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Characterization of glutathione conjugates of pyrrolylated amino acids and peptides by liquid chromatography–mass spectrometry and tandem mass spectrometry with electrospray ionization

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Abstract

High-performance liquid chromatography (HPLC) coupled with electrospray mass spectrometry (ES-MS) and tandem mass spectrometry (MS–MS) was used to identify the products formed upon reaction of lysine-containing peptides with the neurotoxicant 2,5-hexanedione (2,5-HD). In addition, secondary autoxidative reaction products of the resultant alkylpyrroles with the biological thiol, glutathione, were characterized. ES mass spectra of the HPLC-separated conjugates showed intense $[M+H]^+$ ions as well as several ions formed by amide and C-S bond cleavage. The glutathione conjugates of pyrrolylated amino acids and peptides were analyzed by ES ionization and MS–MS, and product-ion spectra showed fragmentation pathways typical of glutathione conjugates. ES-MS–MS analysis of a synthetic nonapeptide modeling a sequence found in neurofilament proteins showed pyrrole formation after incubation with 2,5-HD, and sequence ions were used to assign the position of the pyrrole adduct. Subsequent reaction of the pyrrolylated peptide with reduced glutathione was evidenced by a shift in m/z of the sequence ions of the reaction products with or without prior methylation. The results demonstrate the utility of ES-MS and ES-MS–MS in the characterization of xenobiotic-modified peptides and confirm that stable pyrrole–thiol conjugates are formed by the reaction of biological thiols with pyrrolylated peptides.

Keywords: Amino acids; Peptides; 2,5-Hexanedione

1. Introduction

The neurotoxicity of *n*-hexane has been attributed to its cytochrome P450-catalyzed metabolism to 2,5-hexanedione (2,5-HD) and the subsequent reaction of 2,5-HD with primary amino groups to form 2,5-dimethylpyrrolyl adducts [1–5]. The formation of these adducts in neurofilament proteins is thought to be an obligatory step in the peripheral neuropathy caused by chronic hexane exposure, which is characterized by neurofilament accumulation. Although the underlying mechanisms responsible for neurofilament accumulation have not been established, a prevailing hypothesis is that autoxidative and/or nucleophilic reactions of the 2,5-dimethylpyrrolyl adducts leading to crosslinking of the neurofilament proteins are involved [1,5,6]. A clear understanding of these autoxidative reactions of pyrrolylated peptides and proteins is necessary for our understanding of the mechanisms of *n*-hexane-induced neuropathy.

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To evaluate the role of 2,5-pyrrolyl adducts and their secondary reaction products in neuropathologic changes, sensitive and specific methods for the analysis of these adducts and their subsequent reaction products are required. In recent studies in this laboratory, thermospray high-performance liquid chromatography–mass spectrometry (TSP-HPLC–MS) was used to identify the products of autoxidative reaction of the 2,5-dimethylpyrrolyl derivatives of several primary amines and amino acids [7–9]. Dimers containing pyrrole-to-pyrrole linkages were identified in autoxidative reactions of low-molecular-mass 2,5-dimethylpyrrolyl derivatives. In addition, low- molecular-mass thiol conjugates of 2,5-dimethylpyrrolyl derivatives were observed when biological thiols were included in the reaction mixtures [9]. While these studies provide insight into the types of reactions that might be observed regarding pyrrole formation and the the autoxidative and nucleophilic reactions of peptides and neurofilament proteins, the TSP-HPLC–MS technique is not appropriate for the analysis of the reaction products of these high-molecular- mass compounds. To extend these studies to the reactions of peptides and neurofilament proteins, a less energetic ionization process is required for HPLC–MS analysis.

Electrospray mass spectrometry (ES-MS) has in recent years gained wide use in the structural characterization of peptides and proteins [10–12]. ES ionization with tandem mass spectrometry (MS–MS) can be used to determine the amino acid sequence of proteins [11], as specific sequence ions are formed during fragmentation of the peptide backbone upon collision-induced dissociation (CID). In cases where proteins are modified by normal physiologic processes such as glycosylation and acylation, MS techniques can provide structural information that is difficult to obtain by other techniques [12]. In addition to physiologic processes that result in modifications of protein structure, exposure to various environmental toxicants can result in altered protein and peptide structure through covalent reaction. Many reactive metabolic products of xenobiotics, including ethylene oxide and diol-epoxides derived from polycyclic aromatic hydrocarbons, readily react with protein nucleophiles, and levels of the resulting protein adducts are often viewed as indicators of exposure to environmental toxicants [13–15]. In this study, HPLC in combination with ES-MS and ES-MS–MS was investigated for the analysis of peptide-pyrrole adducts and their subsequent products formed by reaction with glutathione.

