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Non-destructive spectroscopic tracing of simulated formation processes of humic-like substances based on the Maillard reaction

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ABSTRACT

The formation processes of humic-like substances have been simulated by heating glycine and ribose mixed solution (0.1 mol l^{-1}) at 80 °C through the Maillard reaction. Ultraviolet-visible (UV-vis), 3D excitation emission spectroscopy and size exclusion liquid chromatography succeeded in quantitatively tracing increases of the products during the heating of glycine and ribose mixed solution (0.1 mol l^{-1}). 2D correlation spectroscopic analyses suggested that a band area around 280 nm (UV_{280}) and 254 nm absorbance (UV_{254}) can be used as measures of the formation of furfural-like intermediates and humic-like products, respectively. They were monitored by in-situ UV-vis spectroscopy with the original heatable liquid cell at 60-80 °C. Kinetic analyses of the obtained data gave activation energies of 91.4-96.6 kJ mol^{-1}. These non-destructive measurements by in situ spectroscopic method did not require any additional procedures including drying or extracting the solution and they can be effectively used for direct tracing of the reaction progress and/or decomposition.

INTRODUCTION

In Earth's surface environments, geochemical reactions generally take place in the presence of water. Kinetic behavior of such aquatic geochemical reactions has been generally studied by batch experiments analyzing solution and solid products by extracting them from reaction containers. However, detailed changes of chemical species

without their modification from the original aqueous states could not be monitored by these batch methods. Therefore, in situ spectroscopic observation is expected to provide direct non-destructive monitoring of changes in aqueous species with a fine time resolution. In this study, simulated formation processes of humic-like substances by the Maillard reaction were monitored by in situ Ultraviolet-visible (UV-vis) spectroscopy, Three-Dimensional Excitation Emission Matrix (3D-EEM) spectroscopy and size exclusion liquid chromatography. The validity of UV-vis tracing was evaluated by generalized two-dimensional correlation spectroscopic analyses.

"Humic substances (HS)" is a general term of high-molecular-weight, yellow to black colored organic matter in soils and sediments.[1] UV-vis spectroscopy is one of the simplest measurements for HS because of their color. Although UV-vis absorption spectra of HS are often featureless,[2] UV-vis spectroscopy has been conducted for evaluation of their quantity and property.[3-7] In particular, absorbance at 254 nm has been often used as a measure of dissolved organic matter (DOM) and natural organic matter (NOM). [8,9] On the other hand, 3D-EEM and size exclusion liquid chromatography have been also used for monitoring HS in natural water; Nagao et al. [10] used high-performance size exclusion chromatography for characterization of HS in natural water showing some fluorescence peaks in the range of 250-500 nm of excitation wavelength (Ex.) and 300-600 nm of emission wavelength (Em.). Since it is recently pointed out that destructive procedures such as alkaline extraction or drying for HS analyses may limit precise examination of HS,[11] these non-destructive spectroscopic measurements are expected to provide precise properties of HS.

On the other hand, the Maillard reaction is suggested to be one of possible processes forming HS.[12] It is a continuous reaction between an amino group in amino acid and a carbonyl group in saccharides producing polymerized brown organic compounds, melanoidins.[13] It has been studied for long years by various researchers especially in food chemistry because it is an important reaction changing not only colors but also flavors and nutrients of foods.[14] Since the Maillard reaction is a color changing reaction, UV-vis spectroscopy has been often employed for measuring the reaction progress.[15-19] However, most of the Maillard reaction researches have been studied at relatively high temperatures simulating food cooking conditions and there are very little information on time scales of the Maillard reaction in geological environments,[20] except for some limited studies on reactions between amino acids and kerogens (high molecular weight insoluble organic matter) and analyses on dried products[21,12].

Although UV-vis spectroscopy has been used in both the HS and the Maillard reaction researches, the problem is how UV-vis spectra correspond to the Maillard reaction

products including HS-like materials. In order to examine correlation among spectroscopic data, generalized two-dimensional correlation spectroscopy (2D-COS) has been used. It is a mathematical method to visualize correlation between bands in two series of spectra changing in response to a perturbation, for example time, temperature and composition. The two series of spectra can have the same or different spectral variables.[22] Since the first proposal in 1986,[23] the use of 2D-COS in spectroscopy has been increasing.[24] Recently in the HS researches, 2D-COS has been introduced for examining the relationships among spectroscopic data concerning HS.[25,26] In this study, generalized 2D-COS was conducted on spectroscopic data obtained by the following non-destructive direct measurements without any additional procedures including drying or extracting the solution: UV-vis spectroscopy, Three-Dimensional Excitation Emission Matrix (3D-EEM) spectroscopy and size exclusion liquid chromatography.

We first confirmed the progress of the Maillard reaction in the batch heating experiments of 0.1 mol l^{-1} glycine + ribose solutions at 80 °C. In order to examine properties and molecular sizes of the products, the product solutions were analyzed by (1) UV-vis spectroscopy, (2) Three-Dimensional Excitation Emission Matrix (3D-EEM) spectroscopy and (3) size exclusion liquid chromatography. Glycine and ribose are selected here as representative and simple amino and carbonyl bearing compounds. Glycine is the simplest amino acid and ribose is a simple sugar compound, which is used in nucleotides forming RNA and DNA.

Second, generalized 2D-COS were used to examine specific UV absorbances at 280 and 254 nm and their correlations to some of the above spectroscopic data.

Third, in order to evaluate precise kinetic data for the progress of the Maillard reaction by using 280 nm and 254 nm absorbances, in situ UV-Vis spectroscopy with an original heatable liquid cell was conducted on 0.1 mol l^{-1} glycine + ribose mixture solutions heated at 60, 65, 70, 75 and 80 °C for 0-144 hours, for accelerating the reaction.

