Application of Solid Phase Micro Extraction (SPME) and On-Fiber Derivatization

to Detect Precursors of Biomolecules in the study of Proto-metabolism

プロトメタボリズム研究における生体分子前駆体検出のためのマイクロ固相抽出・誘導体化法

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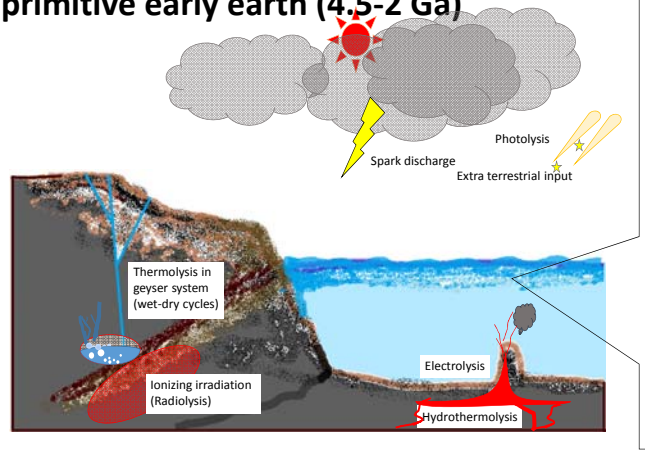
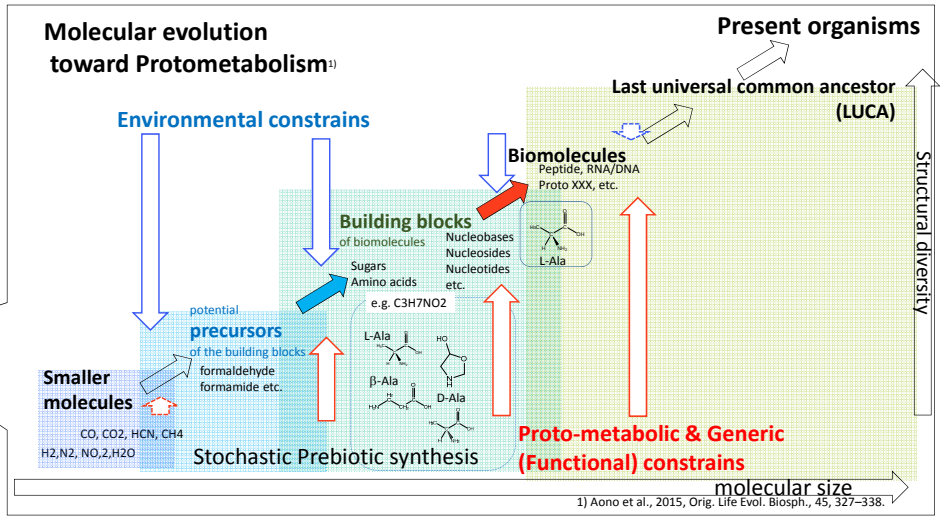
Keywords: proto-metabolism, GC-MS, SPME, on-fiber derivatization

■ Prebiotic organic syntheses on the primitive early earth (4.5-2 Ga)





In the studies of origins of life, plausible synthetic reaction pathways to form biological relevant molecules, or building blocks of proto-bilayer lipids, protein, RNA, and DNA in the primitive environment have been investigated intensively. We are focusing on studying syntheses of primitive molecular set, including **Sugar, amino acid, and their analogues**, which were selected by further functional and informational constrains. Researchers hope to detect those targets, even they were ultra-trace comparing to major components (source molecules and undefined byproducts). Analytical point of the views, **it is the worst situation**.

