Characterization of Organic Aggregates Formed by Heating Products of Simulated Primitive Earth Atmosphere Experiments

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We observed formation of organic aggregates from a solution of complex organics which were abiotically synthesized from a simulated primitive earth atmosphere with high-energy protons. The structure of the complex organics was partly altered under hydrothermal conditions to form organic aggregates. The aggregates possessed properties of combined amino acids, which had high tolerance against heating. Formation of this kind of organic aggregates might have been an important step in the chemical evolution leading to the origin of life.

One of the essential characteristics of life is having a structure that separate itself from the outer environment. At the primitive stage of chemical evolution on the primitive Earth, organic matter could be condensed by formation of self-aggregates or globules, in which chemical evolution was accelerated, and then the first cells were formed. From carbonaceous meteorites, hollow organic globules were found[1] and their relationship to the origin of life has been discussed. From this point of view, some simulation experiments were conducted to make organic aggregates or globules prebiotically from the possible prebiotic materials.[2–6] However, most of them so far used high concentrations of pure chemicals as their starting materials. For example, Yanagawa and Kojima observed numerous microspheres after heating a mixture of 4 amino acids with very high concentration (0.3 M glycine, 0.1 M alanine, 0.3 M valine, and 0.1 M aspartic acid),[5] a condition unlikely to have existed on the primitive Earth.

Great numbers of experiments have been conducted to synthesize amino acids, hydrocarbons, and nucleic acid bases from simple molecules with sources of energy.[7–12] If the primitive Earth atmosphere was not strongly reducing, cosmic rays were one of the major energies for prebiotic synthesis of amino acids.[13–17] Kobayashi and co-workers showed that high-molecular-weight complex organic compounds were synthesized from a mixture of CO, N_2, and H_2O by irradiation with high-energy protons, and various amino acids were detected by acid hydrolysis of these complex organic compounds.[13,14] It is likely that organic compounds were generated in the primitive Earth atmosphere and interstellar environment, and were carried to the ocean on the primitive Earth.[18]

One of the most promising environments for prebiotic chemistry involving the origin of life is submarine hydrothermal systems (SHSs) because of the reduced condition, continuous supply of thermal energy, and the presence of high concentration of transition-metal ions as catalysts.[19] It was suggested that the last universal common ancestor (LUCA) was a kind of hyper-thermophile,[20] which also underscored the importance of SHSs as the sites for the origin of life. In this study, we examined the formation of organic aggregates from aqueous solution of abiotically formed complex organic compounds, by heating in a flow reactor simulating SHSs.

The complex organic compounds were synthesized from a gas mixture of CO, N_2, and H_2O, which were possible constituents of the primitive Earth atmosphere, by irradiation with 3 MeV protons from a Van de Graff accelerator (Tokyo Institute of Technology) or a TIARA tandem accelerator (Takasaki Advanced Radiation Research Institute, Japan Atomic Energy Agency). Hereafter, we call these synthesized complex organic compounds CNW. The aqueous solution of the CNW is slightly yellow, and was characterized as several-thousand-dalton "amino acid precursors" which yielded various amino acids after hydrolysis.[21] We injected aqueous solution of CNW into a supercritical water flow reactor (SCWFR) simulating a hydrothermal system[22] to observe CNW alterations after heating at high temperature and high pressure. We set the reaction temperatures to 150, 200, 250, 300, 350, or 400 °C. The injected CNW solution was heated at each temperature for 2 min and then quenched in a cold water jacket (ca. 0 °C). Fluid pressure in the system was maintained at 25 MPa. After being eluted out from SCWFR, the heated CNW solution was collected. A control sample was also prepared by passing the CNW solution through SCWFR without heating (at room temperature).

