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Geochemical Kinetics of Glucose and Amino Acids Consumption in Laboratory and Lake Inawashiro Sediments

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Consumption rates of *glucose*, *Ala* (*alanine*) and *Gly* (*glycine*) in Maillard reaction were examined and compared with those in sediments. By means of monitoring the concentration change of *glucose*+*Ala* (or *glucose*+*Gly*) in aqueous solution, the reaction order of this condition was determined to be second-order. For the purpose of drawing Arrhenius plots, the measurements of change in the concentration of reactants were conducted at four different temperatures (65, 80, 89 and 94°C) and the apparent activation energies and Arrhenius equations of *glucose*+*Ala* and *glucose*+*Gly* consumption were obtained. Influences on the reaction rates of these systems by $CaCO_3$ and *mont* (*montmorillonite*) were also examined under the same reaction conditions.

The apparent activation energy of *glucose*+*Ala* (E=27.7 kcal/mol) was 8.3% lower than that of *glucose*+*Gly* (E=30.2 kcal/mol). This indicates that the reaction of *glucose*+*Ala* is more likely to occur at ordinary temperature in the surface sediments than that of *glucose*+*Gly*. The apparent activation energies in the presence of *mont* were 7.9~17.2% lower than those in the absence of *mont*, and the frequency factors were also lower in the presence of *mont*. It appears that *mont* apparently plays a role as a weak positive catalyser in this reaction. In order to compare the consumption rates in laboratory with those in sediments, the core sample (10~50 cm) of sediment was collected in Lake Inawashiro, and the concentrations of *glucose*, *Ala* and *Gly* and TOC (Total Organic Carbon) were determined. Assuming possible reaction temperatures and time, the rate constants of decreasing *glucose*, *Ala* and *Gly* concentrations with increasing core depth were estimated to be 0.423, 5.20 and 1.20 ($l\,mol^{-1}\,yr.^{-1}$), respectively. While, each rate constant in laboratory experiments calculated based on Arrhenius equation were 1.18×10^{-2}, 1.18×10^{-2} and 1.21×10^{-2} ($l\,mol^{-1}\,yr.^{-1}$). Reaction rates in sediments were about 10^2 times as fast as those in laboratory experiments. This observation suggests that biodegradation rates of *glucose*, *Ala* and *Gly* by the activity of microorganisms in the surface sediments (~50 cm) is about 10^2 times as fast as condensation rates of them.

Introduction

Since Maillard (1912), it has been suggested that a part of natural humic substances or kerogen precursors in sediments may be produced by condensation reactions between reducing sugars and amino acids. This reaction is regarded as one of the possible pathways to form kerogen precursors. Many geochemists have studied about chemical

features of melanoidins synthesized from free amino acids and reducing sugars, and pointed out the similarities to humic substances or kerogen (Abelson and Hare, 1971; Hoering, 1973; Tissot and Welte, 1984; Rubinsztain et al., 1984).

A few studies have been conducted to evaluate kinetics of Maillard reaction under the geochemical condition (Yamamoto and Ishiwatari, 1989; Rubinsztain et al., 1984). Yamamoto and Ishiwatari (1989) have reported that Maillard reaction of protein (*casein*) with reducing sugar (*glucose*) is more likely to prevail in the natural environment than the reaction of free amino acids with reducing sugar, and the kinetic rate constant of polycondensation was calculated. Since the polycondensation is consecutive reaction, the reaction rate of that is affected by the first reaction which is reducing sugar with amino acid (the form of protein, peptide and free amino acid) reaction, and a part of amino acids is present as free forms in recent sediments (Tissot and Welte, 1984; Kamp and Mudrochova, 1973). Therefore it is also necessary to investigate about the geochemical kinetics of reducing sugar with free amino acid reaction. In the present study, free amino acids are appropriate for studying the kinetics of reducing sugar and amino acid consumption in laboratory, because their amino groups exist not combined and their quantitative treatment in kinetics is relatively simple. Using *Ala* and *Gly* as free amino acids, reaction order, apparent activation energy and Arrhenius equation of glucose-amino acid condensation were examined. Furthermore $CaCO_3$ and *montmorillonite* were added to these systems and their influences on the kinetics of glucose-amino acid reaction were investigated similarly. In the surface sediments, both biodegradation of carbohydrates and amino acids and condensation of them take place at the same time (Koyama et al., 1973a, 1979; Tissot and Welte, 1984). But their reaction rates have not been made clear yet. Therefore consumption rates of *glucose*, *Ala* and *Gly* under laboratory condition were determined and compared with those in Lake Inawashiro sediments.

