

Glucosamine and Galactosamine in Terrestrial Organic Matter and Their Correlation with Other Biochemical Indicators

Yoshinori Takano,* Lallan P. Gupta, Hodaka Kawahata, Katsumi Marumo, Yoji Ishikawa,[1] and Kensei Kobayashi[2,3]

National Institute of Advanced Industrial Science and Technology, AIST Central 7, Higashi 1-1-1, Tsukuba, Ibaraki 305-8567

[1]Bio-Environmental Engineering Department, Technical Research Institute, Obayashi Corporation, 4-640 Shimokiyoto, Kiyose-shi, Tokyo 204-8558

[2]Department of Chemistry and Biotechnology, Yokohama National University, 79-5 Hodogaya-ku, Yokohama 240-8501

[3]Institute of Space and Astronautical Science, 3-1-1 Yoshinodai, Sagamihara, Kanagawa 229-8510

Received August 6, 2003; E-mail: takano.yoshinori@aist.go.jp

Core samples of sediments at depths of 0–300 cm at Rikubetsu, Hokkaido, Japan were analyzed for hexosamine (HA) of glucosamine (Gluam) and galactosamine (Galam). The Gluam and Galam contents were highest at the surface, and drastically decreased with increasing depth: the Gluam and Galam contents were 7.98 and 3.19 μmol/g at a depth of 0–5 cm, respectively. Highly positive correlations for Gluam and Galam versus the microbial cell density, total organic carbon, and total hydrolyzed amino acids were observed. The present results suggest that an exponential degradation for amino compounds on the early stage of diagenesis had occurred, and then the amino compounds decreased asymptotically with time. The degradation rate of amino sugars was slower than that of amino acids in the terrestrial diagenetic pathway. The present study shows the vertical distribution of amino sugars with the trends of other biochemical indicators.

Hexosamines, such as glucosamine (2-amino-2-deoxyglucose) and galactosamine (2-amino-2-deoxygalactose), are major amino sugars in sedimentary organic matter. The linear forms of glucosamine (Gluam) and galactosamine (Galam) consist of a formyl group, a hydroxy group, and an amino group with six carbons. Gluam and Galam (also known as amino sugars) occur as structural components of a broad group of substances, the mucopolysaccharides, and have been found in combination with mucopeptides and mucoproteins.[1] Generally, the amino sugars in surface sediment are of microbial origin and account for 5 to 10% of the N-compounds in the surface layer.[1] Amino sugars have a wide variety of forms, ranging from minor contributions by free monomers to a dominance of polymeric forms, such as chitin,[2] chitin peptide materials,[3] and the bacterial cell wall.[4] The terrestrial organic matter, particularly humins, which have been reported to be rich in amino sugars, could represent a primary source of amino sugars along with chitinaceous remnants.[5] At the early stage of diagenesis in organic matter, the degradation of polypeptides, and oligopeptides to amino acid monomers, takes place.[6] In a previous report, we clarified the vertical profile and correlation coefficient of the microbial population and organic matter in terms of the total organic carbon, amino acids,[7] and phosphatase activity.[8] Concentration of amino sugars might also be correlated with the subterranean microbial activity and organic matter.

Here, we report on the vertical distribution of amino sugar at the early stage of diagenesis, and compare it with the trends of other biochemical indicators. Core samples of sediments at depths of 0–300 cm at Rikubetsu, Hokkaido, Japan were analyzed for Gluam and Galam. Possible epimerization reaction leading to a change of one predominant hexosamine to the other in the diagenetic pathway was also considered.

Experimental

Sampling. Core samples were obtained at Rikubetsu by the Obayashi Corporation in February, 1996. Rikubetsu is a boreal area in Hokkaido, Japan (Fig. 1), located near the center of the island, and is one of the coldest cities in Japan.[7] The altitude of the boring site is 207 m from the mean sea level, and the average annual temperature is 5.8 °C at 43°28′0″ N, 143°44′5″ E. The site is situated in a slightly marshy area, which is seasonally frozen down to a depth of 80 cm, and covered with ice during the winter. The boring was performed to a maximum depth of 300 cm. Radiocarbon dating confirmed the sediment age as being 4420 ± 40 yrBP and 9290 ± 50 yrBP at depths of 62 cm and 162 cm, respectively.[7] Hence, the accumulation rate was estimated as being 0.17 mm/yr for the core sample.