The heated CNW solution was filtered onto 0.2 μm pore size isopore polycarbonate membrane filters (ADVANTEC) and observed with a scanning electron microscope (KEYENCE KE-8800). We found aggregates in the samples heated at 200 °C and over, whereas no aggregates were observed in the control (without heating) nor the sample heated at 150 °C (Figure 1). The aggregates were amorphous measuring around 10 μm. Larger-sized aggregates were also found in the products after heating at 300 °C. The number of observed aggregates in the heated products increased in order of the heating temperature: 300 °C > 250 °C > 350 °C > 400 °C > 200 °C (Table 1). These results indicate that, under hydrothermal conditions around 300 °C, self-aggregates could be formed from a diluted solution of organic compounds abiotically synthesized from the possible primitive Earth atmosphere.
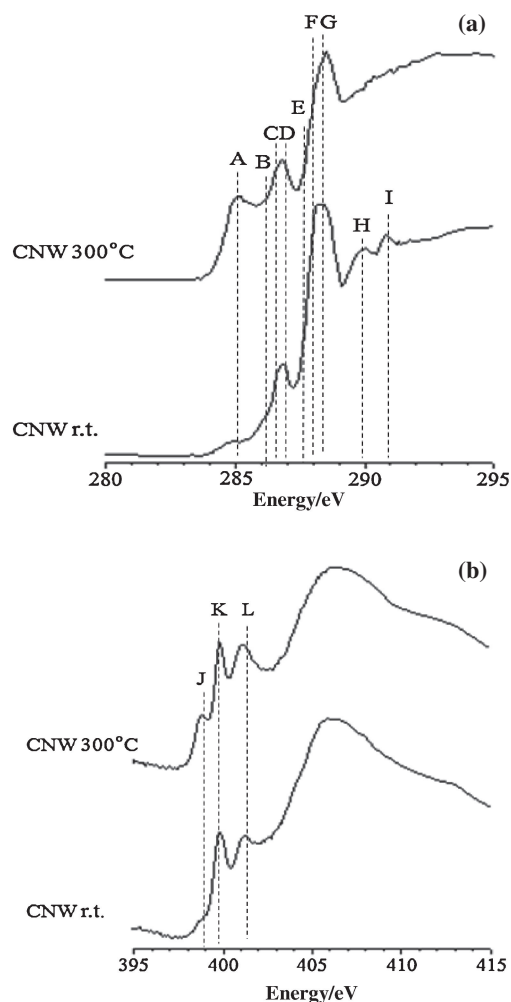
The X-ray absorption near-edge structure (XANES) spectra of the lyophilized fractions of heated (300 °C) and nonheated CNW samples after SCWFR were obtained using the scanning transmission X-ray microscope (STXM) at beamline 5.3.2.2., Advanced Light Source, Lawrence Berkeley National Laboratory. Carbon (C)-XANES spectrum of the nonheated CNW
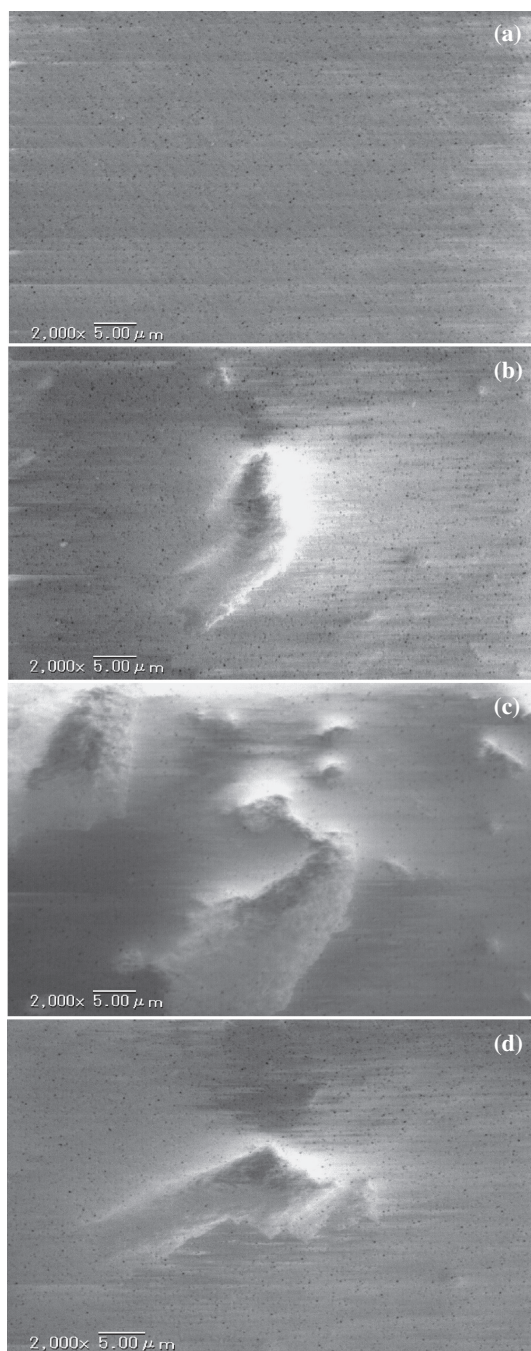
Figure 1. Organic aggregates formed in CNW after being treated with SCWFR for 2 min at (a) room temperature, (b) 250, (c) 300, and (d) 350 °C.

