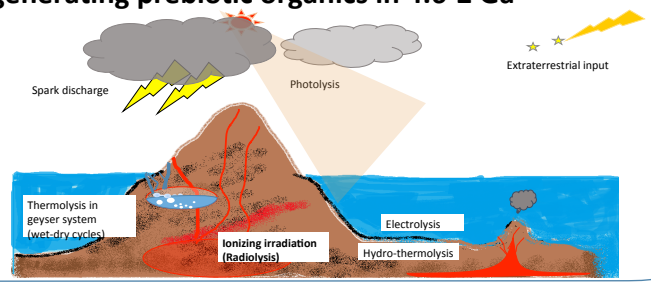
2140 presentation @ 17:30 on TUESDAY 15TH AUGUST
13N:Biogeochemical processes and radioactive waste disposal

Characterization of Analogues of Amino Acids and Sugars in Radiolysis Products using Solid Phase Micro Extraction (SPME)-GC-MS

Y. HONGO[1*], K.YAMADA[2], H.J. HÜBSCHMANN[3], R. Yi[1]
1 Earth-Life Science Institute, Tokyo-Tech., Meguro, Tokyo 152-8511, Japan (*correspondence: yayoi@elsi.jp),
2 AMR, Inc. , Tokyo, Japan, 3 CTC Analytics AG, Osaka, Japan

Radiolysis is potentially important energetic process generating prebiotic organics in 4.6-2 Ga

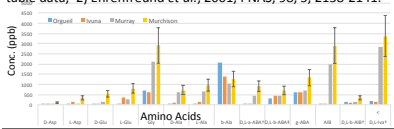


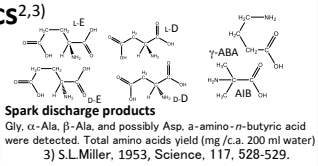
Amino acids, sugars, and their analogues are indispensable targets in the investigation of molecular evolution in the primitive earth leading the protometabolism[1]. Radiolysis is one of the effective mechanisms generating biomolecular building blocks from smaller molecules, however diverse byproduct formations disturb the pseudo-targeted analyses.

1) Aono et al., 2015, Orig. Life Evol. Biosph., 45, 327–338.

Diverse prebiotic organics[2,3]

table data; 2) Ehrenfreund *et al.*, 2001, PNAS, 98, 5, 2138-2141.





Spark discharge products
Gly, α-Ala, β-Ala, and possibly Asp, a-amino-*n*-butyric acid were detected. Total amino acids yield (mg /c.a. 200 ml water)
3) S.L.Miller, 1953, Science, 117, 528-529.

targeted analyses:
Sensitivity first /An authentic compound is available

pseudo-targeted analyses:
Qualification of the products first /
Target molecular structures are unpredictable

Suitable techniques
HPLC with Fluorescence detection*
LCMS with selective reaction monitoring
*chemical tag is required

70eV GC-EI-MS with NIST database search
Powerful for the first characterization
*sample extraction and derivatization

Analytical challenges

Molecular characterization for messy products in γ–radiolysis
Solid phase micro extraction (SPME Arrow™) and on-fiber derivatization.
Online sample prep. using a programmed CTC PAL RTC System & GC-MS.

Radiolysis

1) 15% CH_3CN + 1M NH_4HCO_3 aq.
2) 33mM COH_2 aq.

400 or 800 h
x 2.5 kgr γray (^{60}Co) /h
= 1000 or 2000kgr

Target species
Amino acids and analogues (AAA)
Sugars

Controls were kept in a darkened box at room temperature.

SPME extraction and on-fiber derivatization

SPME Arrow™ fibers

Conventional SPME — Sorption phase 0.6 uL
SPME Arrow™ — 15.3 uL

- Sensitivity
- throughput
- Robustness
- Reproducibility (>200times)

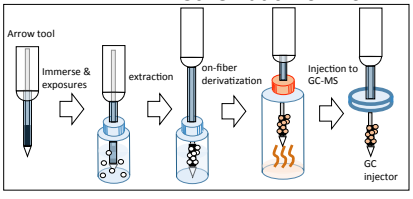
Fiber choice

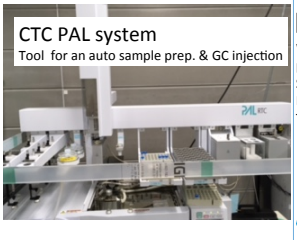
Polyacrylate(PA) — **Sugars**
Absorption of polar compounds
glycolaldehyde dimer (C_2)
glyceraldehyde dimer (C_3)