Experimental

Laboratory experiments

Glucose (*D-glucose*), *Ala* (*L-alanine*) and *Gly* (*L-glycine*) were chosen as the experimental materials, because those are abundant chemical components in algae which are common organisms in marine and lacustrine environment (Hedges 1978), and are readily soluble in water. *Mont* (*montmorillonite*) was prepared by hydraulic elutriation of bentonite (70% *mont*) as a standard reagent. Calcium carbonate ($CaCO_3$) was a powdered reagent of high grade. The aqueous solutions were not buffered to specific pH condition to know the basic mechanisms controlling *glucose* and amino acids consumption and melanoidin formation. Both *glucose* (1.5 mol) and *Ala* (1.5 mol) (or *Gly* (1.5 mol)) were dissolved in distilled water (1000 ml). The reaction solution containing *glucose*+*Ala* or *glucose*+*Gly* was placed in a sealed polyester bottle and stirred gently enery 2 hr. The bottle was heated in the water bath at constant

temperature (65, 80, 89 or 94°C±0.5°C) for 73 min. to 9640 min. under ca. 1 atm pressure.

In order to monitor the concentration change of reactants with increasing heating

Table 1. Concentration changes of *glucose* and *Ala*, and ones under the $CaCO_3$ or *mont* (*montmorillonite*) additional condition with heating time at four different temperatures

Temp	*glucose*+*Ala* solution			*glucose*+*Ala*+*mont* solution			*glucose*+*Ala*+$CaCO_3$ solution		
	Heating time(min.)	*glucose* (mol/l)	*Ala* (mol/l)	Heating time(min.)	*glucose* (mol/l)	*Ala* (mol/l)	Heating time(min.)	*glucose* (mol/l)	*Ala* (mol/l)
	73	1.41	1.42	93	1.32	1.31	80	1.20	1.20
	196	1.27	1.31	218	1.13	1.25	210	0.904	0.832
	359	1.12	1.15	381	0.943	1.03	371	0.685	0.680
94°C	757	1.01	0.718	778	0.727	0.672	766	0.406	0.450
	1458	0.629	0.641	1483	0.414	0.442	1472	0.225	0.234
	2025	0.493	0.524	2040	0.359	0.339	2033	0.217	0.207
				2955	0.270	0.262	2945	0.146	0.140
	179	1.37	1.37	108	1.37	1.42	90	1.30	1.28
	293	1.29	1.29	240	1.31	1.22	220	1.07	0.998
	435	1.20	1.15	333	1.02	1.10	315	0.971	0.934
	735	1.05	1.05	435	0.868	1.04	445	0.812	0.841
	1445	0.826	0.942	755	0.870	1.04	738	0.635	0.618
89°C	1765	0.738	0.746	1470	0.620	0.549	1450	0.375	0.446
	2133	0.675	0.680	1785	0.550	0.521	1770	0.350	0.311
	3140	0.581	0.595	2160	0.498	0.505	2140	0.317	0.292
	4975	0.472	0.500						
	7172	0.324	0.301						
	9630	0.196	0.196						
	257	1.44	1.44	98	1.46	1.46	268	1.29	1.27
	959	1.26	1.26	270	1.37	1.30	970	0.900	1.08
	1450	1.16	1.19	983	1.10	1.14	1563	0.711	0.710
80°C	2633	0.928	0.950	1570	0.940	0.935	2642	0.538	0.459
	4382	0.799	0.805	2647	0.746	0.740	4385	0.380	0.338
	5862	0.673	0.675	4393	0.615	0.515	5877	0.297	0.317
				5883	0.474	0.500	8176	0.225	0.222
				8190	0.346	0.352			
	2000	1.47	1.45	2040	1.31	1.35	2020	1.23	1.22
	3000	1.36	1.32	3040	1.23	1.20	3020	1.10	1.12
65°C	4010	1.33	1.30	4045	1.17	1.16	4025	1.03	1.04
	7005	1.21	1.23	7045	0.977	1.01	7025	0.840	0.834
	8100	1.16	1.17	8140	0.932	0.939	8120	0.783	0.763
	9600	1.12	1.13	9640	0.870	0.861	9620	0.707	0.708

time, 4 ml of reaction solution were transferred to a small glass bottle by a pipette at the time shown in Table 1 and stored in the refrigerator (−10°C). These 4 ml reaction solutions were diluted in 1000~10000 times with distilled water for a colorimetric method, and each reactant color was developed by applying phenol-sulfuric acid reaction for reducing sugars and ninhydrine reaction for amino acids, respectively, and the concentration was determined using UV spectrometric method (using HITACHI-UV323 at 485 nm and 570 nm). The data were obtained on the average of five measurements under the same conditions. These methods have been used after proving the absence of recognizable interference by functional groups presented in the soluble melanoidins. Preliminary experiments showed that the concentration of only *glucose* in aqueous solution (1.5 mol/l) did not change after heating for 5000 min. (80°C). The same results were obtained as the concentrations of *Ala* and *Gly*. These facts support that the consumption by same material reaction (e.g., *glucose*+*glucose*, *Ala*+*Ala*, *Gly*+*Gly*) can be negligible under the present experimental conditions.