MATERIALS AND METHODS

Preparation of batch solutions

0.2 mol l^{-1} glycine and ribose solutions were prepared respectively by dissolving them in pure water (MilliQ: Resistance > 18.2 MΩ cm). These two solutions were mixed to obtain 0.1 mol l^{-1} glycine plus 0.1 mol l^{-1} ribose solution. This glycine + ribose mixture solution was subdivided into 16 polypropylene micro-tubes containing 1.5 ml of 0.1 mol l^{-1} glycine + ribose solutions. One of them was kept at room temperature (heated for 0 hour) and the others were heated at 80 °C in an electric oven for about 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 72, 96, 120, 144, 168 hours in order to accelerate the reaction. After

heating at 80 °C for each period, the solution bottles were taken out from the oven and stored in a refrigerator (6 °C). 0.1 ml of these batch solutions were diluted 100 times by pure water (MilliQ) in order to prevent spectral saturation and fluorescence quenching for the following measurements; (1) ultraviolet-visible (UV-vis) spectroscopy, (2) Three-Dimensional Excitation Emission Matrix (3D-EEM) spectroscopy, and (3) size exclusion liquid chromatography. In order to obtain UV-vis spectra of larger molecular weight components in the 168 hour heated products, another 0.1 mol l^{-1} glycine + ribose mixture solution heated at 80 °C for 168 hours was prepared. It was put into a dialysis tube (MWCO: 3500 Da, in pure water buffer) for 24 hours and dialyzed to obtain fractions over 3500 Da.

Analyses of the batch solutions

(1) Ultraviolet-visible (UV-Vis) spectra were measured in a quartz cell (optical pass length: 10 mm) by an UV-Vis spectrometer (V-570, Jasco). Pure water was measured as a background spectrum, and then the diluted sample solutions were measured. UV-Vis spectra were measured at a scanning speed of 400 nm min^{-1} with a resolution of 1.0 nm in the 190-1100 nm spectral range.

(2) Three-Dimensional Excitation Emission Matrix (3D-EEM) spectra were measured in a fluorescence quartz cell (optical pass length: 2mm, width: 10 mm) by a three-dimensional fluorescence spectrometer (F-7000, HITACH). Excitation wavelength (Ex.) was 250-500 nm (excitation slit: 5 nm) and emission wavelength (Em.) was 300-600 nm (emission slit: 5 nm). Scanning speed was 2400 nm min^{-1} at 5 nm intervals and photomultiplier voltage was 400 V. All of the spectra were corrected based on rhodamine B spectrum.

(3) Size exclusion liquid chromatography was conducted by using a High Performance Size Exclusion Liquid Chromatography (HPLC-SEC) unit (EXTREMA, Jasco) and a SEC column (YMC-Pack Diol 120, YMC, 35 °C). 50 mM sodium acetate/acetic acid solution (pH = 7) was used as a common eluent. The injection volume was 100 µl, the flow rate was 1 ml min^{-1}, and the analysis time was 30 min for each sample. The SEC column and experimental condition were the same as Moriizumi and Matsunaga[27]. Positions of void volume (V_0: retention time 5.5 minutes), total effective column volume (V_e: retention time 12.2 minutes) and retention time of molecular weight standards (ovalbumin: 42 kDa; myoglobin: 17 kDa) in their report are marked in the obtained chromatograms for reference. Because these protein standards and the Maillard reaction products are considered to have different molecular shapes and electrical properties, it should be noted that these values cannot be directly compared.

Chromatograms were recorded with a UV detector (UV-4070, Jasco) at 280 nm without baseline correction and a fluorescence detector (FP-4025, Jasco) at Ex. 345 nm/Em. 430 nm. Obtained data were analyzed by a commercial software (Chromato-Pro, Runtime Instruments).

In situ UV-Vis spectroscopic observation

In order to monitor the progress of the Maillard reaction, in situ UV-Vis spectroscopy with an original heatable liquid cell (Fig. 1) was conducted at 60-80 °C.[28] PTFE spacers of 0.1 mm thick were made from commercial PTFE sheets (AS ONE, 7-358-02) by piercing them with punches of 12 mm (outside diameter) and 8 mm (inside diameter). 0.2 M glycine and 0.2 M ribose solutions were prepared by dissolving them in pure water (MilliQ). These solutions and pure water were mixed to obtain 0.1 mol l^{-1} glycine + ribose mixture solutions. About 3 μl of the solution was injected in the sample chamber of the liquid cell (Fig. 1).

0.1 mol l^{-1} glycine + ribose mixture solutions were heated at 80, 75, 70, 65 and 60 °C for 144 hours (6 days) in an UV-Vis spectrometer (V-530, Jasco). In order to examine reproducibility, 80 °C measurement was conducted three times. The heatable liquid cell was mounted on the sample position of the UV-Vis spectrometer and the reference position was left blank. UV-Vis spectra were measured every 5 minutes at a scanning speed of 400 nm min^{-1} with a resolution of 1.0 nm in the 200-1100 nm spectral range.

Generalized Two-dimensional Correlation Spectroscopy (2D-COS)

Principles of 2D-COS are described in detail in a reference by Noda and Ozaki[22]. Here, only an outline of the principle is shown.