2. Experimental

2.1. Reagents and chemicals

2,5-HD was purchased from Eastman Organic Chemicals (Rochester, NY, USA). N^{α}-Acetyllysine (NAL) and N^{α}-acetyl-Gly-Lys, methyl ester, acetate salt (GK-dipeptide) were obtained from Bachem Bioscience (Philadelphia, PA, USA). Trifluoroacetic acid (TFA), reduced glutathione (GSH) and oxidized glutathione (GSSG) were obtained from Sigma (St. Louis, MO, USA). HPLC-grade CH_3CN was obtained from Fisher (Phillipsburg, NJ, USA), and HPLC-grade water was purchased from J.T. Baker (Fair Lawn, NJ, USA). The nonapeptide N^{α}-acetyl-Ala-Glu-Ala-Lys-Ser-Pro-Ala-Glu-Ala was synthesized by staff of the Wadsworth Center Peptide Synthesis Facility using standard solid-phase Fmoc chemistry and cleavage with TFA in the presence of appropriate scavengers.

2.2. Synthesis of pyrrolylated derivatives and GSH conjugates

Pyrrolylated NAL and GK-dipeptide, and their GSH conjugation products were synthesized by incubating 2,5-HD (100 m*M*) and GSH (10 m*M*) with either NAL or GK-dipeptide (100 m*M*) in 200 m*M* sodium phosphate, pH 7.4, at 37°C under air atmosphere in the dark (standard incubation conditions) for up to 40 h. Pyrrolylated nonapeptide was prepared by incubation of 2,5-HD (100 m*M*) with nonapeptide (100 m*M*) under standard conditions followed by HPLC-based purification. Pyrrolylated nonapeptide (1.59 m*M*) was mixed with GSH (15.9 m*M*) under standard conditions to yield the GSH conjugate.

2.3. HPLC of pyrrolylated products and their conjugates

HPLC separation of all products was performed on a Waters system (Waters Associates, Milford, MA,

USA) composed of two Model 510 pumps, a Model 740 gradient controller, a Model 680 multiwavelength detector and a NovaPak C_{18} (15×0.39 cm I.D., 4 μm particle size) column. The column was eluted with a gradient of increasing CH_3CN in water with TFA constant throughout at 0.06% (v/v). The CH_3CN concentration was increased from 0% intitially to 30% (v/v) at 7 min, 55% (v/v) at 20 min, 70% (v/v) at 30 min and 80% (v/v) at 35 min; all steps were linear. The flow-rate was 1 ml/min.

2.4. Electrospray mass spectrometry

ES-MS was performed on a Fisons VG Quattro-SQ (Micromass, Manchester, UK) equipped with a Megaflow ESI interface. The HPLC effluent was split 1:10 for introduction into the MS, with nebulizing N_2 flow at 80 l/h and bath gas at 350 l/h. ES-MS–MS was performed with a Finnigan MAT TSQ-700 (Finnigan MAT, San Jose, CA, USA) equipped with a Finnigan MAT electrospray ion source. Salt-free sample compounds in 0.06% TFA were introduced into the mass spectrometer at a flow-rate of 1 to 3 μl/min by using a Harvard Apparatus Model 22 syringe pump (Harvard Apparatus, South Natick, MA, USA). Product-ion spectra were obtained with argon as the collision gas. In some cases, peptide carboxylic acid groups were converted to methyl esters by a previously described methanolysis procedure [16].

3. Results and discussion

The reactivity of primary amines and oligopeptides with 2,5-HD to form pyrrole derivatives and crosslinked products has been established [7,8,17]. In the present series of experiments, the reactivity of pyrrolylated amino acid and peptide derivatives with the biological thiol GSH was investigated using HPLC with ES-MS and ES-MS–MS. Analysis of the reaction products of a 40-h incubation mixture containing NAL, 2,5-HD, and GSH by HPLC–ES-MS is shown in Fig. 1. The total-ion current (TIC) chromatogram exhibited a peak (A1) corresponding to the unreacted NAL (retention time 2.3 min) and a major non-polar peak at 21.1 min (A6) that corresponded to the pyrrole derivative of NAL, with $[M+H]^+$ at *m/z* 267 as identified in previous studies