Each aqueous solution system of *glucose*+*Ala*+*mont*, *glucose*+*Gly*+*mont*, *glucose*+*Ala*+$CaCO_3$ and *glucose*+*Gly*+$CaCO_3$ was similarly heated in the water bath under the constant temperatures as Table 1. Preliminary experiments showed that the consumption rates of *glucose* and amino acids with increasing heating time were unstable and dependent on the amount of *mont* or $CaCO_3$. However, it was observed that the rate was stable with more than 80 g/l of *mont* and 55 g/l of $CaCO_3$. Thus, 85 g/l of *mont* and 60 g/l of $CaCO_3$ were chosen for further experiments.

For the purpose of examining about the affection of absorption by *mont*, another preliminary experiments were performed: *glucose*+*Ala* (1.5 mol/l)+*mont* (85 g/l) and *glucose*+*Ala* (1.5 mol) (or *glucose*+*Gly*+*mont* and *glucose*+*Gly*) in polyester bottle were stirred and left at 25°C. After 2 hours the concentration of *glucose* and *Ala* (or *glucose* and *Gly*) were measured using the same method described above. The result indicated that the concentration of *glucose*, *Ala* and *Gly* were not decreased. Since the concentrations of *glucose*, *Ala* and *Gly* in the presence of mont were equal to those in the absence of *mont* at 25°C, the adsorption of them and interference in colorimetric method by *mont* were able to be negligible under present experimental conditions.

Lake Inawashiro samples

The core sample of sediment was collected near the center of Lake Inawashiro in Fukushima prefecture, Japan (Fig. 1). Records of sediment sampling by gravity core sampler at St1 were as follows: longitude 140°5.7′E; latitude 37°27.8′N; core length 10~50 cm (0~10 cm was disorganized and was not used for analysis); water depth 89 m. Temperature and pH were measured immediately after sampling through the sampler pipe windows. The sample was stored in refrigerator (−10°C) until analysis. The core sample of sediment was cut horizontally into 5 cm sections. The amount of TOC (Total Organic Carbon wt%) was measured (using YANACO CHN corder MT-2). By X-ray diffraction examination, *mont* was detected significantly. The sediment samples

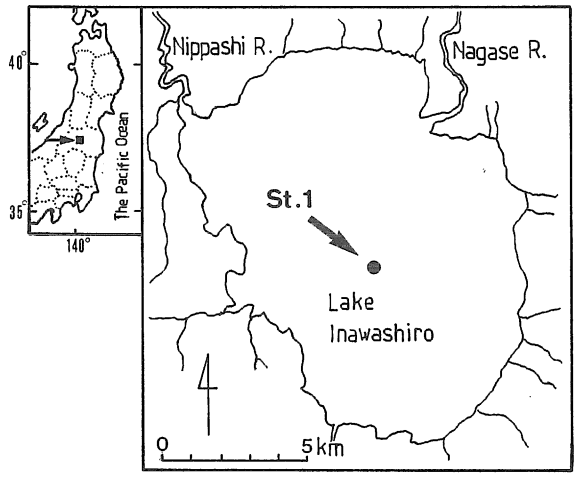

Fig. 1. Sampling location at Lake Inawashiro in northeast Japan.
St. 1●: Sampling site

were hydrolyzed under reflux condition of 6N-HCl. *Glucose*, *Ala* and *Gly* were separated by paper chromatography and analyzed by colorimetric method described above.

