, and one or more equivalent(s) of $[(\eta^5\text{-}C_6H_7)\ Fe(CO)_3]BF_4$ (Fed^+) added. The ESMS of these solutions were then recorded using a Fisons/VG Biotech Quattro mass spectrometer after mixing with 50% aqueous CH_3CN (with or without 1% HCOOH). The solvent stream was 50% aqueous methanol and a skimmer potential of 20V was employed in all cases.

Table 1 *In situ* electrospray mass spectra of peptides labelled with $[Fed]^+$ in water

peptide		Initial pH[a]	Final pH[b]	$[Fed^+]$:[peptide]	Major ions in ESMS (*m/z*)
N-Acetyl-histidine	**3**	7.0	4.6	1	416([N-Ac.His + Fed]$^+$,25%), 219([Fed]$^+$,30%),198 (N-Ac.His + H]$^+$,100%).
p-Glu-his-pro	**4**	7.5	5.9	1	582([peptide + Fed]$^+$,50%), 554([peptide + Fed-CO]$^+$, 1%),364([peptide]$^+$,71%), 219([Fed]$^+$,100%).
p-Glu-his-gly-NH_2	**5**	8.0	5.7	1	541([peptide + Fed]$^+$,52%), 513([peptide + Fed-CO]$^+$, 1%),323([peptide]$^+$,54%), 219([Fed]$^+$,100%).
Glutathione (γ-Glu-cys-gly)	**6**	3.4	3.2	1	526([glut + Fed + H]$^+$,84%), 307([glut + H]$^+$,12%),219 ([Fed]$^+$,100%).
p-Glu-his-trp-ser-try-gly -leu-arg-pro-gly (LHRH)	**7**	7.9	4.9	1	702([peptide + Fed + 2H]$^{2+}$, 23%),593([peptide)]$^{2+}$,59%), 219([Fed]$^+$,100%).
		9.2	5.8	2	702([peptide + Fed + 2H]$^{2+}$, 12%),593([peptide + 2H]$^{2+}$, 35%),219([Fed]$^+$,100%).
p-Glu-his-trp-ser-try-gly -leu-arg-pro-gly-cys (Cys-11 LHRH)	**8**	9.4	4.8	3	862([peptide + 2 Fed + H]$^{2+}$, 4%),858(2%),753([peptide + Fed + 2H]$^{2+}$,11%),644 ([peptide + 2H]$^{2+}$, 3%), 219 ([Fed]$^+$,100%).

[a] Free peptide after pH adjustment with either NH_4OH (peptides **1**, **2**, and **4**) or acetic acid (peptide **3**). [b] pH of mixture after addition of one equivalent of Fed^+.



1 2

With *N*-acetylhistidine, **3**, and each of the tripeptides *p*-glu-his-pro **4**, *p*-glu-his-gly NH_2 **5**, and glutathione **6**, a 1:1 molar ratio of Fed^+ and the appropriate amino acid or peptide was employed. Very clean ES mass spectra were obtained, exhibiting medium to high intensity $[peptide + Fed]^+$ molecular ions, confirming organometallic adduct formation in each case (Table 1). Strong peaks were also invariably observed for the Fed^+ cation at *m/z* 219 and for the particular free amino acid or peptide. These latter ions are believed to arise from partial fragmentation of the adduct molecular ions *via* collision activation in the mass spectrometer source rather than incomplete adduct formation, since independent IR and ^1H NMR spectral studies[1] confirmed quantitative adduct formation under the conditions employed (pH 7.0–8.0 for substrates **3–5** and pH 3.4 for glutathione). The only other evidence for fragmentation in the ESMS of these organometallic peptide adducts was the presence of very weak ions (rel. intensity 1%) at *m/z* 554 and 513