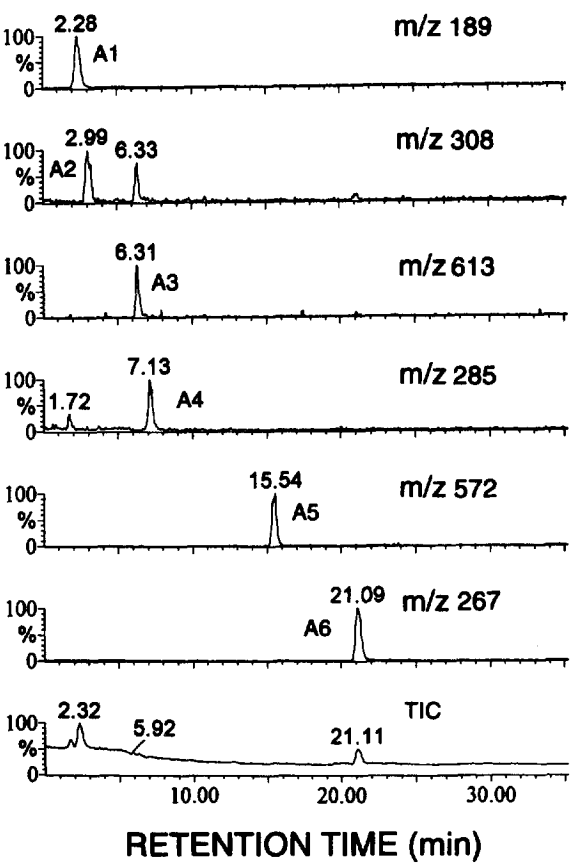

Fig. 1. HPLC–ES-MS reconstructed-ion chromatogram of the reaction products of 2,5-HD, NAL and GSH. 2,5-HD (100 m*M*), NAL (100 m*M*) and GSH (10 m*M*) were incubated under the standard conditions for 40 h. An aliquot (3 μl) of the reaction mixture was analyzed by HPLC–ES-MS with an acetonitrile gradient and a 1:10 split for ES–MS. The identity of the peaks labeled A1–A6 is discussed under Section 3.

[8,9]. Reconstructed-ion chromatograms showed that, in addition to unreacted NAL and pyrrolylated NAL, several other components (A2–A5) were present in the reaction mixture (Fig. 1). Tabulations of the principal ion intensities of mass spectra of these compounds, peak areas of the assigned $[M+H]^+$ ions and the proposed structural assignments are presented in Table 1.

The mass spectrum of the component eluting with retention time 15.5 min (A5) is shown in Fig. 2. On the basis of the intense peak at *m/z* 572, assigned to $[M+H]^+$, and ions that appear to be derived from cleavage of an amide bond and the two C–S bonds, this compound can be assigned the structure of the

Table 1
Characterization of the reaction products of 2.5-HD, NAL and GSH by HPLC–ES-MS

Peak[a]	HPLC retention time (min)	ES-MS, *m/z* (relative intensity)	Peak area[c]	Structural identification
A1	2.3	377(9), 189(100)[b], 147(15) 130(36), 129(77)	1229	NAL
A2	3.0	308	28	GSH
A3	6.3	635(8), 613(86)[b], 484(25), 355(22), 307(100), 235(15), 231(35), 179(28), 177(48), 129(35)	19	GSSG
A4	7.1	285(100)[b], 209(17), 151(19)	56	unknown
A5	15.5	594(14), 572(100)[b], 443(42), 297(15), 287(13), 265(10)	54	GSH adduct of pyrrolylated NAL
A6	21.1	531(11), 267(100), 189(9), 225(22), 162(24)	812	pyrrolylated NAL

[a] As indicated in Fig. 1.
[b] Assigned to $[M+H]^+$.
[c] From the reconstructed-ion chromatograms of the assigned $[M+H]^+$ ions (Fig. 1), expressed in arbitrary units ($\times 10^{-3}$).

glutathione adduct of pyrrolylated NAL (Fig. 2, inset). In a similar manner, the products of the incubation of 2,5-HD, GK-dipeptide, and GSH were analyzed by HPLC–ES-MS with the same gradient elution, and pyrrolylated GK-dipeptide (retention time 22.5 min, $[M+H]^+$ at *m/z* 338) and the putative glutathione conjugate (retention time 16.0, $[M+H]^+$ at *m/z* 643) were identified (data not shown). In comparison with analyses by HPLC–TSP-MS [9], HPLC–ES-MS was found to be 10- to 50-fold more sensitive for the analysis of these compounds. In addition, the HPLC solvent system used in the present study to aid ES–MS ionization (0.06% TFA in acetonitrile–water) also proved better for the HPLC resolution of these compounds than when 0.1 *M* ammonium acetate was included in the mobile phase to aid in thermospray ionization.

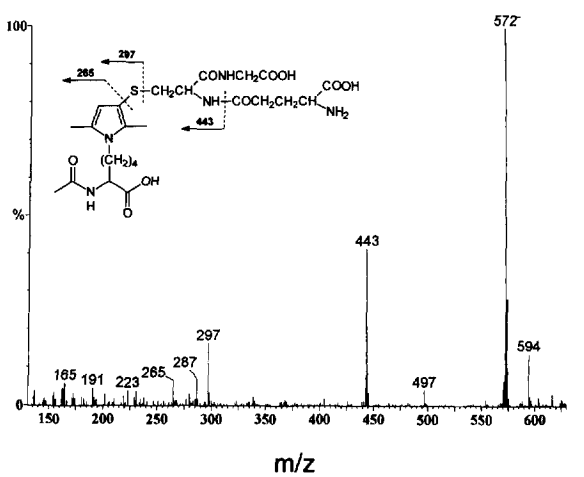

Fig. 2. ES-MS of GSH conjugate of pyrrolylated NAL. The mass spectrum of the component eluting with retention time 15.54 min in Fig. 1 is shown. Inset: proposed structure and fragmentation pattern of the conjugate.