Analytical Methods. Approximately 1.0 g of dried sample was hydrolyzed with 3 mL 6 M HCl in a test tube for 22 h at 110 °C. The hydrolyzate containing Gluam and Galam was cooled to room temperature and then filtered through a membrane filter (0.45 μm pore size) using a disposable syringe. The ampoule was washed twice with Milli-Q water, which was also filtered through the same filter. The filtrate was then evaporated to dryness under vacuum at 40 °C. About 2 mL Milli-Q water was added to the dried residue, dissolved, and evaporated again to dryness to ensure complete removal of HCl from the sample. The residue was then dissolved in 1 mL of diluant buffer (pH 2.2). Ten microliters of sample were injected with an auto injector into a Shimadzu amino acid analyzer.

Published on the web April 2, 2004; DOI 10.1246/bcsj.77.729

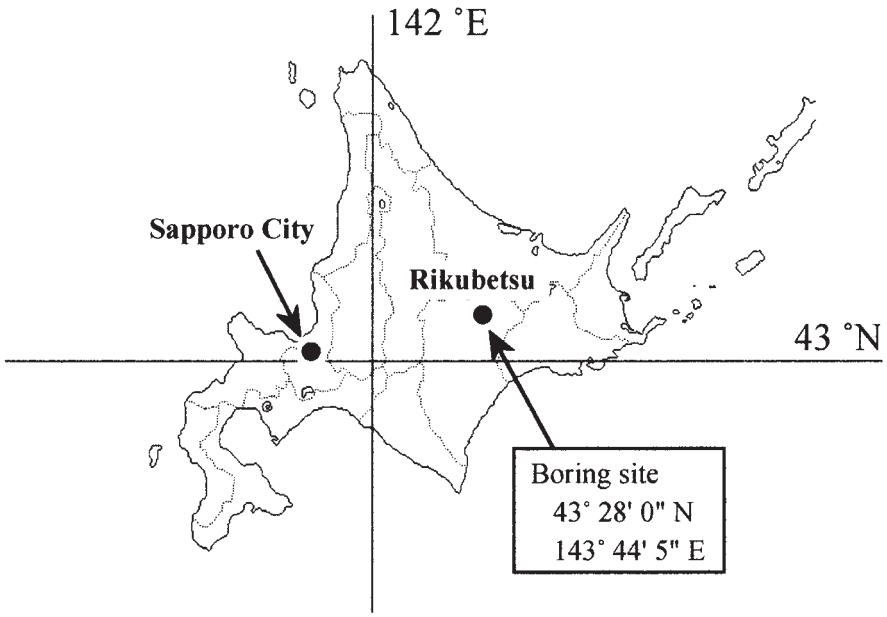

Fig. 1. The geological location of Rikubetsu, Hokkaido, Japan. The boring site was 143°44′5″ E, 43°28′0″ N.

A fraction of unhydrolyzate was also prepared as follows: Approximately 1.00 g of dried sample was extracted by shaking with 3 mL of ion-exchanged water for an hour at room temperature. The extraction was repeated four times and the extracted solutions were combined together. After evaporation to dryness, the fraction was then dissolved in 1 mL diluant buffer (pH 2.2).

The concentrations of Gluam and Galam were determined by an ion-exchange HPLC system, which was composed of three high-performance liquid chromatograph pumps (Shimadzu LC-9A), and a strongly acidic cation exchange column (Shim-pack Amino-Na column, I.D. 6.0 mm, 10 cm long).[9] The column was maintained at 60 °C during analysis and preceded by a precolumn for reducing the effect of ammonia and other unwanted chemicals. Post-column derivatization of Gluam and Galam were achieved with two reaction reagents, the phthalaldehyde (OPA) reagent and carbonic acid–boric acid buffer solution (pH 10.0) supplied by a peristaltic pump, Shimadzu PRR-2A, in a chemical reaction box at 60 °C. The resulting fluorescent complexes were detected and estimated by a Shimadzu RF 550 fluorescence detector (Excitation wavelength: 355 nm and Emission wavelength: 397 nm). Ten microliters of standard, containing 1 nmol of each Gluam and Galam were also analyzed after every 5th sample. The resulting peak areas were computed with a Shimadzu C-R4A Chromatopac integrator. The detection limit in this method was ca. 1 nmol/g for the hexosamines. The data reproducibility in the present study was better than ±5% for relative molar concentrations of the hexosamines.