For constructing generalized 2D-COS, dynamic spectra are first calculated by subtracting the parent spectra from the reference spectrum (time-average spectrum in the present case). Second, the dynamic spectra measured in time domain are transformed in those in frequency domain by Fourier transform. Another series of dynamic spectra from the reference spectrum (time-average spectrum in the present case) is calculated in the same way. These two series of dynamic spectra were converted to 2D-COS spectra by using a synthesized correlation function. The 2D-COS spectra consist of real and imaginary components derived from the Fourier transform, and the real and imaginary components respectively give synchronous and asynchronous correlation intensity. In a synchronous spectrum, positive (or negative) peaks imply that the pairs of the spectral variable change corresponding to the perturbation in the same (or opposite) direction. In an asynchronous spectrum, if positive (negative) peaks appear at the same position as

synchronous correlation peaks, that implies changes at the spectral variable on the horizontal axis occurring earlier (later) than those on the vertical axis.

In this study, generalized 2D-COS was conducted on UV-vis spectra, emission spectra at a certain excitation wavelength in 3D-EEM spectra and SEC chromatograms for the batch solutions. The spectra were generated by using a free software available at https://sites.google.com/site/shigemorita/home/2dshige (last accessed date; March 27, 2017), 2DShige (Shigeaki Morita, Kwansei-Gakuin University, 2004-2005).

RESULTS

Results of batch measurements

Representative UV-Vis spectra for 0.1 mol l^{-1} glycine + ribose mixture solutions heated at 80 °C for 0, 24, 48, 72, 96, 120, 144 and 168 hours (diluted 100 times) are shown in Fig. 2(a). A band around 280 nm increased with time. Moreover, absorbance at shorter wavelength increased more for longer heating durations. Fig. 2(b) shows a spectrum for the solution heated for 168 hours and that after dialysis (> 3500 Da). Absorption in the UV region generally decreased after dialysis, in particular around 280 nm. However in the visible region, broad and featureless absorption remained.

Representative 3D-EEM spectra for 0.1 mol l^{-1} glycine + ribose mixture solutions heated at 80 °C for 0, 96 and 168 hours (diluted 100 times) are shown in Fig. 3(a), (b) and (c), respectively. Fluorescence maxima at Ex. 345 nm/ Em. 430 nm can be recognized for spectra at 96 and 168 hours (Fig. 3(b), (c)).

Representative size exclusion liquid chromatograms by 280 nm absorbance on the product solutions show, from left to right, large (5.7 minutes), medium (10, 11 minutes) and small (18.8 minutes) molecular weight components based on markers (ovalbumin: 42 kDa; myoglobin: 17 kDa) (Fig. 4(a)). It should be noted that this chromatogram can include not only the peak around 280 nm but also a tail of broad absorption in the ultraviolet range because the detection was without baseline correction. On the other hand, representative chromatograms by fluorescence intensity at Ex. 345 nm/Em. 430 nm show peaks around retention time of 10 to 17 minutes (Fig. 4(b)), corresponding to medium molecular weights observed by 280 nm absorbance chromatograms (Fig. 4(a)).

Results of in situ measurements

Reaction progress of the Maillard reaction was monitored by in situ UV-Vis spectroscopy with the original heatable liquid cell (Fig. 1) at 60-80 °C. Representative UV-vis spectra for 0.1 mol l^{-1} glycine + ribose mixture solutions heated at 80 °C for 0-

144 hours are shown in a supplemental figure (Fig. s1). A band around 280 nm increased with time, in the same way as the batch experiments.

It should be noted that the batch measurements were done on 10 mm cell for 100 times diluted solutions, while the in situ measurements were on about 0.1 mm thickness with initial solutions. Therefore, absorbance values by the both methods can be expected to be in the same order. However, 280 nm absorbance on the 144 hours spectrum in the in situ measurements at 80 °C are about 2.5 times larger than those in the batch experiments. These differences are considered to be due to thicker solution thicknesses than the spacer of the in situ cell and the different references (blank for in situ vs. pure water for batch). The solution escape from the same in situ cell have been found to be minor by our in situ IR spectroscopy showing the stable absorbance of water around 5200 and 7000 cm^{-1}.[28] Unfortunately, absorption by water at 960 nm in the near-IR range is too weak to be monitored for the water loss. It should be also noted that glycine only and ribose only solutions showed almost no absorption increases in the UV-Vis range at 80 °C.

DISCUSSION

Generalized 2D Correlation Spectroscopic Analyses and their Interpretations

Generalized 2D synchronous and asynchronous spectra were generated from UV-vis spectra, emission spectra at a certain excitation wavelength in 3D-EEM spectra and SEC chromatograms for the batch solutions by using 2DShige.

Generalized 2D synchronous correlation UV-vis spectra for the product solutions of 0.1 mol l^{-1} glycine + ribose mixture solutions heated at 80 °C for 0-168 hours (batch solutions) show generally strong positive correlations around 200 and 280 nm (red portions in Fig. 5(a)). Asynchronous correlation spectra indicate sequential order of spectral changes: absorption around 320 nm firstly increased and 200, 280 and >350 nm absorption followed in this order (from reddish to bluish regions in Fig. 5(b)).

Fig. 5(c) and (d) shows generalized 2D (c) synchronous and (d) asynchronous correlation spectra (horizontal axis: UV-vis spectra, vertical axis: emission spectra excited at 345 nm), respectively. The fluorescence maxima around 430 nm have strong correlations to absorbance around 200 and 280 nm (Fig. 5(c)). This correlation extends to 500 nm in the visible region. In asynchronous correlation spectra, the fluorescence maxima around 430 nm increased at the same time as absorptions around 250 nm and 400 nm (white regions in Fig. 5(d)). Although changes in the emission spectra excited at 345 nm are assumed to have correlation with UV absorbance changes at 345 nm, Fig. 5(c) showed the relatively weak correlation among them. This is possibly because the fluorescent components less contributed to UV-vis spectra than other UV absorbing

components. In fact, the fluorescent components having chromatographic peaks around retention time of 10 to 17 minutes (Fig. 4(b)) showed much weaker absorption at 280 nm than the components at retention time of 18.8 minutes (Fig. 4(a)) contributing mainly to changes in the 280 nm absorption band.