The compounds that were resolved by HPLC in Fig. 1 were recovered from the HPLC effluent and further analyzed by ES-MS–MS. The CID product-ion spectra of several glutathione conjugates have been investigated in detail [18–22], and, if the structural assignments of the current study as based on HPLC-ES–MS are correct, CID product-ion spectra of the protonated GSH conjugates should show ions that can be assigned to the **a** through **h** ions (see Fig. 3) of the glutathione fragmentation series [18,19]. The low-energy product ion spectrum derived from collisional activation of the protonated pyrrolylated dipeptide–GSH conjugate ($[M+H]^+$ at *m/z* 643) is shown in Fig. 3, and the fragmentation data are summarized in Table 2.

Three major decomposition pathways that are in confirmation of the proposed glutathione conjugate structure were observed by MS–MS analysis. The ion at *m/z* 514 resulted from the loss of the γ-glutamyl moiety (M_r 129) of GSH (**e** ion). The ion at *m/z* 568 resulted from the elimination of glycine (M_r 75) to form an **a**-series ion which, by subsequent loss of X (pyrrolylated dipeptide moiety; M_r 336), gave

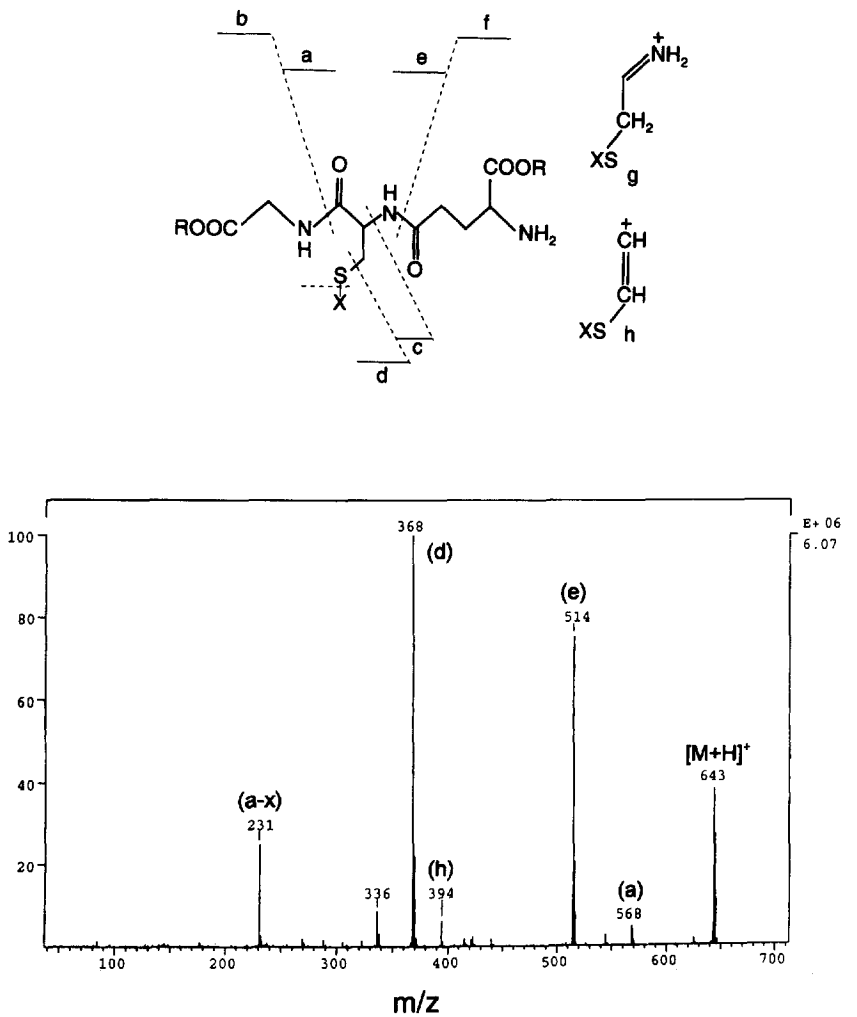

Fig. 3. Low-energy CID fragmentation pattern of GSH conjugates. The nomenclature system of Haroldsen et al. [18] is illustrated above, and the MS–MS spectrum obtained by CID of the $[M+H]^+$ ion at m/z 643 of the GSH conjugate of the pyrrolylated GK-dipeptide is shown below with the ions of glutathione fragmentation pathways assigned.