Analytical challenges

- Qualification of unpredictable products
  70eV EI fragmentation combined with NIST data base was the best way to get structural information.=> **GC-MS**
- Messy matrix (metal/buffer)
  Pretreatment is must before GC-MS:
  - Desalt and metal elimination
  - Molecular screening (chemical prop./ size)
  - Chemical derivatization

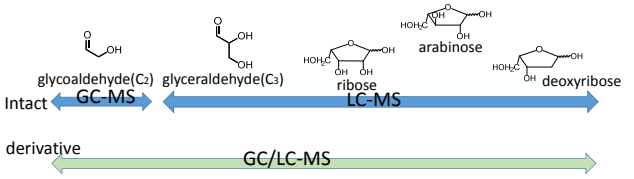
  } requiring much time and effort

=>

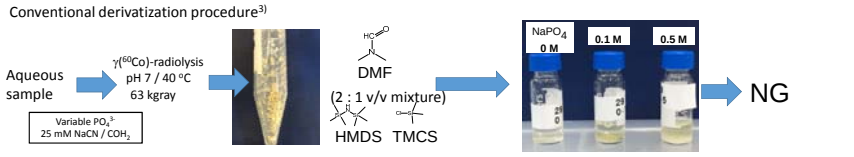
An automated technique using a large volume SPME (**SPME Arrow**[TM]) mounted on a **CTC-PAL system was** applied.

Prebiotic product analyses demand new analytical approaches.

Simultaneous analysis of Potential products in Formose reaction (1861)[2]



Conventional derivatization procedure[3]

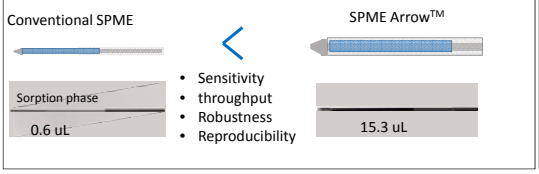


Salts, inorganics, unexpected diverse products containing polymers disturbed the effective reactions.

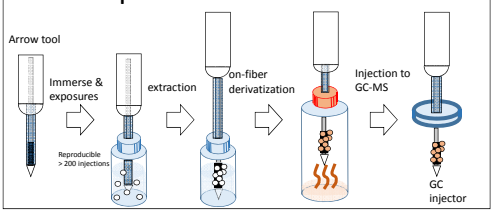
2) Butlerov, A. C. R.,1861, Acad. Sci., 53, 145-147 3) Borate Minerals Stabilize Ribose A. Ricardo, et al. 2004, Science, 303, 196

■ SPME Arrow extraction and on fiber derivatization

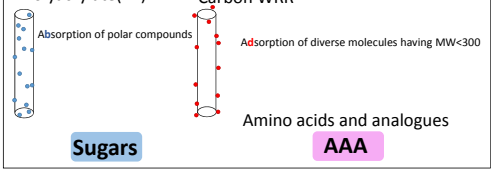
SPME Arrow[TM] fibers



The simplest schematic workflow



Fiber choice



Standard experiments

GCMS: SHIMADZU QP-2010 Ultra split/splitless injector GC2010 system DB-5 column (Agilent, 30 m, 0.25 mm I.D., 0.25 mm df).

GC conditions

Sugars

Injection Temperature	270 °C
Split Flow	64.3 ml/min
Injection Mode	Splitless
Carrier Gas, Column Flow	He, 1.20 ml/min
Oven Temperature	65 °C hold 2 min 1°C/min to 100°C 100°C/min to
	280°C
	200°C/min to hold 2min
320°C	
Interface Temperature	210 °C

AAA

Injection Temperature	295 °C
Split Flow	64.3 ml/min
Injection Mode	Splitless
Carrier Gas, Column Flow	He, 1.20 ml/min
Oven Temperature	65 °C hold 4 min 27°C/min to 280oC 180°C/min to 320 oC hold 2min
Interface Temperature	250 oC

EI-MS conditions

Sugars **AAA**

Retention times and reference ions (standard)