UV-vis absorbances around 280 nm on the horizontal axis were determined as spectra above the baseline (245-315 nm) (Fig. 2(a)). Generalized 2D (e) synchronous and (f) asynchronous correlation spectra (horizontal axis: spectra around 280 nm above the baseline (245-315 nm), vertical axis: chromatograms by 280 nm absorbance) are shown in Fig. 5(e) and (f). The chromatograms around retention time of 5.7, 10, 11 and 18.8 minutes ($A_{5.7}$, A_{10}, A_{11} and $A_{18.8}$) show synchronous correlations around 260-300 nm (Fig. 5(e)). A_{10} and A_{11} increased earlier than UV-vis spectral changes and $A_{5.7}$ and $A_{18.8}$ increased later (blue and red regions in Fig. 5(f), respectively). The spectra around 280 nm above the baseline show strong and simultaneous correlations with $A_{18.8}$ (red region in Fig. 5(e) and white region in Fig. 5(f), respectively).

Generalized 2D synchronous correlation spectra (horizontal axis: UV-vis spectra, vertical axis: chromatograms by fluorescence intensity at Ex. 345 nm/Em. 430 nm) indicate that the components with 10-17 minutes retention time show strong correlation with 200-300 nm absorbance (red regions in Fig. 5(g)). Asynchronous correlation spectra indicate earlier increases than UV-vis spectral changes of the components with retention times at 10 and 11 minutes (blue regions in Fig. 5(h)). The components with longer retention time (12-17 minutes) show later changes (red regions in Fig. 5(h)).

Implications from UV-vis and 3D-EEM Spectroscopy and Size Exclusion Liquid Chromatography

UV-vis and 3D-EEM spectroscopy and size exclusion liquid chromatography succeeded in quantitatively tracing increases of the products. These measurements did not require any additional procedures including drying or extracting the solution. In the following, implications of these spectral changes including their 2D correlation spectroscopic analyses are discussed.

UV-vis spectra (Fig. 2(a)) and chromatograms by 280 nm (Fig. 4(a)) suggested the formation with time of small components with 280 nm absorption. These are considered to include furfural, which has an absorption peak around 280 nm.[29] The disappearance of peak at 280 nm in the spectrum of the dialyzed solution (> 3500 Da) (Fig. 2(b)) can be understood by the small size of furfural.

By 2D correlation spectroscopic analyses, a band around 280 nm above the baseline of 245-315 nm can be taken as a measure of the intermediates including furfural (Fig.

5(e) and (f)). Spectral changes in the visible region (> 350 nm: browning) followed after the 280 nm increases (Fig. 5(d)). Therefore, in this study, the band area around 280 nm with a baseline of 245-315 nm (UV_{280}) will be used for kinetic analyses of the formation of furfural-like intermediates.

On the other hand, humic-like products were indicated by UV-vis spectra (Fig. 2), 3D-EEM spectra (Fig. 3) and chromatograms by fluorescence intensity at Ex. 345 nm/Em. 430 nm (Fig. 4(b)). The fluorescence maxima in Fig. 4(a), (b), (c) resemble to those of natural humic solutions reported in a previous research.[10] Therefore, humic-like fluorescent products are considered to be produced from glycine and ribose by heating them at 80 °C.

By 2D correlation spectroscopic analyses, it was suggested that 250 nm absorbance and visible absorbance can be employed as a measure of the humic-like products with fluorescence. In this study, 254 nm absorption with a one-point base at 600 nm (UV_{254}) will be used for kinetic analyses of the formation of humic-like products. It should be noted that UV_{254} include not only tails of 280 nm absorption band but also broad UV tails (Fig. 2).

Other components at retention times at 5.7 and 10-17 minutes (Figs. 5(e). (f), (g) and (h)) could not be assigned to UV-vis spectral changes. It should be noted that changes in chromatograms at retention time 5.7 and 12-17 minutes were later than UV-vis spectral changes and those at 10 and 11 minutes were earlier. Moreover, no shift in the chromatographic peaks (no shift in the molecular weight) may imply increases with time of similar molecular weight components (Fig. 4(a) and (b)).

Since the Maillard reaction has many pathways and products possibly including non-UV-active and non-fluorescent products,[30] some components in the Maillard reaction cannot be traced by UV-vis and fluorescence spectroscopy. In order to characterize the products, we tried additional infrared (IR) spectroscopy, Hetero-nuclear Single Quantum Coherence (HSQC) Nuclear Magnetic Resonance (NMR) spectroscopy and Matrix Assisted Laser Desorption/Ionization Time-of Flight Mass Spectrometry (MALDI-TOF-MS). However, many components and residual reactants, glycine and ribose in the product solution prevented accurate measurements and they provided little information on details of products, except for the results that the products may include aliphatic and aromatic components. These are difficulties of characterizing complex products of simulated geochemical processes.