Results and Discussion

Reaction order

The determination of reaction order was examined to obtain rate constants at each temperature. The concentration of *glucose*+*Ala* shown in Fig. 2 decreased rapidly in the early stage of reaction at 89°C. The tendency of *glucose* concentration change in Fig. 2 was almost the same with that of *Ala*. It is obvious that the contribution of *glucose* and *Ala* to this reaction was in the ratio of 1:1. In order to determine the reaction order, the method of integration was chosen. A reaction order is mainly regarded as an integer, which is usually less than three. The correlation coefficients were calculated between the first-, second- and third-order reaction plots of *glucose*+*Ala* concentrations and heating times at 89°C. Each correlation coefficient (R) were 0.979 (first-order), 0.989 (second-order) and 0.920 (third-order), respectively. Therefore the second-order plots were the most fitful to the straight line. It is evident that the reaction of *glucose*+*Ala* in aqueous solution was second-order reaction. The same result was obtained in case of *glucose*+*Gly* (R=0.982, 0.985 and 0.918, respectively), and this also indicated that *glucose*+*Gly* in aqueous solution was second-order reaction. Thus the reaction rate in these systems can be written as follows:

$$r=k[A]\,[B]\,, \tag{1}$$

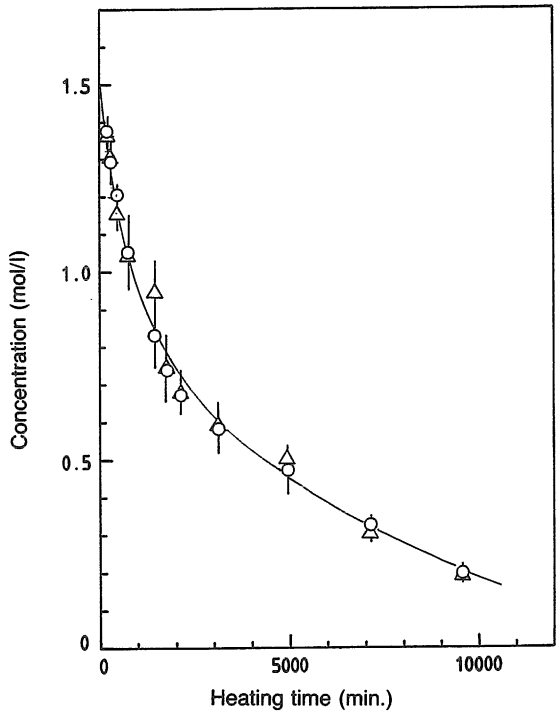

Fig. 2. Concentration changes of *glucose* and *Ala* in *glucose*+*Ala* aqueous solution with increasing heating time.
○: *glucose* △: *Ala*

where r is the reaction rate, k is the rate constant, $[A]$ and $[B]$ are the concentrations (mol/l) of A and B, A is *glucose* and B is *Ala* or *Gly*. These results can be applied only to the first reaction in successive melanoidin formation. This is evident from following reasons. According to Yagi (1974), Maillard reaction can be written in a concept as follows:

$$A+B\rightarrow C\,, \tag{2}$$

$$C\rightarrow X_1\rightarrow X_2\rightarrow\cdots\cdots X_n\,, \tag{3}$$

where A is reducing sugar, B is amino acid, C is the initial product (amino-carbonyl reaction product), X_1, X_2 and X_n are melanoidins (consecutive polycondensation products), and (3) is a very complex reactions. This suggests that Maillard reaction is consecutive reaction. In the present study, the obtained reaction order is considered to be due to the first step reaction (2). In regard to the reaction order of (3), Yamamoto and Ishiwatari (1989) have reported that the formation of alkali-soluble melanoidin by the reaction of protein (*casein*) with reducing sugar (*glucose*) follows the apparent firts-order kinetic equation at least in the first few days (at 50~80°C). Thus the

reaction order of reducing sugar with free amino acid (2) can be distinguished from that of polycondensations (3).

Rate constant and apparent activation energy

In order to obtain Arrhenius equations, the measurements of the concentration change of reactants were conducted at four different temperatures (65, 80, 89 or 94°C). Fig. 3 shows the second-order plots of *Ala* (*glucose*+*Ala*) at each temperature. The correlation coefficients between second-order plots at each temperature and heating times were from 0.985 to 0.999. Each rate constant of *glucose*+*Ala*, *glucose*+*Gly*, *glucose*+*Ala*+*mont*, *glucose*+*Gly*+*mont*, *glucose*+*Ala*+$CaCO_3$ and *glucose*+*Gly*+