Sugars

Standard Peaks tR(min)	Standard sugars	Reference peaks (m/z)
5.595	2TMS-Glycoaldehyde (C2)	117 116 191 73 59
9.415	3TMS-Glyceraldehyde (C3)	73 147 191 218 307 103
9.570	3TMS-Glyceraldehyde (C3)	73 103 147 217 307 103
9.665	3TMS-Glyceraldehyde (C3)	73 103 147 217 307 103
8.585	4TMS-Ribose (C5)	73 217
8.66	4TMS-Ribose (C5)	73 217
8.705	4TMS-Ribopyranose (C5)	73 147 191 204 217
8.840	4TMS-Ribofranose (C5)	73 147 191 204 217
8.430	4TMS-Arabinose (C5)	73 147 191 204 217
8.670	4TMS-Arabinpyranose (C5)	73 147 191 204 217
8.865	4TMS-Arabinofranose (C5)	73 147 191 204 217
7.430	3TMS-Deoxyribopyranose (C5)	73 75 101 103 116 129 133 147 191 204 217 260
7.550	3TMS-Deoxyribose (C5)	73 75 101 103 116 129 133 147 191 204 217 260

AAA

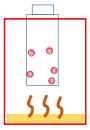
Standard Peaks tR(min)	Standard amino acids	Reference peaks (m/z)
6.540	2TBDMS-pyrvate	73 147 189 261
6.84	2TBDMS-Glycine	73 147 218 206
6.760	2TBDMS-Alanine	73 147 158 232 260 302
7.220	2TBDMS-beta Alanine	73 145 147 218 260 302
8.640	3TBDMS-Serine	73 147 288 302 362 390 432

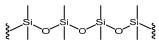
PAL tasks and typical parameter settings

		Mount Arrow tool	Fiber exposure in GC inset		Sample extraction	drying	derivatization	desolvation	injection
T [°C]	Sugars	Polyacrylate 100 µm	270	Aqueous samples 0.1-1 mM 200 uL	Room temperature	60	45 DMF/HDMS&TMSC=1/1	60	280
	AAA	Carbon WR/PDMS 120 µm	295				60 MTBSFTA/CH3CN=1/1		295
Duration [min]	Sugars		2		60	0.5	10	10	4
	AAA		4		20		20		

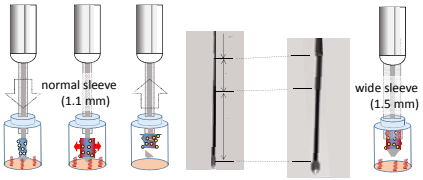
Swelling character of PDMS based fiber

The fiber was broken in the derivatization step





Unexpected swelling of PDMS contained in Carbon WR fiber caused serious damage to the fiber in the derivatization step. It was mechanical problem, so wide sleeve fiber was applied.



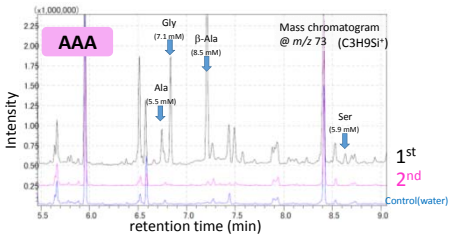
Fiber was swelled by the reagents in derivatization.

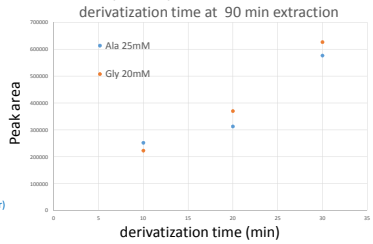
The expanded surface area would enhance the capacity retaining molecules.[4]Chemical property of PDMS (log Ko/w (a logarithm of octanol-water partitioning coefficient) > 3.0) [5] can be modified with appropriate solvents. Wide sleeve fiber is profitable to various applications.

4) Ochiai N., Samamoto K. David F, Sandra P. *J Chromatogr A.* 2016, 15, 1455, 45-56.
5) J. Li, Y.-B. Wang, K.-Y. Li, Y.-Q. Cao, S. Wu, L. Wu *Trends Anal. Chem.*, 2015, 72, 141–152.