Kinetic analyses on the results of in situ UV-Vis spectroscopic observation

In the in situ UV-Vis spectroscopic measurements, UV-vis spectra of 0.1 mol l^{-1}

glycine + ribose mixture solutions heated at 60, 65, 70, 75 and 80 °C for 0-144 hours were obtained. The continuous measurements at a short time interval (5 minutes) and the stability of temperature (±1 °C) of the present in situ spectroscopy enable detailed quantitative analyses of kinetic data. Changes of (a) UV_{280} and (b) UV_{254} with time during the in situ measurements at each heating temperature are plotted in Fig. 6(a), (b). These changes can be divided into the early induction stage and the later progress stage after Song et al.[15]. In this study, the later stage where $UV_{280} > 1$ or $UV_{254} > 0.095$ were fitted as steady changing states for kinetic analyses. Changes with time of the Maillard reaction can be fitted by both the zeroth and the first order reactions. UV_{280} and UV_{254} changes in the later stage were fitted by both of the zeroth and first order fittings and they gave almost the same kinetic parameters.[15-17] In the following analyses, only the first order reaction model is presented. This is because of exponential shapes of UV_{280} increases (Fig. 6(a)), possible comparison of rate constant values (unit: s^{-1}) and easiness of using half-life values. The fittings of the data by

$$UV = C_1 (1 - k (t - \tau)) + C_2 \quad (1)$$

give apparent first order reaction rate constants k_{280} and k_{254}, where τ is a starting time of each later stage when $UV_{280} = 1$ and $UV_{254} = 0.095$, C_2 is 1 for UV_{280} and 0.095 for UV_{254}, and C_1 is a fixed parameter determined by preliminary fittings. The preliminary fittings were conducted on the changes at 80 °C by Eq. 1 (fitting parameters: k, C_1). Since 80 °C in situ measurement was conducted three times for replicability, average value of C_1 was employed. C_1 is 49.2 for UV_{280} and 3.23 for UV_{254}. The fitting curves are shown in Fig. 6 on the UV_{280} and UV_{254} changes. k_{280}, k_{254} and correlation coefficient r values obtained by the first order fittings are listed in Table 1.

The obtained apparent reaction rate constants increased with heating temperatures. They can be described by Arrhenius equation:

$$\ln k = \ln A - \frac{E_a}{RT} \quad (2)$$

, where A is the frequency factor (s^{-1}), E_a is the activation energy (kJ mol^{-1}), R is the gas constant (8.31 J mol^{-1} K^{-1}) and T is the absolute temperature (K). Arrhenius plots of k_{280} and k_{254} and T (333-350 K) (Fig. 7) show relatively good linear trends, where average values of ln k at 80 °C are employed and their standard deviations (0.14 for k_{280} and 0.10 for k_{254} at 80 °C) are indicated by error bars. The fitting lines of these experimental data give E_a and A values for the reactions in the induction and the first reaction stages of 280 nm band area and 254 nm absorbance during the heating of 0.1 mol l^{-1} glycine + ribose mixture solutions (Table 2). These activation energy E_a values (91.4 and 96.6 kJ mol^{-1}) are in the reported range (60-145 kJ mol^{-1}) for the Maillard reaction in aqueous solutions

indicating the validity of the present in situ UV-Vis spectroscopic method with an original heatable liquid cell.[20] UV_{254} with one point base at 600 nm can reflect not only the shoulder of 280 nm peak but also a tail of broad UV absorptions. In fact, the first order rate constants for increases of the band area around 280 nm (k_{280}) are larger than those for the 254 nm absorbance (k_{254}) with a slight difference in the activation energy values (E_a) (Table 1,2, Fig.6,7).

The obtained E_a and A values would enable extrapolation of k_{280} and k_{254} values to lower and higher temperatures by the Arrhenius equation (Eq. 2). For example, half-lives for k_{280} and k_{254} values at 15 °C are 30 and 98 years. Considering the early induction stage, time scales of the whole reaction may be longer. The present spectroscopic method and obtained kinetic parameters can be used in evaluating time scales of the Maillard reaction both in Earth surface environment and food chemistry with further detailed studies.

In future experiments, more complex amino and carbonyl groups in proteins and polysaccharides should be used. Moreover, minerals should be added to these organic compounds for simulating more realistic geological environments. In order to conduct these kinds of experiments, the present in situ heatable cell can be placed under infrared and visible/Raman microspectrometers for monitoring changes in IR and visible spectra with time.[31, 32] In the presence of proteins, polysaccharides and minerals, which can be solid phases, solid, liquid and interface portions under the microscopes can be monitored separately. These in situ micro-spectroscopic methods would enable direct tracing of the reaction progress and/or decomposition without extraction procedures.

CONCLUSION

In order to evaluate the progress of the Maillard reaction, we first conducted batch heating experiments of 0.1 mol l^{-1} glycine + ribose solutions at 80 °C directly in solution states. The product solutions were analyzed by (1) ultraviolet-visible (UV-vis) spectroscopy, (2) Three-Dimensional Excitation Emission Matrix (3D-EEM) spectroscopy and (3) size exclusion liquid chromatography. Generalized two-dimensional correlation spectroscopy (2D-COS) were also used to examine correlations among some of the above spectroscopic data.

UV-vis spectra and chromatograms by 280 nm suggested the formation with time of small components with 280 nm absorption. These are considered to include furfural, one of reported intermediates of the Maillard reaction having an absorption peak around 280 nm. By 2D correlation spectroscopic analyses, a band around 280 nm above the baseline of 245-315 nm can be taken as a measure of the formation of furfural-like intermediates.

Larger products were indicated by UV-vis spectra, 3D-EEM spectra and

chromatograms by fluorescence intensity at Ex. 345 nm/Em. 430 nm. The fluorescence maxima resemble to those of natural humic solutions reported in a previous research. By 2D correlation spectroscopic analyses, it was suggested that 254 nm absorbance with a one-point base at 600 nm (UV_{254}) can be employed as a measure of the humic-like products.

In order to evaluate precise kinetic data for the progress of the Maillard reaction by using 280 nm and 254 nm absorbances, in situ UV-Vis spectroscopy with an original heatable liquid cell was conducted on 0.1 mol l^{-1} glycine + ribose mixture solutions heated at 60, 65, 70, 75 and 80 °C for 0-144 hours. Kinetic analyses of the obtained data gave activation energies of 91.4-96.6 kJ mol^{-1}.