Result and Discussion

Vertical Distribution of Amino Sugars. As shown in Table 1, the concentrations of Gluam and Galam varied from 0.06 to 7.98 μmol/g and from 0.04 to 3.19 μmol/g, respectively. Their concentrations decreased drastically with increasing

Table 1. The Vertical Profiles of Hexosamines (HA) and Other Biochemical Indicators of the Core Samples in Rikubetsu, Hokkaido, Japan

Depth/cm	Hexosamine (HA)			Other biochemical indicators			THAA/HA
	Gluam	Galam	Gluam/Galam	TOC	THAA	Cell density	
	μmol/g	μmol/g		wt %/g	μmol/g	10^8 cell/g	
0–5	7.98	3.19	2.50	2.55	61.84	8.3	5.54
5–10	2.05	0.48	4.30	1.26	28.65	4.1	11.37
20–30	1.91	0.35	5.45	0.66	11.82	0.5	5.24
30–40	0.72	0.37	1.97	0.27	2.85	0.2	2.61
50–75	0.21	0.15	1.40	0.23	0.84	0.3	2.30
75–100	0.74	0.15	5.05	0.22	1.86	0.2	2.10
100–125	0.06	0.05	1.28	0.15	1.21	0.3	10.59
125–150	0.30	0.05	5.93	0.17	1.30	1.7	3.65
150–175	0.12	0.04	2.84	0.14	0.54	0.1	3.34
175–200	0.16	0.04	4.08	0.09	0.66	0.2	3.22
200–250	0.60	0.07	8.12	0.15	0.71	0.3	1.06
250–300	0.63	0.08	8.12	0.09	0.65	0.2	0.92

Abbreviations; Gluam: Glucosamine, Galam: Galactosamine, TOC: Total organic carbon, THAA: Total hydrolyzed amino acids. The data of TOC, THAA, and microbial cell density are referred from Ref. 7.