Carbon (SPME) WR — **AAA**
Adsorption of diverse molecules having MW<300

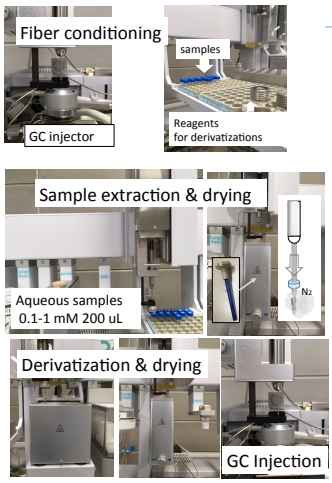
TBDMS
TMS
(TBDMS)

Schematic workflow





PAL tasks and typical parameter settings



	AAA	Sugars
Arrow fiber	Carbon WR/PDMS 120 μm	Polyacrylate 100 μm
Fiber conditioning T [°C]	280	270
Fiber conditioning Duration [min]	4	2
Extraction T [°C]	Room temperature	
Extraction Duration [min]	30	2
Drying T [°C]		
Drying Duration [sec]	30	30
Derivatization	MTBSFTA/CH3CN=1/1	DMF/HDMS&TMSC=1/1
Derivatization T [°C]	60	60
Derivatization Duration [min]	30	10
Drying T [°C]	Room temperature	
Drying Duration [min]	10	10
Sample Desorption	3	3

GC separation DB-5 0.25um I.D 30 m — split20 / splitless, temperature gradient
ionization — 70eV EI Source T:200°C Interface T:250°C

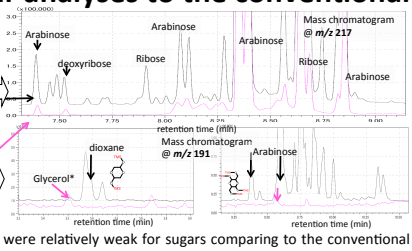
Comparison of sugar analyses to the conventional technique

Sugars Conventional off-line technique

200 uL sample solution containing
Glycoaldehyde 2100 μM
Glyceraldehyde 586μM
D-(-) Arabinose 410μM
D-(-) Ribose 350μM
D-deoxy D-Ribose 400μM
→ Dry up → Add 200uL Deriv. reagent → 1 uL inject

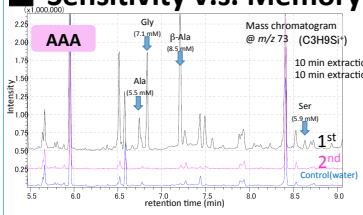
Automatic sample prep.

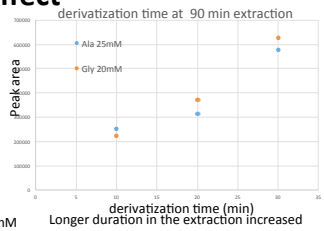
This method



Though GC sampling yields in the new method were relatively weak for sugars comparing to the conventional method, dynamic isomerization would be reduced in on-fiber extraction and derivatization.

Sensitivity v.s. Memory effect



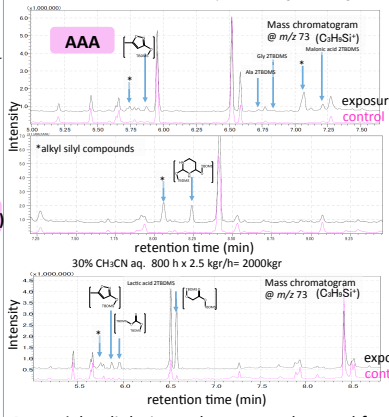


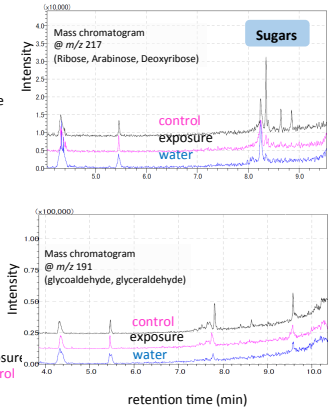
Signals were decreased in the 2nd analyses effectively for c.a.10 mM samples, indicating that 10min was enough to accumulate 2 umol NET AA in 200 uL aqueous solutions.

Longer duration in the extraction increased derivatization time. Imbalance between extraction and derivatization causes serious memory effect)

Analyses of radiolysis products

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





Potential radiolysis products were detected for both 30% acetonitrile and 15% acetonitrile + 1M NH4HCO3. TBDMS-AA, Ala and Gly, were detected only in 15% acetonitrile + 1M NH4HCO3 sample. Using specific fragment to derivative structures at *m/z* 73 ($C3H9Si^+$), we can search the potential target having similar structures to those of amino acids. 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 irradiation system. This analytical 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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