These non-destructive measurements by in situ spectroscopic method did not require any additional procedures including drying or extracting the solution and they can be effectively used for direct tracing of the reaction progress and/or decomposition.

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SUPPLEMENTAL MATERIALS

The supplementary material (Figs. S1) can be accessed at the journal web site.

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FIGURE AND TABLE

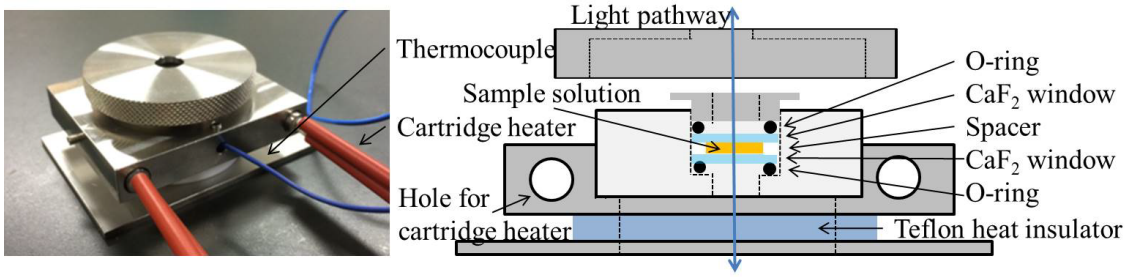

Fig. 1. The overview and cross section of an original heatable liquid cell.

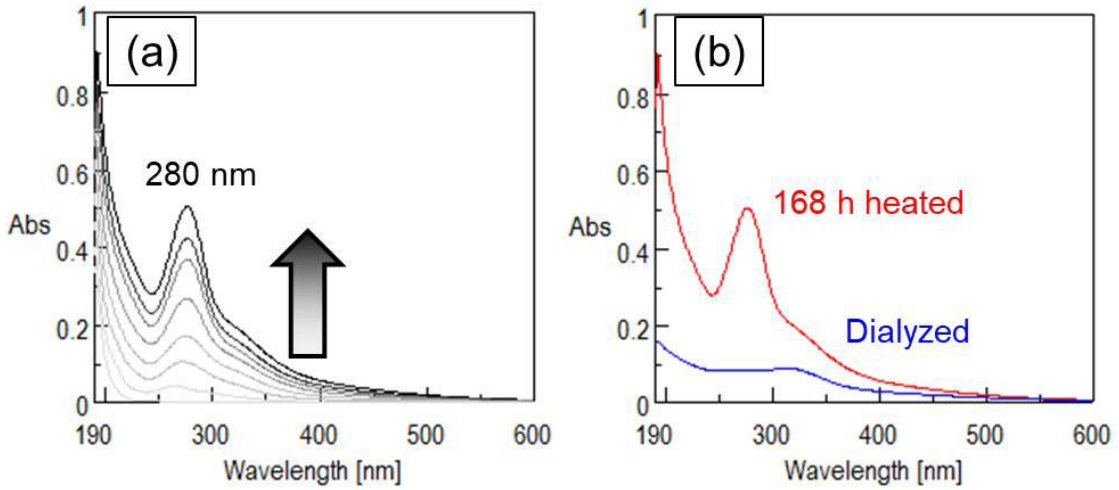

Fig. 2. (a) Representative UV-Vis spectra for the product solutions of 0.1 mol l^{-1} glycine + ribose mixture solutions heated at 80 °C for 0, 24, 48, 72, 96, 120, 144 and 168 hours (diluted 100 times) and (b) spectra for the 168 hours heated solution and for the dialyzed solution (> 3500 Da) (diluted 100 times).

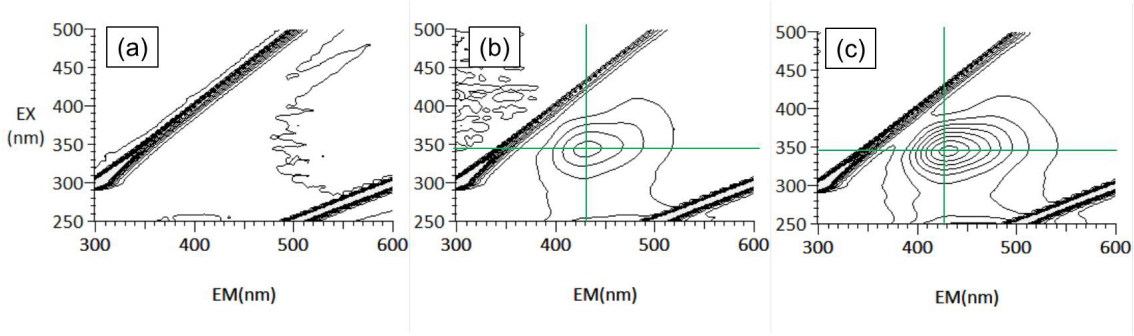

Fig. 3. Representative 3D-EEM spectra for the 0.1 mol l^{-1} glycine + ribose mixture solutions heated at 80 °C for (a) 0, (b) 96 and (c) 168 hours. All the solutions were diluted 100 times. Contour intervals are 2.5 intensity units. Fluorescence intensity maximum positions at Ex. 345 nm/ Em. 430 nm are indicated.