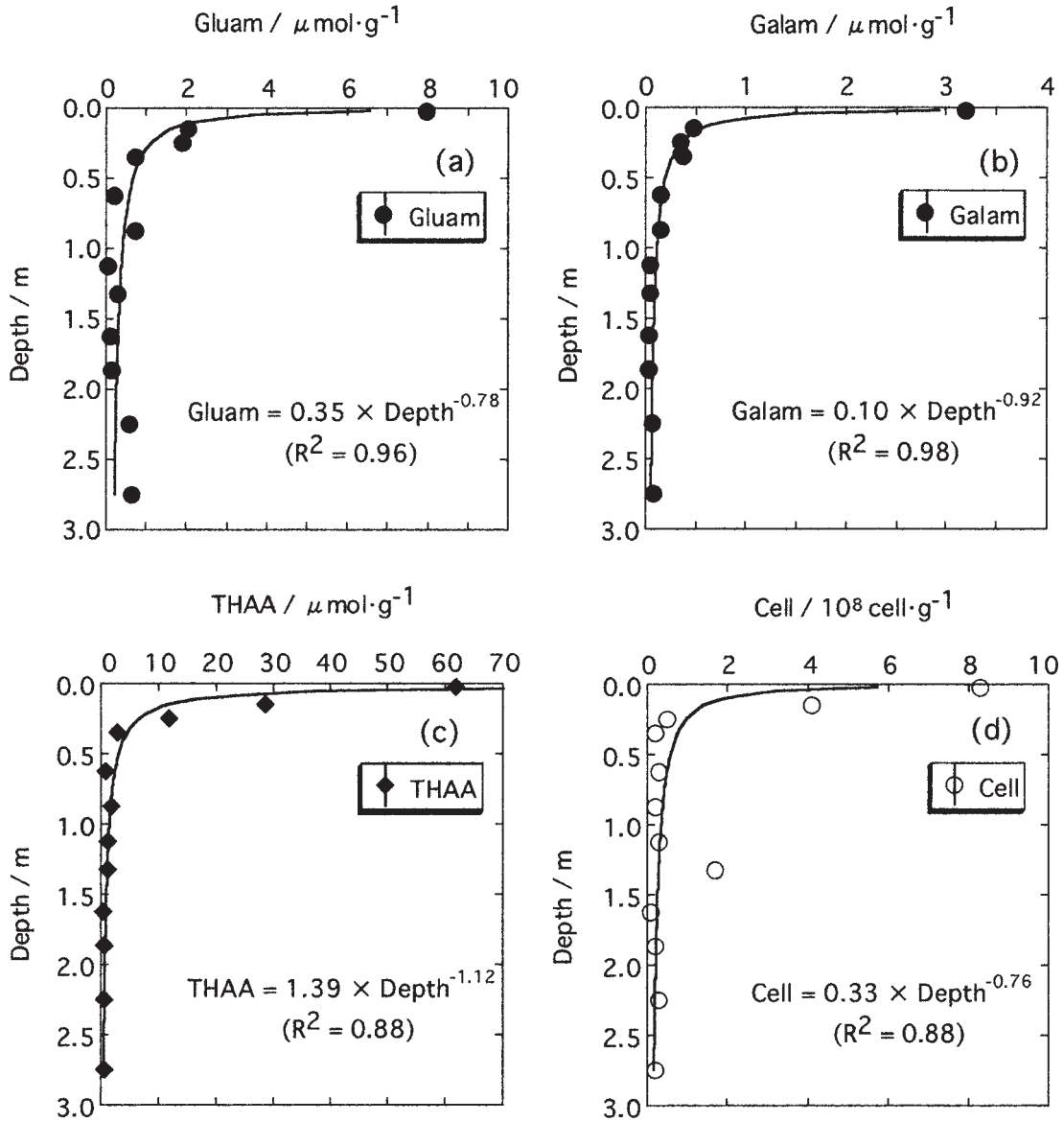

Fig. 2. Vertical distribution of (a) glucosamine (Gluam), (b) galactosamine (Galam), (c) total hydrolyzed amino acids (THAA), and (d) microbial cell density of the sediment core samples. The data of THAA and microbial cell density are referred from Ref. 7.

depth by two orders of magnitude, as shown in Figs. 2-(a), -(b). These figures show an exponential distribution for Gluam and Galam: The logarithmic slopes shown are −0.78 and −0.92 with the depth.

The exponential decrease in Gluam and Galam contents is in sharp contrast to what has been reported for core sediments obtained from the estuarine region of a subtropical river, which showed lower contents in the upper layer and higher contents in the deeper layers.[10] It is evident from the Figs. 2-(a) and (b) that two steps of the degradation stage are plausible: at first, the degradation pathway for amino compounds under an oxidative surface environment proceeds at an exponential rate; next, degradation pathway for amino compounds proceeds asymptotically with time after burial below the surface layer.

From a stereo chemical perspective, Gluam and Galam are epimeric amino sugars to each other. The concentration profiles showed that Gluam is more predominant than Galam, on the average. Thus, an apparent epimerization from predominant Gluam to Galam in the diagenetic pathway did not take place. As shown in Fig. 2-(c), the distribution of amino acids also yielded a logarithmic slope of −1.12 with the depth. The higher slope value of amino acids suggests that the degradation rate of amino acids with the depth might be faster than that of amino sugars.

Both Gluam and Galam did not occur in free, water-soluble form; strong acid hydrolysis was required for their release from organic matter. Only a trace amount of Gluam and Galam were detected in some sequences of the sediment. This result suggests that combined amino sugars and not free amino sugars were major components in organic matter. Consequently, combined form analogs in the sedimentary matrix might be more stable than free-form analogs.

Correlation among Amino Sugars and Other Biochemical Indicators. The vertical profile of Gluam is positively correlated with that of Galam, as can be seen in Fig. 3. Since the microbiological activity is maximum at the sediment water in-

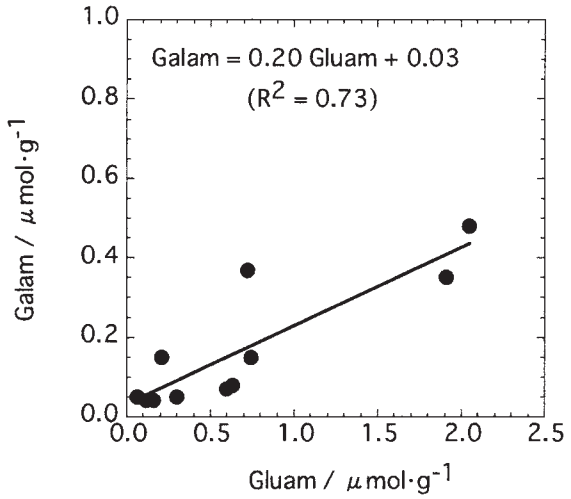

Fig. 3. Concentration of Galam and Gluam in terrestrial digenetic pathway.

