Cysteine and Acetylcysteine Conjugated Acylcarnitines Revealed by LC-MS/MS

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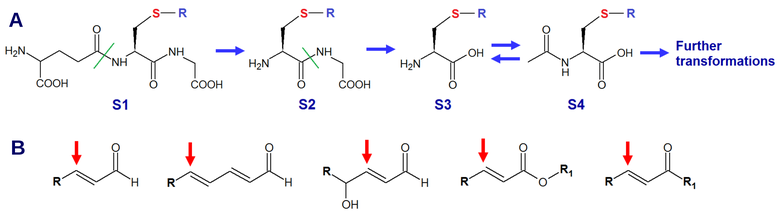
1. Introduction

Acylcarnitine (AC, $C_nH_mNO_k$) is one of the major classes of urinary metabolites and can be modified as a complex compound with a composition "$C_nH_mN_iO_kS_j$" by phases I and II bio-transformations. The modified species have not been reported before. Missing information on these modifications can hinder a complete profiling of a biological sample and important biomarkers for diagnosing and monitoring diseases. This report presents identifications of bio-transformed ACs, in particular cysteine and acetylcysteine conjugated ACs, obtained from LC ESI-MS/MS data of urine materials. The identified MS/MS spectra will be incorporated into a mass spectral library of ACs.

2. Background

Glutathione (GSH), a tripeptide, *γ*-L-glutamyl-L-cysteinyl glycine, exists in high concentration (millimolar) in the intracellular fluid of mammalian systems. GSH targets electrophilic compounds or groups during transformation, and finally results in cysteine, acetylcysteine conjugates, etc., see Figure 1A. For acylcarnitines, the relevant electrophilic groups are presented in Figure 1B.

Figure 1. **A**. This figure presents a GSH-conjugated compound **R** (**S1**) and its enzymatic metabolites: **S2 –** cysteinyl glycine conjugate, **S3 –** cysteine conjugate, **S4 –** *N*-acetylcysteine conjugate, and oxidations on the sulfur atom of cysteine; **B**. The red arrows point to the most possible GSH-conjugate positions on metabolites with double bond [1-3].



3. Methods

Six NIST urine standard reference materials served as the source of acylcarnitines for this study. HCD spectra were acquired using reverse phase C18 columns coupled with a Thermo Lumos mass spectrometer in positive electrospray and data dependent modes. Resolutions were set at 120,000 for full MS scan, and 30,000 for MS/MS scan. Nine normalized collision energies (NCE) were used to acquire tandem spectra. A computer program was developed to detect bio-transformed acylcarnitines.

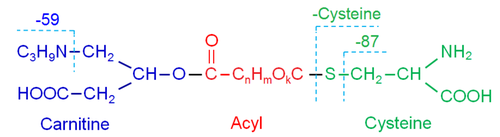
4. Results

Around 80 modified acylcarnitines were identified by the analyses of high resolution HCD spectra acquired from 108 LC-MS/MS runs of six urine samples. This work revealed that glutathione conjugated acylcarnitines are the major subclass among the bio-transformed acylcarnitines in the studied urine samples. Therefore, this poster concentrates on glutathione (i.e., cysteine and acetylcysteine) conjugated acylcarnitines.

4.1 Conjugated acylcarnitine structure and major fragments

For acylcarnitine, glutathione attacks an electrophilic C of the acyl chain and forms a S-C bond, see Figure 2. The most significant tandem mass spectral fragments of the conjugated ACs are the neutral loss peaks of C_3H_9N + cysteine for cysteinylated ACs and C_3H_9N + acetylcysteine for acetyl-cysteinylated ACs from their precursors.

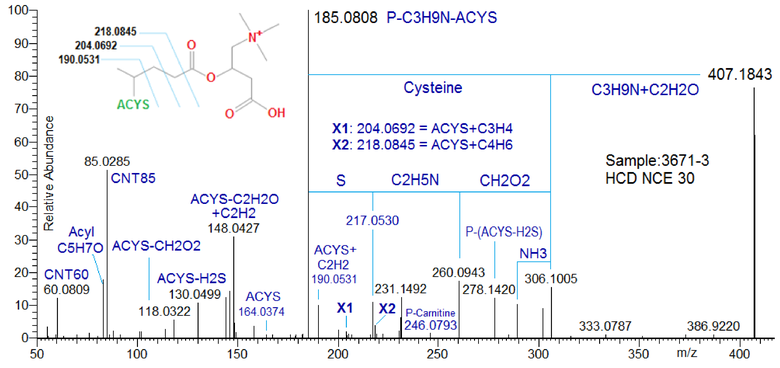
Figure 2. **Three moieties of cysteinylated acylcarnitine and labile bonds.**



4.2 HCD spectra

The HCD mass spectra of conjugated acylcarnitines contains feature fragments for recognizing their chemical class. Some spectra may provide enough structural data, an example is given in Figure 3. This spectrum (NCE 30) indicates a possible structure by the consecutive cleavages between C–C bonds of the ion acyl moiety, but without the fragment ACYS+CH_2, indicating that the S atom of ACYS does not bond to a terminal C of the acyl.

Figure 3. A HCD spectrum of acetyl-cysteinylated acylcarnitine C5.



4.3 Retention times (RT) of conjugated and un-conjugated acylcarnitines

As expected, cysteinylated ACs eluted much earlier than that of the corresponding un-conjugated ACs; some examples are provided in Table 1. However, acetyl-cysteinylated ACs eluted later than or similar to that of corresponding un-conjugated ACs; see Table 2.