Sensitive analyses v.s. Memory effect

Recovery check for 10 min extraction 10 min extraction(60oC)

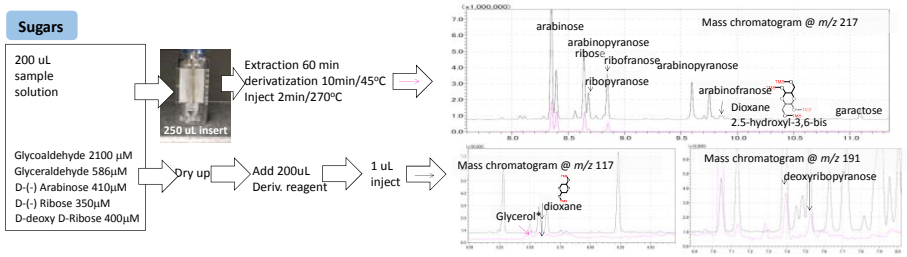




Signals were substantially decreased in the 2nd analyses for c.a.10 mM samples, indicating that 2 umol NET AA in 200 uL aqueous solutions can be accumulated for 10 min.

Longer duration in the extraction enhanced signal intensities, and required longer duration in derivatization. Imbalance between long extraction and short derivatization cause serious memory effect.

■ Comparison between conventional and this technique for 200 uL sugar solution samples.

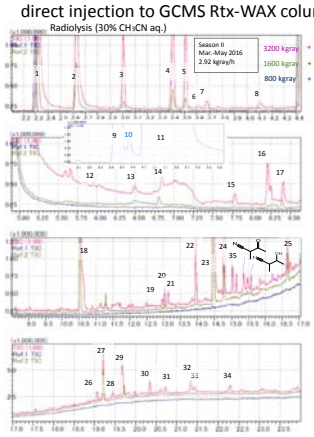


γ (^{60}Co)-radiolysis sample analyses

The radiolysis sample was prepared by aqueous CH_2O (33 mM) and CH_3CN (30%)/(15%) with NH_4HCO_3. Dose rates of gamma radiation from a ^{60}Co source at the Tokyo Institute of Technology's 60Co Gamma Radiation Facility, and the control vials were kept in a darkened box at room temperature as a control.

Preliminary product search

direct injection to GCMS Rtx-WAX column

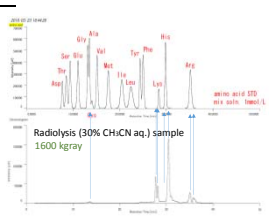


Similarity search

1 2.315/---/---/---/	Carbon dioxide
2 2.63 /---/---/---/	Acetaldehyde
3 3.005 /---/---/---/	Acetone
4 3.41 /---/---/---/	Methyl Alcohol
5 3.51 /---/---/---/	2-Butanone
6 3.605 /---/---/3.59/	4-Penten-2-ol
7 3.66/3.7/3.695	Ethanol
8 4.095/4.115/4.125	Acetic acid ethenyl ester
9 4.535 /---/---/ 4.54	Acetonitrile
10 4.555/4.595/4.61	Hydrogen cyanide
11 5.05 /---/---/---/	Water
12 5.585/5.615/5.615	3-Butenenitrile
1 6.485 /---/---/ 6.515	2-Butenenitrile
14 6.825/6.865/6.87	Cyclobutylamine
15 7.77/7.795/7.79	Acetaldoxime
16 8.19/8.22/8.17	1,3,5-Triazine2,4,6-trimethyl
17 8.385/8.44/8.44	Acetoin
18 10.56 /---/---/---/	Acetic acid
19 12.95/12.965/12.985	DL-Asparagine
20 13.04 /---/---/ 13.055	Acetamide N-methyl-
21 13.14 /---/---/ 13.165	Formamide N-methyl-
22 13.92/13.98/13.98	2,5-Dimethylbenzoxazole
23 14.475 /---/---/---/	Acetamide
24 14.775 /---/---/ 14.795	Formamide
25 16.645/16.655/16.675	DL-Alanine-acetyl-
26 19.04/19.085/19.09	1H-Imidazole1,2-dimethyl-
27 19.215 /---/---/ 19.245	succinonitrile
28 19.44 /---/---/ 19.475	6-Methyl uracil
29 19.685 /---/---/ 19.695	3,4, 5-Trimethylpyrazole
30 20.325/20.36/20.375	Propanamide 2-hydroxy-
31 20.735 /---/---/ 20.745	DL-Alanine N-acetyl-
32 21.325 /---/---/ 21.365	1-Pyrrolidinamine
33 21.43 /---/---/ 21.455	N-ethylidene-3-Amino-4 6-dimethylpyridone-2(1H)
34 22.22	carbamic acid, methyl ester
35 15.03	propaneamide