Figure 1a

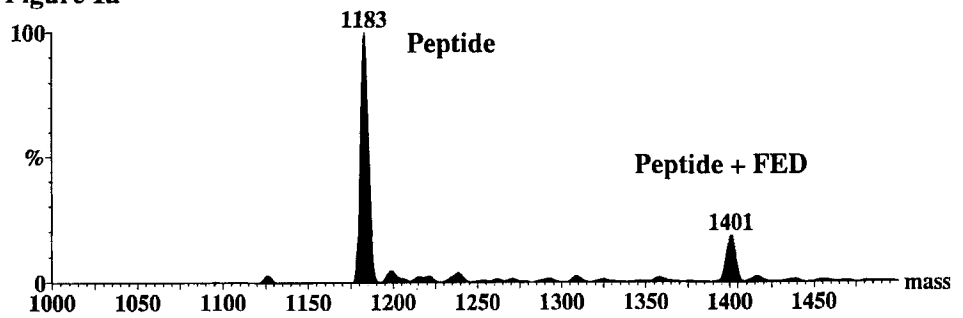


Figure 1b

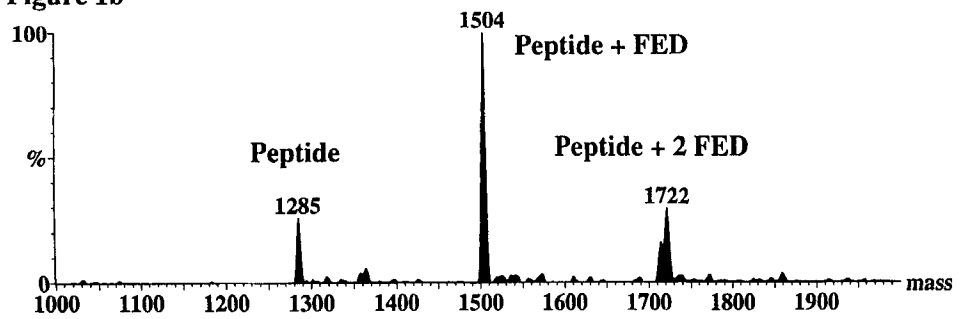


Figure 1 Transformed ESMS spectra of: a) a 1:1 mixture of Fed^+ and peptide **7** (LHRH) at pH 7.9; b) a 3:1 mixture of Fed^+ and peptide **8** (cys-11 LHRH) at pH 9.4.

with peptides **4** and **5**, respectively, which can be assigned to [peptide + Fed-CO]$^+$ ions (Table 1).

Binding of Fed$^+$ to *N*-acetylhistidine and the tripeptides **4** and **5** was accompanied by a sharp decrease in pH (see Table 1). This drop in pH is consistent with the loss of a proton from the imidazole group in the histidine residue in each case. ESMS data also indicate that the extent of adduct formation between Fed$^+$ and the tripeptides **4** and **5** is pH dependent. For example, with *p*-glu-his-pro at pH = 4.8 and an equimolar amount of Fed$^+$, only a relatively weak (rel. intensity 10%) [peptide + Fed]$^+$ molecular ion was observed at *m/z* 582, compared with the strong (rel. intensity 50%) molecular ion noted for the same system at pH 7.5 (Table 1). These observations are consistent with the greater nucleophilicity towards Fed$^+$ expected for the tripeptide containing the deprotonated histidine at higher pH.

In contrast, the ESMS data in Table 1 suggest that adduct formation between Fed$^+$ and glutathione is almost complete even at the low natural pH of 3.4 for the tripeptide. Adduct formation in this case was accompanied by only a small drop in pH, due to a buffering effect of the glutathione. These results are again consistent with our earlier NMR studies.[1]

ESMS has proved particularly convenient and informative for characterising the interaction between Fed$^+$ and the larger peptides **7** (luteinizing hormone releasing hormone, LHRH) and its cysteine derivative **8** (cys-11 LHRH). These latter peptides were examined using various [Fed$^+$]:[peptide] ratios (Table 1) to investigate the possibility of sequential addition of Fed$^+$ to the different potential binding sites on the peptide chains (his, arg in **7** and his, arg, cys in **8**). With LHRH (pH 7.9–9.2) similar ES mass spectra were obtained using either equimolar [peptide]: [Fed$^+$] or a two-fold molar excess of the Fed$^+$ cation (Table 1). In both cases medium intensity ions were observed at *m/z* 702 and 593, which could be assigned to the dicationic protonated mono-adduct [peptide + Fed + 2H]$^{2+}$ and the diprotonated free peptide ion [peptide + 2H]$^{2+}$, respectively. There was also a weaker peak at *m/z* 1183 due to the corresponding singly charged peptide. The ES mass spectrum for the 1:1 sample transformed to a mass scale is shown in Figure 1a. Significantly, with a two-fold molar excess of Fed$^+$, no ions were observed which could be attributed to [peptide + 2Fed]$^{n+}$ di-adduct species. This suggests that at the pH values employed (7.9–9.2) only the histidine residue of peptide **7** is targeted, and that no addition at the arginine side chain occurs.

In contrast, with peptide **8** which contains an additional terminal cysteine residue, the addition of a second Fed$^+$ group to the peptide chain is clearly indicated from the ES mass spectrum when a three-fold molar excess of Fed$^+$ is employed (Table 1). The ES mass spectrum showed a peak due to the dication of the mono-adduct [peptide + Fed + 2H]$^{2+}$ at *m/z* 753, and a peak at *m/z* 862, which can be attributed to the di-adduct [peptide + 2Fed + 2H]$^{2+}$. This is demonstrated more clearly in Figure 1b which shows the ES mass spectrum for this sample transformed from a *m/z* to a mass scale. These data strongly support addition of Fed$^+$ to both the cysteine and histidine residues of peptide **8**. In earlier 2D NMR studies[1] of the reaction of Fed$^+$ with peptide **8** at much lower pH (6.8), we could confirm only preferential addition at the cysteine residue since spectra were too complex to interpret in the presence of excess Fed$^+$.

The above results show that ESMS provides an extremely facile, flexible and informative method for characterising the nature of the interaction of organometallic reagents with peptides. The technique very usefully complements 2D NMR

studies, and for more complex peptides may provide information not readily available by NMR. A major attraction of ESMS is its convenience and the low sample quantities required compared with NMR spectroscopy. We have therefore commenced an extensive study of the interaction of Fed^+ and related organometallic reagents with a wide range of amino acids and peptides, in various pH and [complex]/[peptide] regimes, to establish the parameters controlling peptide labelling with these new reagents.

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