Table 1. Retention times of cysteinylated and corresponding unconjugated ACs.
CYS-AC: cysteinylated AC; Ion formula: cysteinylated AC ion formula; EOAC: estimated original ACs before conjugation; RT: retention time in second.

Cysteine (CYS) conjugated				Unconjugated	
CYS-AC *m/z*	EOAC	CYS-AC formulas	RT/s	AC	RT/s
351.1584	C4:1	$C_{14}H_{27}N_2O_6S$	66	C4:1	193
365.1741	C5:1	$C_{15}H_{29}N_2O_6S$	164	C5:1	434
407.2210	C8:1	$C_{18}H_{35}N_2O_6S$	673	C8:1	1007
421.2367	C9:1	$C_{19}H_{37}N_2O_6S$	758	C9:1	1218
431.2210	C10:3	$C_{20}H_{35}N_2O_6S$	674	C10:3	1170
433.2367	C10:2	$C_{20}H_{37}N_2O_6S$	858	C10:2	1192

Table 2. **Retention times of acetyl-cysteine (ACYS) conjugated and unconjugated ACs.**

Acetyl-cysteine conjugated				Unconjugated	
ACYS-AC *m/z*	EOAC	ACYS-AC formulas	RT/s	AC	RT/s
393.1690	C4:1	$C_{16}H_{29}N_2O_7S$	367	C4:1	193
407.1846	C5:1	$C_{17}H_{31}N_2O_7S$	470	C5:1	434
449.2316	C8:1	$C_{20}H_{37}N_2O_7S$	1117	C8:1	1007
473.2316	C10:3	$C_{22}H_{37}N_2O_7S$	1176	C10:3	1170
475.2472	C10:2	$C_{22}H_{39}N_2O_7S$	1188	C10:2	1192
477.2629	C10:1	$C_{22}H_{41}N_2O_7S$	1202	C10:1	1226

4.4 Abundances of conjugated and un-conjugated acylcarnitines

We found that the conjugated acylcarnitine abundances can be smaller or larger than or similar to these of corresponding unconjugated acylcarnitines. See examples in Table 3.

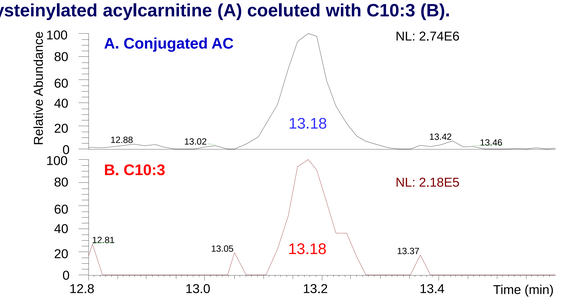
Table 3. **Acylcarnitine and conjugated acylcarnitine median abundances -** based on the total ion chromatograms of 18 runs of one urine sample. (Note: only the values of the most abundant isomers are listed here.)

Acylcarnitines without conjugations		Acylcarnitines with conjugations	
Acylcarnitines	Abundances	Conjugated Acylcarnitines	Abundances
C4:1	1.18e+8	C4:1 + Cysteine	4.31e+8
		C4:1 + Acetylcysteine	1.44e+7
		C4 + SO_2	5.84e+7
C5:1	5.93e+8	C5:1 + Cysteine	3.64e+8
		C5:1 + CH_4OS	2.08e+7
		C5:1 + Acetylcysteine	7.29e+6
C6:1	6.85e+7	C6:1 + SH	1.00e+8
C8:1	8.74e+9	C8:1 + Cysteine	2.03e+7
		C8:1 + Acetylcysteine	2.32e+7
C10:2	8.81e+8	C10:2 + Cysteine	1.04e+8
		C10.2 + Acetylcysteine	1.56e+8

4.5 Conjugated acylcarnitines and dimers?

The analysis of the urine data indicates that conjugated acylcarnitines may form dimers with unconjugated acylcarnitines in the urine sample solution. For example, acylcarnitine C10:3 ($C_{17}H_{28}NO_4$) coelutes with cysteinylated acylcarnitine $C_{20}H_{35}N_2O_6S$ at 13.18 min, see Figure 4, while all other isomers of C10:3 eluted after 19.40 min. The two ions' average RT difference and median of 18 runs from one sample are 0.40 and 0.34 seconds, respectively. The analysis of MS1 and MS2 spectra indicates that the coeluted C10:3 is not an in-source product, suggesting the presence of a dimer that can be dissociated.

Figure 4. **Cysteinylated acylcarnitine (A) coeluted with C10:3 (B).**



5. Summary

This research reveals the presence of phase II modified acylcarnitines in the studied urine samples, and describes mass spectra, retention times, abundances and dimers of cysteine and acetylcysteine conjugated acylcarnitines. These transformed acylcarnitines are important for describing a more complete picture of acylcarnitines in a biological sample, as well as for understanding the functions of bio-transformations. These findings are valuable for developing metabolite identification strategies.

Reference

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3. Andrew Parkinson and Brian W. Ogilvie. Biotransformation of Xenobiotics, page 161, chapter 6 in Casarett and Doull's Toxicology - the Basic Science of Poisons (editor: Curtis D. Klaassen), Seventh edition, McGraw-Hill, New York, 2008.