Table 2
MS–MS fragmentation of GSH-pyrrole conjugates

GSH conjugate of	$[M+H]^+$, m/z (relative intensity)	Fragment ions[a], m/z (relative intensity)						
		a	**e**	**d**	**h**	X	**a**−X	other
Pyrrolylated NAL	572(42)	497(6)	443(42)	297(100)	323(4)	265(10)	231(18)	351(8)368(3)
Pyrrolylated NAL, methylated	614(68)	525(7)	471(73)	311(100)	337(6)	279(8)	245(9)	365(2)382(1)
Pyrrolylated GK-dipeptide	643(40)	568(5)	514(76)	368(100)	394(7)	336(10)	231(25)	269(3)
Pyrrolylated GK-dipeptide, methylated	671(100)	582(7)	528(54)	368(75)	394(2)	336(6)	245(10)	269(2)

[a] Fragmentations are illustrated in Fig. 3.

rise to the $[\mathbf{a}-X+H]^+$ ion at m/z 231. The pathways **e** and **a** represent the primary fragmentation pathways of native GSH and N-derived GSH conjugates respectively [18,19]. A glutathione fragmentation **d**-series ion, $[XS+H]^+$, resulting from the cleavage of the cysteinyl C–S bond, was observed at m/z 368. The presence of a **d**-series ion is considered to be a characteristic of aromatic thio-ether conjugates [20]. The observation of a **d** ion as the base peak in the product-ion spectra of the GSH-pyrrole conjugates is evidence that the sulfur atom of GSH is bound to carbon 3 of the pyrrole ring; this structural assignment is further supported by the lack of **c**- and **g**-series ions. In addition to the prominent ions, several minor fragment ions were also generated by collisional activation of the m/z 643 ion, including an **h**-series ion at m/z 394 an ion at m/z 336 representing the pyrrolylated dipeptide moiety. The results of other MS–MS analyses of the glutathione conjugate of pyrrolylated NAL and of various methyl esters of the glutathione conjugates of pyrrolylated NAL and GK-dipeptide are also summarized in Table 2.

The rat neurofilament proteins NF-H and NF-M show selective pyrrole adduction in their carboxyl-terminal domains in in vitro incubation studies with 2,5-HD [23]. These domains contain numerous repeats of the sequence Lys-Ser-Pro, which are putative phosphorylation sites for NF-specific kinases [24]. Since they are potential adduction target sites for pyrrolylation by 2,5-HD, a nonapeptide was synthesized that corresponds to a consensus sequence present in multiple copies in rat NF-H protein [25]. The sequence of the synthetic N^{α}-acetylated nonapeptide was confirmed by electrospray MS–MS. Upon CID of a $[M+H]^+$ ion at m/z 915, the complete *b* series [26] and most of the *y*-series ions were observed (Fig. 4).

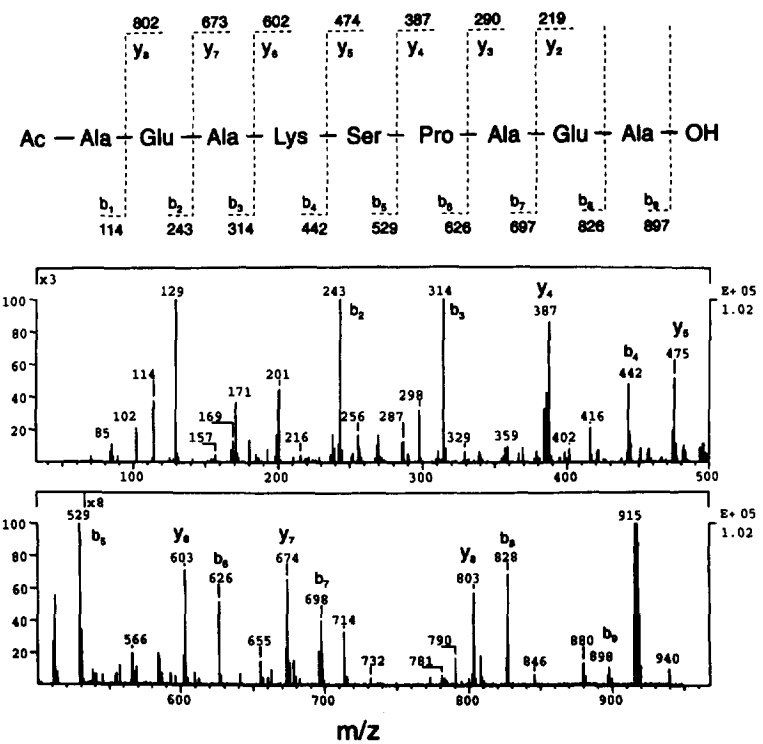

Fig. 4. Sequence analysis of N^{α}-acetylated nonapeptide by ES-MS–MS. The product-ion spectrum from collisional activation of the $[M+H]^+$ ion at m/z 915 is shown together with the assignments for the *b*- and *y*-series ions.