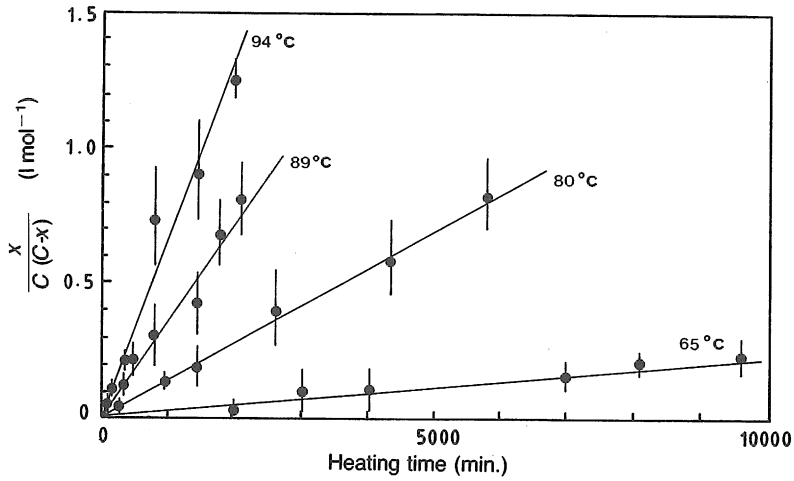

Fig. 3. Second-order reaction prots of *Ala* in *glucose*+*Ala* aqeous solution at four different temperatures.

Table 2. Rate constant (k) of each association solution at four different temperatures

Rate constant (k) (l mol^{-1} min.$^{-1}$)			
Temp	*glucose*+*Ala*	*glucose*+*Ala*+*mot*	*glucose*+*Ala*+$CaCO_3$
94°C	6.45×10^{-4}	1.08×10^{-3}	2.19×10^{-3}
89°C	3.98×10^{-4}	6.63×10^{-4}	1.29×10^{-3}
80°C	1.38×10^{-4}	2.59×10^{-4}	4.70×10^{-4}
65°C	2.37×10^{-5}	5.02×10^{-5}	7.81×10^{-5}
Temp	*glucose*+*Gly*	*glucose*+*Gly*+*mot*	*glucose*+*Gly*+$CaCO_3$
94°C	6.18×10^{-4}	1.16×10^{-3}	1.98×10^{-3}
89°C	3.58×10^{-4}	7.15×10^{-4}	1.13×10^{-3}
80°C	1.15×10^{-4}	2.87×10^{-4}	3.95×10^{-4}
65°C	1.63×10^{-5}	5.61×10^{-5}	6.10×10^{-5}

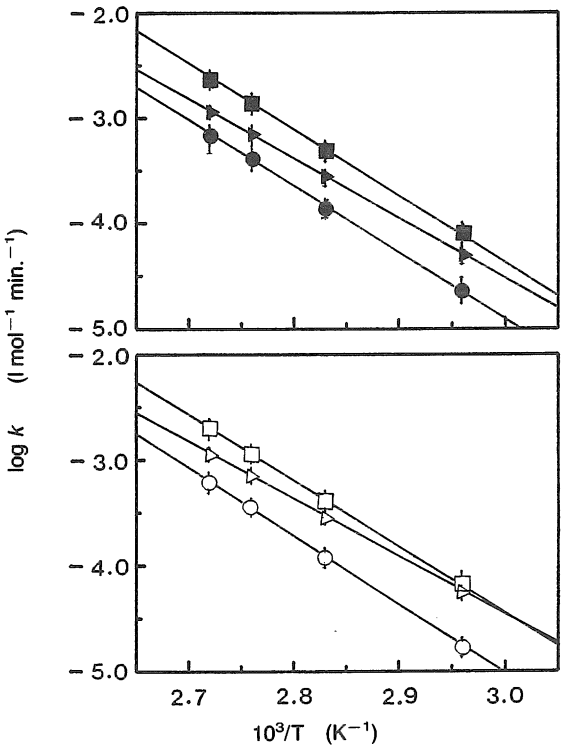

Fig. 4. Arrhenius plots of log k versus $10^3/T$ in various association solutions.
●: *Ala* (*glucose*+*Ala*)
▶: *Ala* (*glucose*+*Ala*+*mont*)
■: *Ala* (*glucose*+*Ala*+$CaCO_3$)
○: *Gly* (*glucose*+*Gly*)
▷: *Gly* (*glucose*+*Gly*+*mont*)
□: *Gly* (*glucose*+*Gly*+$CaCO_3$)

Table 3. Apparent activation energy and frequency factor obtained by Arrhenius plots of *Ala* and *Gly*

	Activation energy (kcal) : E Frequency fractor (l mol^{-1} min.$^{-1}$) : A		
	glucose+*Ala*	*glucose*+*Ala*+*mot*	*glucose*+*Ala*+$CaCO_3$
E:	27.7±1.7*	25.5±2.1	27.7±3.1
A:	2.00×10^{13}	1.58×10^{12}	6.31×10^{13}
	glucose+*Gly*	*glucose*+*Gly*+*mot*	*glucose*+*Gly*+$CaCO_3$
E:	30.2±3.9	25.0±4.7	28.8±4.0
A:	6.31×10^{14}	7.94×10^{11}	2.51×10^{14}