AA analysis using conventional HPLC fluorescence post column derivatization (OPA)

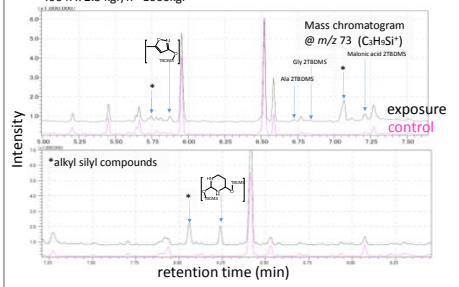
Detected Peaks Radiolysis Sample tR(min)	Amino acids standard mixture tR(min)
13.2 uncharacterized	7.7 L-Aspartic Acid
13.6 uncharacterized	8.6 L-Threonine
24.6 uncharacterized	9.4 L-Serine
25.3 uncharacterized	11.1 L-Glutamic Acid
25.8 uncharacterized	13.2 Glycine
27.7 uncharacterized	13.6 L-Alanine
28.1 uncharacterized	14.1 L-Cystine
30.4 NH4+	15.2 L-Valine
31.0 uncharacterized	17.6 L-Methionine
35.1 uncharacterized	20.5 L-Isoleucine
35.7 uncharacterized	22.4 L-Leucine
	24.3 L-Tyrosine
	25.0 L-Phenylalanine
	28.4 L-Lysine,
	29.8 L-Histidine,
	30.4 NH4+
	35.1 L-Arginine



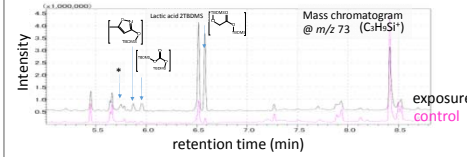
SPME extraction and on-fiber derivatization

AAA

15% CH_3CN + 1M NH_4HCO_3 aq.
400 h x 2.5 kgr/h= 1000kgr

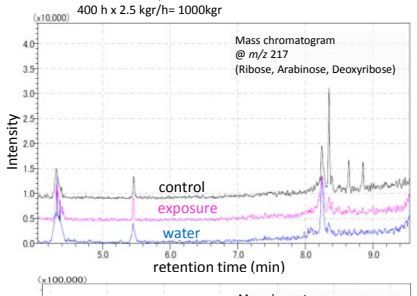


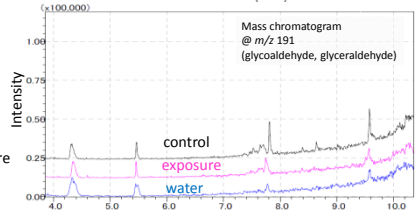
30% CH_3CN aq.
800 h x 2.5 kgr/h= 2000kgr



Sugars

33mM COH_2 aq.
400 h x 2.5 kgr/h= 1000kgr





Potential radiolysis products were detected for both 30% acetonitrile and 15% acetonitrile + 1M NH_4HCO_3. Potential TBDMS-AA, Ala and Gly, were detected only in 15% acetonitrile + 1M NH_4HCO_3 sample. Using specific fragment to derivative structures at *m/z* 73 ($C_3H_9Si^+$), we can search the potential target having similar structures to those of amino acids. Carbon WR seemed to eliminate smaller and semi-polar organics detected in direct GC injection using RtX WAX column.
For γ-ray exposure experiment on 33mM formaldehyde, no sugars were detected. The sugar syntheses in radiolysis has not been confirmed. Sugars potentially break under high energy system.
This procedure would work in the first screening of the stochastic reaction products. I will apply this technique to not only prebiotic synthetic samples but also biological samples.

Acknowledgements

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