The pyrrolylated derivative of the nonapeptide was prepared by incubation with 2,5-HD followed by purification by HPLC. The ES mass spectrum showed a series of peaks in the molecular-ion region; $[M+K]^+$ at m/z 1031 (18% relative intensity), $[M+Na]^+$ at m/z 1016 (11%), $[M+H]^+$ at m/z 994 (10%), $[M+2K]^{2+}$ at m/z 535 (20%) and $[M+K+H]^{2+}$ at m/z 516 (100%) (data not shown). The product-ion spectrum obtained upon CID of the protonated molecule afforded a series of sequence ions that indicated the position of the pyrrole moiety (Fig. 5). The ions b_4, b_5, b_7, and b_8 were apparent at m/z 520, 607, 775 and 904, respectively, each at an increment of 78 Da over the corresponding b-series ions in the product-ion spectrum of the unadducted nonapeptide (Fig. 4). The ion at m/z 475 is assigned to y_5, a fragment that includes the five C-terminal amino-acid residues of the peptide. This y_5 ion is identical to that observed in the parent-ion spectrum of the unreacted peptide, indicating that the pyrrole adduct must be in the Ala_1 to Lys_4 portion of the peptide. The assignment of the ion at m/z 680 as the y_6 ion, which is 78 amu higher than the y_6 ion of the unreacted peptide, places the pyrrole adduct at Lys_4.

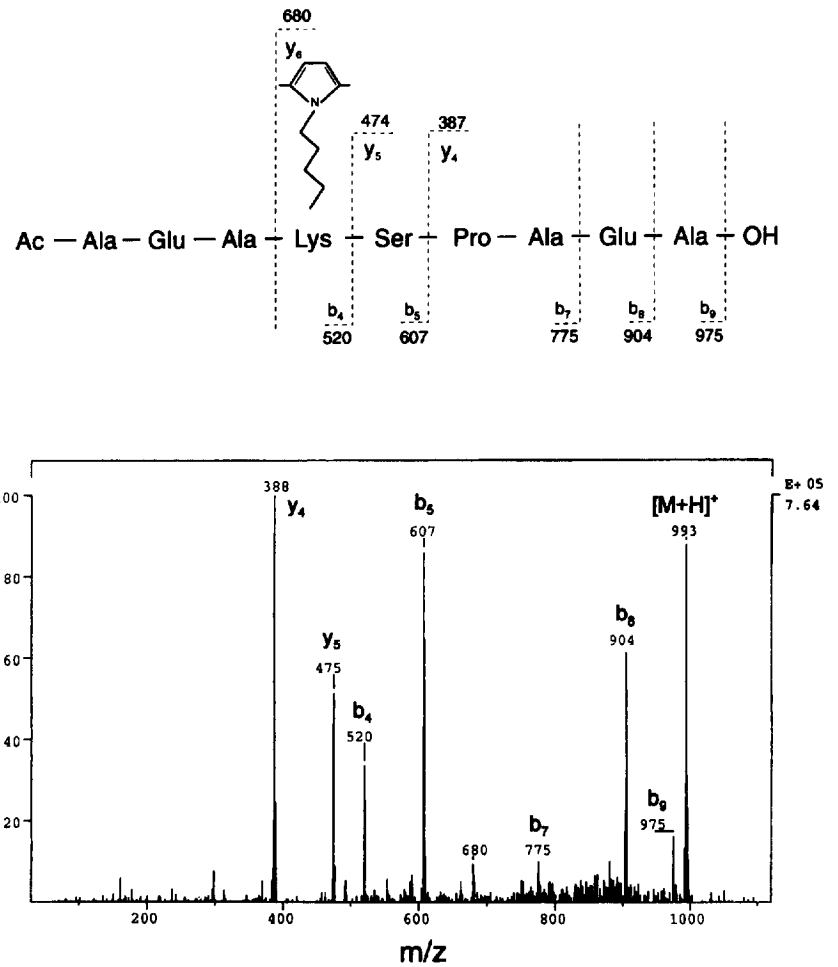

Fig. 5. Structural characterization of pyrrolylated nonapeptide by ES-MS–MS. The product-ion spectrum from collisional activation of the $[M+H]^+$ ion at m/z 993 is shown together with the assignments for b- and y-series ions.

The pyrrolylated nonapeptide was incubated with GSH under standard conditions. After 18 h incubation, analysis by HPLC–UV showed that an additional compound was produced (Fig. 6, peak B2). This component was recovered from the HPLC eluent, lyophilized, and esterified by methanolysis prior to analysis by ES-MS and ES-MS–MS. The ES mass spectrum of the product showed a series of singly- and multiply-charged ions consistent with protonated and salt-adducted species of the fully methylated nonapeptide–pyrrole–GSH conjugate (data not shown).