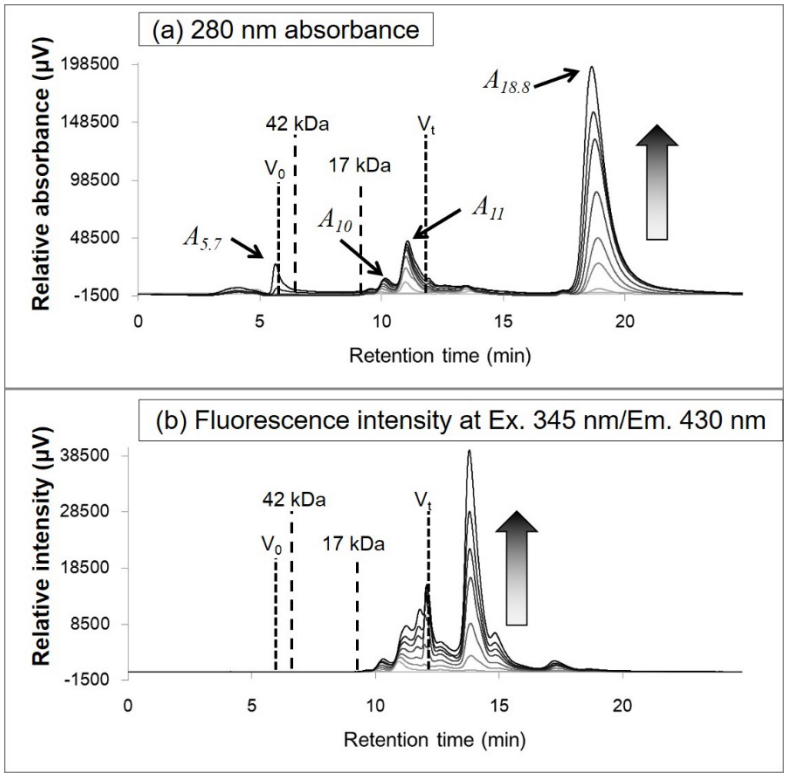

Fig. 4. Representative chromatograms by (a) 280 nm absorbance and (b) fluorescence intensity at Ex. 345 nm/Em. 430 nm on the product solutions of 0.1 mol l^{-1} glycine + ribose mixture solutions heated at 80 °C for about 0, 24, 48, 72, 96, 120, 144 and 168 hours (100 times diluted solutions further diluted 10 times by an eluent).