* 2σ

$CaCO_3$ at four different temperatures was shown in Table 2. The correlation coefficients between log k (k: rate constant) and $10^3/T$ (T: absolute temperature), which were shown in Fig. 4, were from 0.998 to 0.999. These correlation coefficients indicate the good correlation. Each pair of apparent activation energy and frequency factor is presented in Table 3. The apparent activation energy of *glucose*+*Ala* (E=27.7 kcal/

mol) is 8.3% lower than that of *glucose*+*Gly* (E=30.2 kcal/mol). This tendency was also recognized in the relationship between *glucose*+*Ala*+$CaCO_3$ (E=27.7 kcal/mol) and *glucose*+*Gly*+$CaCO_3$ (E=28.8 kcal/mol). From these differences in the apparent activation energy, it can be expected that the reaction of *glucose*+*Ala* is more likely to occur in ordinary temperature in sediments than that of *glucose*+*Gly*. Next, comparing *glucose*+*Ala* (E=27.7 kcal/mol) with *glucose*+*Ala*+*mont* (E=25.5 kcal/mol), the apparent activation energy of the latter was 7.9% lower than that of the former, and the frequency factor of the latter is also lower than that of the former. The same tendency was obtained in the relationship between *glucose*+*Gly* (E=30.2 kcal/mol) and *glucose* +*Gly*+*mont* (E=25.0 kcal/mol), the apparent activation energy of the latter was 17.2% lower than that of the former. Generally positive catalyst has the ability which decrease activation energy and frequency factor (Keii, 1983). Since *mont* has a broad surface area, it seems that *mont* apparently plays a role of weak positive catalyser in these association solutions. Taguchi and Sampei (1986) reported that the rate of mealnoidin formation in Maillard reaction was higher in the presence of *mont*. Therefore it appears that *mont* plays a role of weak positive catalyser in reducing sugar and amino acids consumption (first step in Maillard reaction) and polycondensation (subsequent successive steps in Maillard reaction).

In regard to *glucose*+*Gly* (E=30.2 kcal/mol) and *glucose*+*Gly*+$CaCO_3$ (E=28.8 kcal/mol), the activation energy of the latter is 4.6% lower than that of the former. But the activation energy of *glucose*+*Ala* and *glucose*+*Ala*+$CaCO_3$ are almost the same (E=27.7 kcal/mol). This suggests that $CaCO_3$ did not necessarily play a role as a positive catalyser in these reactions examined.

On the basis of the kinetics described above, following Arrhenius equations were obtained:

$$\textit{glucose}+\textit{Ala}: \quad k=2.00\times10^{13}\cdot\exp(-1.40\times10^{4}/T), \tag{4}$$

$$\textit{glucose}+\textit{Gly}: \quad k=6.31\times10^{14}\cdot\exp(-1.53\times10^{4}/T), \tag{5}$$

$$\textit{glucose}+\textit{Ala}+\textit{mont}: \quad k=1.58\times10^{12}\cdot\exp(-1.28\times10^{4}/T), \tag{6}$$

$$\textit{glucose}+\textit{Gly}+\textit{mont}: \quad k=7.94\times10^{11}\cdot\exp(-1.26\times10^{4}/T), \tag{7}$$

$$\textit{glucose}+\textit{Ala}+CaCO_3: \quad k=6.31\times10^{13}\cdot\exp(-1.40\times10^{4}/T), \tag{8}$$

$$\textit{glucose}+\textit{Gly}+CaCO_3: \quad k=2.51\times10^{14}\cdot\exp(-1.45\times10^{4}/T), \tag{9}$$

where k is the rate constant ($l\,mol^{-1}\,min.^{-1}$) and T is the absolute temperature (K). Assuming that these Arrhenius equations are applicable in the temperature range less than 65°C, at actual temperature their consumption rates in sediments can be examined.

Analysis of Lake Inawashiro samples

Lake Inawashiro sediments were collected to compare the results in the laboratory

experiments with the fact in natural environment. The results of the measurements of pH, TOC and concentration of *glucose*, *Ala* and *Gly* in Lake Inawashiro sediments are shown in Table 4. Temperature was 7.1°C at the top of the core sample. According to X-ray powder diffraction examination, *mont* was detected significantly in the core samples. The pH of pore water decreased with increasing depth. TOC was in the narrow range of 1.14% to 3.93% (Fig. 5). The concentrations of *glucose*, *Ala* and *Gly* normalized by TOC (1%) at each depth were shown in Fig. 6. *Glucose* and *Gly* tend to decrease with depth. *Ala* more rapidly decreased with depth. The fitting curve lines of (I)~(III) in Fig. 6 and their rate constants were obtained by least squares method