A second product was also produced by methylation of the lyophilized product, one which by ES-MS analysis appeared to have four rather than five methylated carboxyl groups. ES-MS–MS analysis of this second product was performed by collisional activation of an ion with m/z 678, which was assigned to $[M+2H]^{2+}$ of the tetramethyl GSH adduct. The resulting product-ion spectrum (Fig. 7) indicated that the free carboxyl group was present in the glutathione moiety. Both y-series and b-series ions were observed, as well as additional ions at m/z 626, 533 and 613 which were assigned to $[b_8+H]^+$, $[b_9-b_2]^+$ and $[y_6-y_4]^+$ respectively. When the b- and y-series assignments were made, the mass differences between the b_4 and b_3 and the y_6 and y_5 ions were both 525 amu, which equals the mass of the methylated GS–pyrrole–Lys fragment. Thus, the product-ion spectrum was entirely consistent with the reaction of GSH with the pyrrolyl adduct at Lys_4.

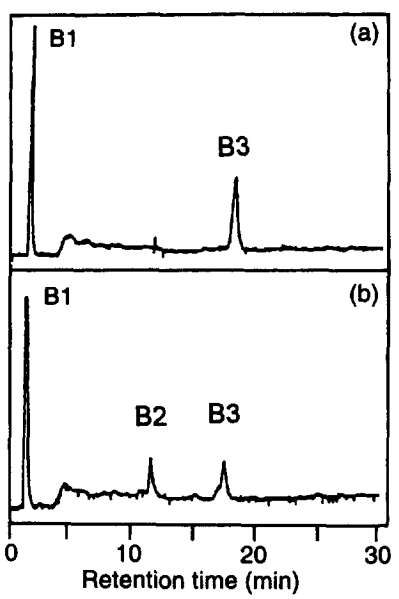

Fig. 6. HPLC separation with UV detection of a mixture of pyrrolylated nonapeptide and GSH, as described in Section 2.3, incubated for zero h (a), and for 18 h under air (b). Peaks B1 and B3 represent unreacted GSH and pyrrolylated nonapeptide, respectively. Peak B2 represents the GSH conjugate of pyrrolylated nonapeptide.

4. Conclusion

This study demonstrates the utility of ES-MS and ES-MS–MS for the analysis of the products of primary and secondary adductive reactions of 2,5-HD, an oxidative metabolite of n-hexane, with polypeptides. A nonapeptide modeling a neurofilament peptide sequence that is a suspected target in n-hexane-induced neuropathy was found to react readily with 2,5-HD to form a pyrrole adduct. Peptides containing pyrrole adducts were readily analyzed by ES-MS, and ES- MS–MS was utilized to determine the position of the adduct in the peptide sequence. The results also showed that stable secondary adducts can be formed by reaction of GSH with pyrrolylated peptides, and that these can be analyzed by ES-MS–MS. These results raise the possibility that the stable secondary adducts formed by the reaction of GSH with pyrrolylated peptides and proteins may more readily serve as potential biomarkers for n-hexane exposure than the highly reactive pyrrolyl adducts.

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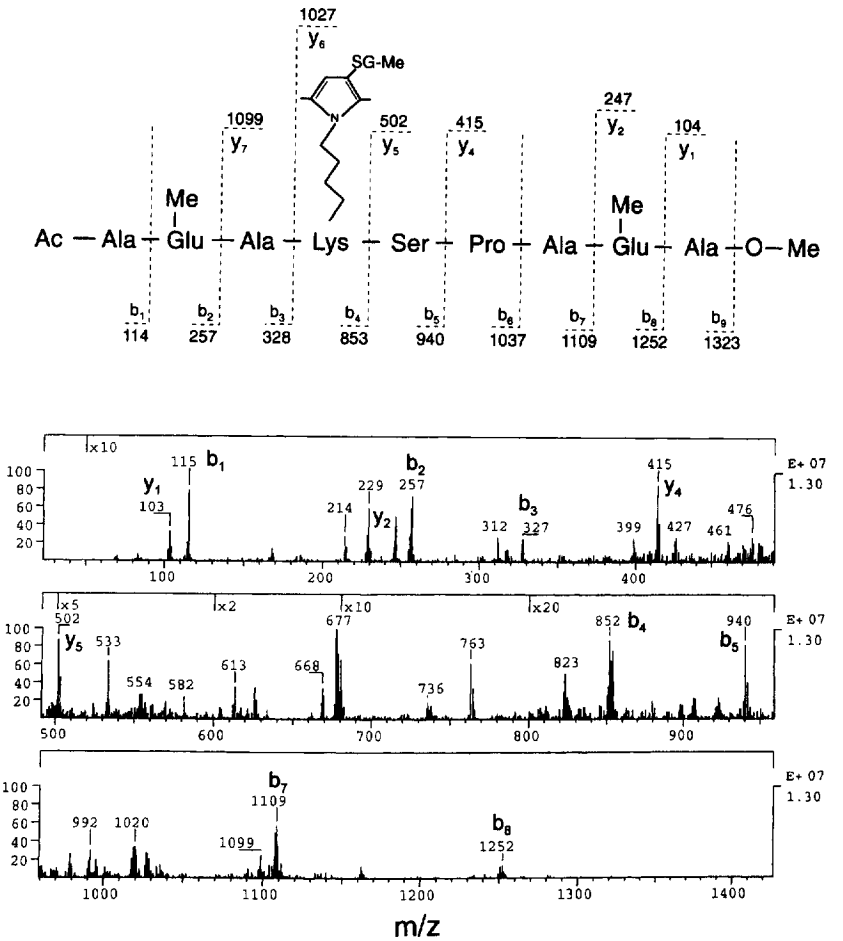