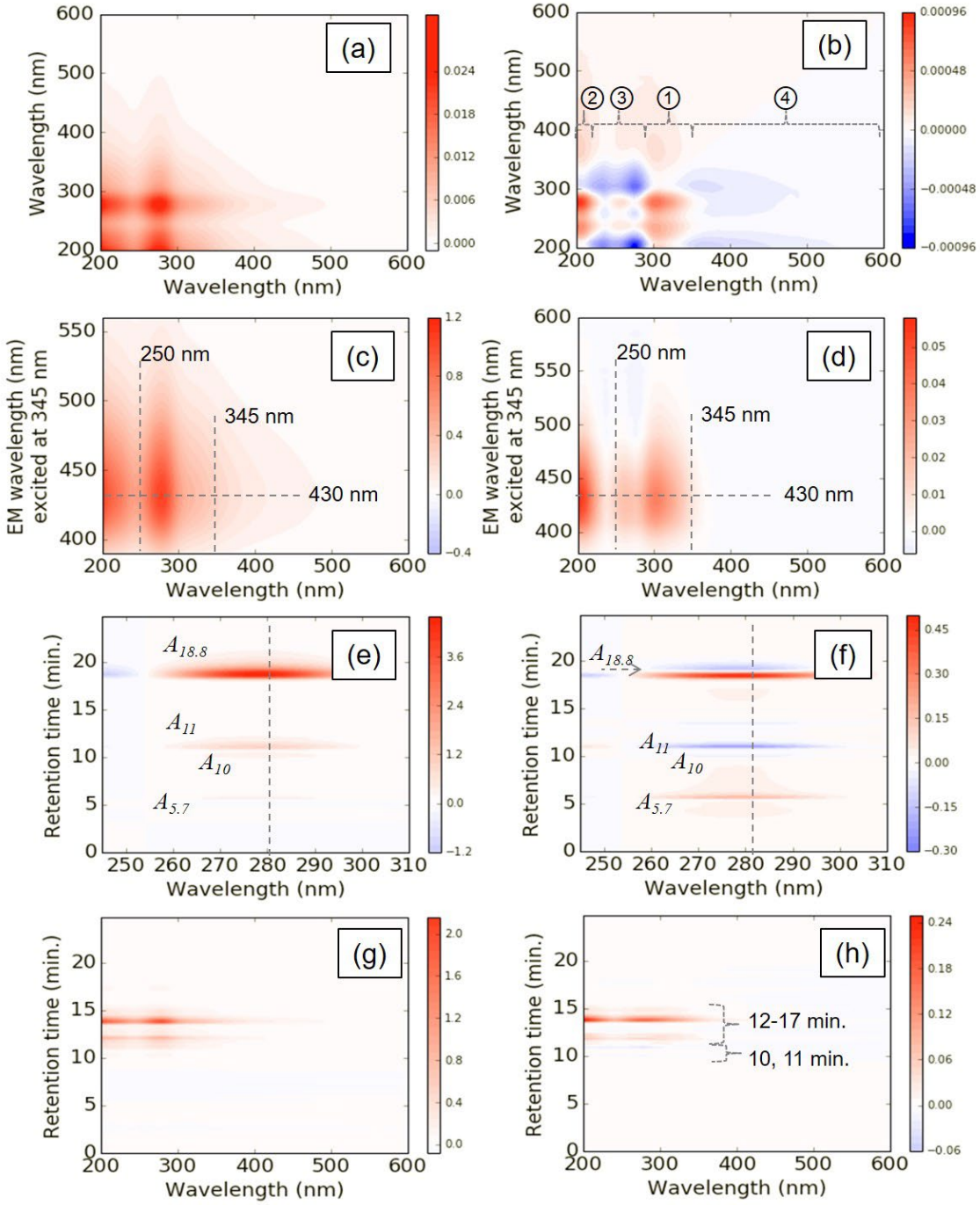

Fig. 5. Generalized 2D (a) synchronous and (b) asynchronous correlation UV-vis spectra for the product solutions of 0.1 mol l^{-1} glycine + ribose mixture solutions heated at 80 °C for 0-168 hours. Numbers of the regions in (b) corresponds to the order of spectral changes. Generalized 2D (c) synchronous and (d) asynchronous correlation spectra (horizontal axis: UV-vis spectra, vertical axis: emission spectra excited at 345 nm) for the product solutions of 0.1 mol l^{-1} glycine + ribose mixture solutions heated at 80 °C for 0-168 hours. Generalized 2D (e) synchronous and (f) asynchronous correlation spectra

(horizontal axis: spectra around 280 nm above the baseline (245-315 nm), vertical axis: chromatograms by 280 nm absorbance). Generalized 2D (g) synchronous and (h) asynchronous correlation spectra (horizontal axis: UV-vis spectra, vertical axis: chromatograms by fluorescence intensity at Ex. 345 nm/Em. 430 nm). Color scale bars are indicated for each figure. These scales are variation ranges of each correlation and cannot be compared each other.

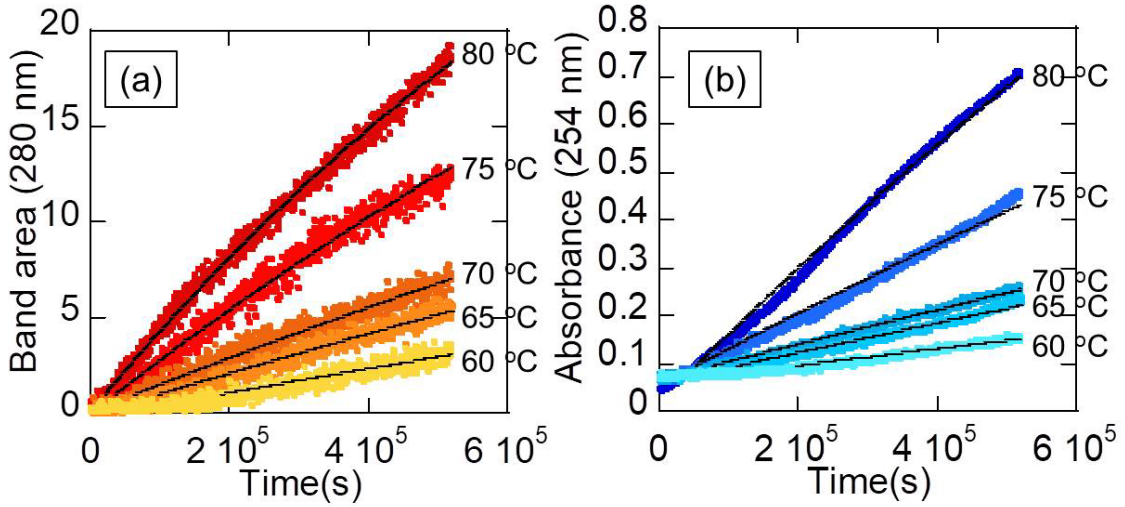

Fig. 6. Changes of UV_{280} and UV_{254} with time during the in situ measurements of 0.1M glycine + ribose mixture solutions heated at 60, 65, 70, 75, and 80 °C with the fitting curves (the first-order reaction).

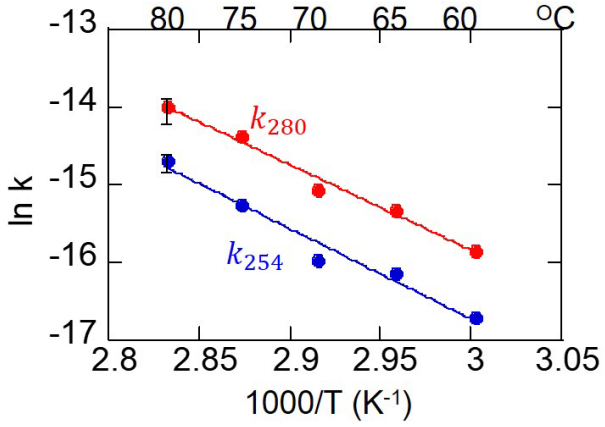

Fig. 7. The Arrhenius plots of k_{280} and k_{254} values and their fitting lines.

Table 1. k_{280}, k_{254} and correlation coefficient r values of the first order reaction fittings in the heating experiments of 0.1 mol l^{-1} glycine + ribose mixture solutions at 60, 65, 70, 75 and 80 °C.

	(a) 280 nm		(b) 254 nm	
	k_{280} (10^{-7} s^{-1})	r	k_{254} (10^{-7} s^{-1})	r
80 °C [1]	8.73	0.997	4.45	0.997
80 °C [2]	9.59	0.998	4.57	0.999
80 °C [3]	6.9	0.997	3.64	0.993
75 °C	5.72	0.99	2.35	0.993
70 °C	2.88	0.979	1.14	0.993
65 °C	2.21	0.976	0.978	0.987
60 °C	1.29	0.927	0.547	0.967

Table 2. Obtained E_a and A values for the reactions in the induction and the first reaction stages of (a) 280 nm band area and (b) 254 nm absorbance in the heating experiments of 0.1 mol l^{-1} glycine + ribose mixture solutions at 60, 65, 70, 75 and 80 °C.

	E_a (kJ mol^{-1})	A (10^8 s^{-1})
(a) 280 nm	91.4	2.79
(b) 254 nm	96.6	7.68