Table 4. Analytical results of the core sample of sediment in Lake Inawashiro

No	Depth (cm)	Water content (%)	pH	TOC* (%)	concentration (mol/l) *glucose*	*Ala*	*Gly*
1.	10~15	71.0	7.01	3.39	3.80×10^{-2}	2.50×10^{-2}	1.66×10^{-3}
2.	15~20	73.0	7.22	2.61	4.81×10^{-3}	1.69×10^{-3}	2.82×10^{-4}
3.	20~25	71.9	7.16	1.14	4.91×10^{-3}	3.52×10^{-4}	9.68×10^{-5}
4.	25~30	70.0	7.05	3.23	6.00×10^{-3}	6.79×10^{-4}	3.95×10^{-4}
5.	30~35	69.9	7.08	3.90	5.79×10^{-3}	4.97×10^{-4}	6.50×10^{-4}
6.	35~40	72.2	7.04	2.82	5.59×10^{-3}	3.32×10^{-4}	3.66×10^{-4}
7.	40~45	68.7	7.06	3.93	5.19×10^{-3}	4.74×10^{-4}	3.50×10^{-4}
8.	45~50	68.3	7.02	3.04	—	6.46×10^{-4}	5.44×10^{-4}

* TOC: Total Organic Carbon

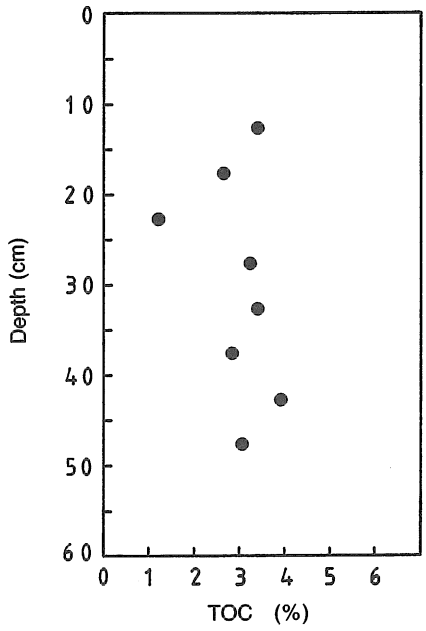

Fig. 5. Plots of Total Organic Carbon in Lake Inawashiro core sediments versus depth.

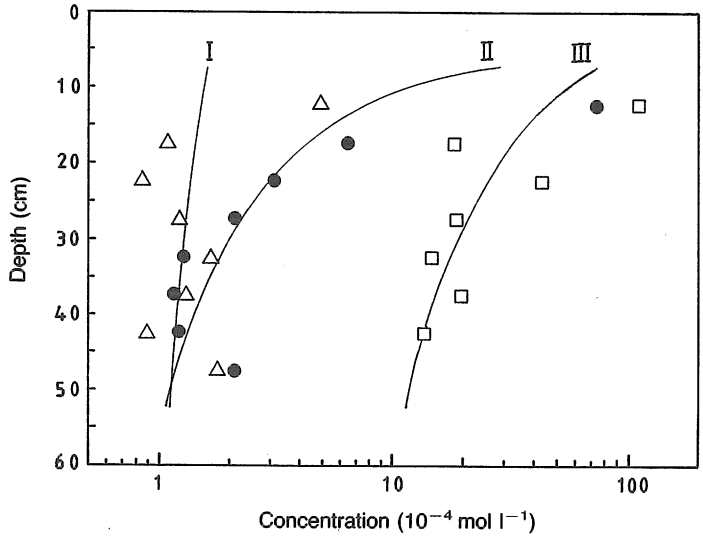

Fig. 6. Plots of *glucose*, *Ala* and *Gly* concentration normarized by TOC in Lake Inawashiro core sediments versus depth, and each fitting curve (I~III) of concentration change.
△: *Gly* (I) ●: *Ala* (II) □: *glucose* (III)

assuming that each concentration change is second-order reaction apparently. In calculating, following equation was used:

$$\frac{x}{C(C-x)}=kt, \tag{10}$$

where x is the consumed concentration (mol/l), C is the initial concentration (mol/l), k is the rate constant ($l\,mol^{-1}\,yr.^{-1}$) and t is the time (yr.). Each rate constant of the decreasing *glucose*, *Ala* and *Gly* concentration is calculated to be 0.423 (*glucose*), 5.02 (*Ala*) and 1.20 (*Gly*) ($l\,mol^{-1}\,yr.^{-1}$), respectively. The sedimentation rate in Lake Inawashiro is estimated to be 0.25 mm/yr. (10 cm/400 yr.) (Suzuki K. et al., 1981).