Fig. 7. Structural characterization of the tetramethylated glutathione conjugate of pyrrolylated nonapeptide by ES-MS–MS. The product-ion spectrum from collisional activation of the $[M+H]^{2+}$ ion at m/z 678 is shown together with the assignments for *b*- and *y*-series ions.

References

[1] A.P. DeCaprio, E.J. Olajos and P. Weber, Toxicol. Appl. Pharmacol., 65 (1982) 440.

[2] D.G. Graham, D.C. Anthony, K. Boekekheide, N.A. Maschmann, R. G. Richards, J.W. Wolfram and B.R. Shaw. Toxicol. Appl. Pharmacol., 64 (1982) 415.

[3] L. Perbellini, M.C. Amantini, F. Brugnone and N. Frontali, Arch. Toxicol., 50 (1982) 203.

[4] A.P. DeCaprio and E.A. O'Neill, Toxicol. Appl. Pharmacol., 78 (1985) 235.

[5] M.B. Genter St. Clair, V. Amanrath, M. A. Moody, D.C. Anthony, C.W. Anderson and D.G. Graham. Chem. Res. Toxicol., 1 (1988) 179.

[6] A.P DeCaprio, Mol. Pharmacol., 30 (1986) 452.

[7] M. Zhu, D.C. Spink, S. Bank, X. Chen and A.P. DeCaprio, J. Chromatogr., 628 (1992) 37.

[8] M. Zhu, D.C. Spink, B. Yan, S. Bank and A.P. DeCaprio, Chem. Res. Toxicol., 7 (1994) 551.

[9] M. Zhu, D.C. Spink, B. Yan, S. Bank and A.P. DeCaprio, Chem. Res. Toxicol., 8 (1995) 764.

[10] J.B. Fenn, M. Mann, C.K. Meng, S.K. Wong and C. Whitehouse, Science, 246 (1989) 64.

[11] J.A. Loo, C.G. Edmonds and R.D. Smith, Science, 248 (1990) 201.

[12] S.A. Carr, M.E. Hemling, M.F. Bean and G.D. Roberts, Anal. Chem., 63 (1991) 2802.

[13] M. Törnqvist, J. Mowrer, S. Jensen and L. Ehrenberg, Anal. Biochem., 154 (1986) 255.

[14] V.E. Walker, J.P. MacNeela, J.A. Swenberg, M.J. Turner and T.R. Fennell, Cancer Res., 52 (1992) 4320.

[15] J.C. Erve, M.L. Deinzer and D.J. Reed, Chem. Res. Toxicol., 8 (1995) 414.
[16] D.F. Hunt, J.R. Yates, J. Shabanowitz, S. Winston and C.R. Hauer, Proc. Natl. Acad. Sci. USA, 83 (1986) 6233.
[17] V. Amarnath, W.M. Valentine, K. Amarnath, M.A. Eng and D.G. Graham, Chem. Res. Toxicol. 7 (1994) 56.
[18] P.E. Haroldsen, M.H. Reilly, H. Hughes, S.J. Gaskell and C.J. Porter, Biomed. Environ. Mass Spectrom., 15 (1988) 615.
[19] P.G. Pearson, M.D. Threadgill, W.N. Howald and T.A. Baillie, Biomed. Environ. Mass. Spectrom., 16 (1988) 51.
[20] P.G. Pearson, W.N. Howald and S.D. Nelson, Anal. Chem., 62 (1990) 1827.
[21] T.A. Baillie and M.R. Davis, Biol. Mass Spectrom., 22 (1993) 319.
[22] C. Fenselau and P.B.W. Smith, Xenobiotica, 22 (1992) 1207.
[23] A.P. DeCaprio and J.H. Fowke, Brain. Res., 586 (1992) 219.
[24] G. Shaw, in R.D. Burgoyne (Editor), Neurofilament proteins, The Neuronal Cytoskeleton, Wiley-Liss, New York, 1991, pp. 185–214.
[25] S.S.M. Chin and R.K.H. Liem, J. Neurosci., 10 (1990) 3714.
[26] P. Roepstorff and J. Fohlman, Biomed. Mass Spectrom., 11 (1984) 601.