Table 2. The Correlation of Gluam, Galam, Total Hydrolyzed Amino Acids (THAA), Total Organic Carbon (TOC), and Microbial Cell Density

	Gluam	THAA	TOC	Cell density
Gluam	—	0.970	0.969	0.930
Galam	0.984	0.944	0.942	0.918

The fundamental data of THAA, TOC, and microbial cell density were referred from Ref. 7.

terface, and decreases exponentially with the depth,[7] the decreasing trends for Gluam and Galam versus the microbial cell density were similar to each other, as can be seen in Fig. 2-(d). Different microorganisms produce different amino sugars.[11] Gluam is common in fungal and micro arthropod chitin.[12,13] Although the origin of Galam is less clear, Sowden and Ivarson (1974)[14] showed that little, if any, Galam was found in fungi-inoculated incubation experiments, whereas it was synthesized by bacteria. Thus, the microbial cell density in the subterranean layer also a showed positive correlation with the Gluam and Galam profiles as 0.92 and 0.93, respectively. The total hydrolyzed amino acids (THAA)[7] to the total hexosamines (HA) ratio varied from 0.9 to 11.3 (mean 4.3), and the ratio of Gluam/Galam varied from 1.3 to 8.1 (mean 4.2). These ratios are lower than those (THAA/HA, 6.7–25.9 and Gluam/Galam, 1.3–10.3) reported for core sediments obtained from a river flood plain.[10] Consequently, the ratios of THAA/HA and Gluam/Galam varied in the difference of sedimentary environments, while the correlation efficient for the organic matter concentrations are similar to each other.

As shown in Table 2, the correlation efficient for Gluam and Galam versus THAA is 0.97 and 0.94, respectively. The vertical distribution of amino sugars, total organic carbon, amino acids and microbial activity in the terrestrial core samples are all positively correlated.

Conclusions

The present study shows the following characteristics with regard to hexosamine in diagenetic pathway:

1) The Gluam and Galam contents were highest at the surface, and drastically decreased with increasing depth: The Gluam and Galam contents were 7.98 and 3.19 μmol/g at a depth of 0–5 cm, respectively. The concentration of Gluam was higher than that of Galam on the average.

2) Highly positive correlations for Gluam and Galam versus total organic carbon and total hydrolyzed amino acids were observed. The results from this study are in agreement with predictions in previous works,[15] which suggest an exponential decay trend for amino compounds in sediments. This means that after the early stage of diagenesis, amino compounds decrease asymptotically with time. The degradation rate of amino sugars was slower than that of amino acids. Hence, amino sugars were more stable than amino acids in the terrestrial diagenetic pathway.

The authors would like to thank Dr. Yukishige Kawasaki, Mitsubishi Kagaku Institute of Life Science for his sincere cooperation. This research was partly funded by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) through the Special Co-ordination Fund for the Archaean Park Project; an international research project on interaction between the sub-vent biosphere and the geo-environment.

References

1 F. J. Stevenson, "Humus Chemistry. Genesis, Composition, Reactions," John Wiley and Sons (1982).

2 M. B. Flannery, A. W. Stott, D. E. G. Briggs, and R. P. Evershed, *Org. Geochem.*, **32**, 745 (2001).

3 B. A. Stankiewicz, M. Mastalerz, C. H. J. Hof, A. Bierstedt, M. B. Flannery, D. E. G. Briggs, and R. P. Evershed, *Org. Geochem.*, **28**, 67 (1998).

4 S. R. Hagan, *J. Chromatogr.*, **632**, 63 (1993).

5 M. Schnitzer, "Short Course in Environmental Geochemistry," ed by M. E. Fleet, Mineralogical Association of Canada, London (1984), p. 237.

6 R. Ogasawara, R. Ishiwatari, and A. Shimoyama, *Geochem. J.*, **35**, 439 (2001).

7 Y. Takano, J. Kudo, T. Kaneko, K. Kobayashi, Y. Kawasaki, and Y. Ishikawa, *Geochem. J.*, in press.

8 H. Mori, Y. Kawasaki, Y. Ishikawa, and K. Kobayashi, *Viva Origino*, **28**, 40 (2000).

9 L. P. Gupta and H. Kawahata, *Deep-Sea Res., Part I*, **47**, 1937 (2000).

10 L. P. Gupta, *J. Asian Earth Sci.*, **19**, 727 (2001).

11 X. Zhang, J. Wang, H. Xie, J. Wang, and W. Zech, *Appl. Soil Ecol.*, **23**, 147 (2003).

12 L. K. Kenne and B. Lindburg, "Bacterial Polysaccharides," In "The Polysaccharides," ed by G. O. Aspinall, Academic Press, New York (1983), Vol. 2, p. 287.

13 J. W. Parsons, "Chemistry and Distribution of Amino Sugars in Soils and Soil Organisms," In "Soil Biochemistry," ed by E. A. Paul and J. N. Ladd, Marcel Dekker, New York (1981), Vol. 5, p. 197.

14 F. J. Sowden and K. C. Ivarson, *Can. J. Soil Sci.*, **54**, 387 (1974).

15 G. Belluomini, M. Branca, G. Calderoni, and M. Schnitzer, *Org. Geochem.*, **9**, 127 (1986).