On the other hand, the rate constants obtained from equations (6) and (7) (T= 280.1 K (7.1°C) was used) were 1.18×10^{-2} (*glucose*), 1.18×10^{-2} (*Ala*) and 1.21×10^{-2} (*Gly*) ($l\,mol^{-1}\,yr.^{-1}$), respectively. The equations (6) and (7) were tentatively used because of the presence of *mont* in the sediment samples. The decreasing rates of *glucose*, *Ala* and *Gly* in sediments are about 10^2 times as fast as those in laboratory. These decreasing rates in sediments are too fast to think that *glucose* and amino acids are consumed by Maillard reaction. The biodegradations are generally very active in the surface sediments (Koyama and Handa, 1974). The rapid decrease of *glucose*, *Ala* and *Gly* by the biodegradation can be expected. If this decrease observed in Lake Inawashiro sediments is mainly due to the biodegradation, the rate of reducing sugar and amino acids consumption by biodegradation is estimated to be about 10^2 times as

fast as that by glucose-amino acids condensation in the surface sediments. Koyama and Handa (1974) have reported that biodegradations of amino acids were quite fast in the surface sediments, but in relatively deep sediment zone in Lake Biwa those were very slow because of inactively microorganisms. They pointed out that the decreasing of amino acids at ~200 m depth in Lake Biwa was approximately following first-order reaction and its rate constant was calculated to be $n\times10^{5}$ yr.$^{-1}$ (n: integer). This rate constant was compared with ones obtained in the present study. However it has no significance to compare directly the rate constant of first-order reaction with that of second-order one, each reaction rate was compared as follows:

$$r_1=k_1[A]\,, \tag{11}$$

$$r_2=k_2[A]\,[B]\,, \tag{12}$$

where r_1 is the reaction rate by first-order reaction, r_2 is the reaction rate by sccond-order reaction, k_1 is the rate constant by Koyama and Handa 1974; $n\times10^{-5}$ (yr.$^{-1}$) (n is integer), k_2 is the rate constant by the equation (6); 1.18×10^{-2} (l mol^{-1} yr.$^{-1}$), $[A]$ is the *Ala* concentration/TOC by Lake Inawashiro data (25~30 cm); 2.1×10^{-4} (mol/l) and $[B]$ is the glucose concentration/TOC by Lake Inawashiro data (25~30 cm); 1.86×10^{-3} (mol/l). The r_1 and r_2 were calculated to be $2.1\cdot n\times10^{-9}$ (mol l^{-1} yr.$^{-1}$) and 4.6×10^{-9} (mol l^{-1} yr.$^{-1}$), respectively. The r_1 and r_2 are not so much different from each other. It is considered that the consumption of *glucose* and amino acids in relatively deep sediment zone is attributed to the chemical reactions like Maillard reaction examined in the present study.

In the present study, although free amino acids were used for experiments, Ishiwatari (1985) reported that melanoidin was more rapidly formed by the reaction of *casein* with *glucose*, and much kerogen in actually are present in surface sediments (Tissot and Welte, 1984). Further geochemical kinetic studies of various systems including protein are required.

Conclusion

Geochemical kinetics of *glucose*, *Ala* and *Gly* consumption in Maillard reaction were examined, and the results were compared with the data of Lake Inawashiro sediments.

The results can be summarized as follows:

(1) The consumptions of *glucose*+*Ala* and *glucose*+*Gly* in Maillard reaction were second-order reaction.

(2) The apparent activation energy of *glucose*+*Ala* (E=27.7 kcal/mol) is 8.3% lower than that of *glucose*+*Gly* (E=30.2 kcal/mol), therefore the reaction of *glucose*+*Ala* is more easy to occur at ordinary temperature in sediments than that of *glucose*+*Gly*.

(3) The apparent activation energy in the presence of *mont* was 7.9～17.2% lower than that in the absence of *mont*. It appears that *mont* apparently plays a role of weak positive catalyst in *glucose*+*Ala* (or *Gly*) consumption in Maillard reaction.

(4) Rate constants of the decreasing *glucose*, *Ala* and *Gly* concentration in Lake Inawashiro sediments were about 10^2 times as fast as those in Maillard reaction in present experiments. On the basis of the result, it is concluded that in the surface sediments in Lake Inawashiro the biodegradation rates of *glucose*, *Ala* and *Gly* are about 10^2 times as fast as the condensation rates of them. However, it seems that in relatively deep sediments zone the chemical reaction like Maillard reaction is the predominant consumption reaction of reducing sugars and amino acids.

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