Figure 2. (a) C-XANES and (b) N-XANES spectra of CNW after treatment with SCWFR for 2 min at room temperature and 300 °C. Peak A: 1s–π^* transition at ca. 285 eV for aromatic carbon, peaks B and C: 1s–π^* transition at 286.1–286.5 eV for vinyl-keto carbon, peak D: 1s–π^* transition at ca. 286.7 eV for nitrile, peak E: 1s–3p/s* transition at ca. 287.5 eV for aliphatic carbon, peak F: 1s–π^* transition at ca. 288.2 eV for carbonyl carbon in amide group, peak G: 1s–π^* transition at ca. 288.5 eV for carbonyl carbon in carboxy or ester group, peak H: 1s–π^* transition at ca. 290 eV for carbamoyl carbon, peak I: 1s–π^* transition at ca. 291 eV for carbonic carbon, peak J: 1s–π^* transition at ca. 398.9 eV for imine, peak K: 1s–π^* transition at ca. 399.8 eV for nitrile, and peak L: 1s–3p/s* transition at 401.2 eV for amide or amino. Peak assignments are referred to Cody et al. (2008).[23]

Table 1. The number of aggregates (mL^{-1}-initial solution) observed in CNW after being heated at various temperatures for 2 min in SCWFR

	Heat temp/°C				
	200	250	300	350	400
Aggregates	3×10^6	8×10^6	10×10^6	6×10^6	5×10^6

(Figure 2a) shows a broad intense peak at 287–289 eV, which is likely a mixture of aliphatic carbon (peak E, 287.5 eV), carbonyls in amide (peak F, 288.2 eV), and in carboxy or ester groups (peak G, 288.5 eV). The spectrum also exhibits peaks at 286.7, 290, and 291 eV, which correspond to nitrile, carbamoyl, and carbonic carbons (peaks D, H, and I), respectively. Although the peak shoulders of aromatic (peak A, 285.1 eV) and vinyl-keto (peak B, 286.1 eV) carbons are identified, their intensities are very weak. In the C-XANES spectrum of the heated CNW, the peak of aromatic carbon (A) is better developed than those of

nonheated CNW. Its peak broadness reflects the formation of a variety of aromatic molecular structures (e.g., different ring numbers and the presence of heterocyclic moieties). Development of peak B in the heated CNW is supported by that of peak C which is also assigned to vinyl-keto. On the other hand, carbamoyl and carbonic carbon (peaks H and I, respectively) are absent in the heated CNW, and the abundances of aliphatic and amide carbonyls are relatively lowered compared to nonheated CNW. These differences between nonheated and heated CNW are also confirmed by their nitrogen (N)-XANES spectra (Figure 2b). Increase of aromatic carbon in the heated CNW is supported by a well-developed peak of imine nitrogen (peak J, 398.9 eV). The most stable functional group is nitrile: its peak intensities (D and K) are not largely changed before and after heating. Amide (or amino) appears to be relatively stable, but the absence of a broad shoulder at 401.5–402.5 eV in the heated CNW may indicate a partial loss of the functional groups due to heating. The results suggest that a part of the structure of CNW was altered by heating at 300 °C, and the increase of hydrophobicity, such as nitrogen- and oxygen-based hetero-aromatic carbon, of the products lead formation of the aggregates.

The amounts of amino acids contained in the aggregates and liquid fractions of SCWFR-treated CNW were analyzed. The aggregates and liquid fraction were separated by filtration using the 0.2 μm pore size polycarbonate membrane. The membrane and the residue (aggregates) on it were dissolved together in tetrahydrofuran (THF). Both the filtrates (aqueous fraction of the products) and the THF-dissolved residues were hydrolyzed with 6 M HCl at 110 °C for 24 h prior to amino acid analysis by high-performance liquid chromatography (Shimadzu LC-10A) with a cation-exchange column (Shimadzu Shim-Pack ISC-07/1504Na). Table 2 shows the amount of glycine in the filtrates and the residues of CNW after heating at each temperature with the SCWFR. Glycine is the predominant amino acid contained in the CNW.[21] The amount of glycine in the residue of the CNW treated at room temperature (the control) was almost the same as the background level which was detected from the polycarbonate membrane dissolved in THF without sample. In the residues of heated CNW, larger amounts of glycine were detected than that in the control. The higher the heating temperature was, the more glycine was recovered from the residue after acid hydrolysis, and the less glycine was found in the filtrate (Table 2). The ratio of glycine in the residue to that in the filtrate was 19% and 29% in the CNW heated at 300 or 350 °C, respectively. These results show that the aggregates of the CNW formed by heating in the simulated SHSs contained combined amino acids and that the amino acids bound to the aggregates had higher stability against heat than those dissolved in the aqueous phase.

In conclusion, we showed the formation of organic aggregates from a diluted aqueous solution of complex organic compounds which were synthesized from the simulated Earth atmosphere by irradiation with high-energy protons. Although the shapes of organic aggregates were different from the reported microspheres which were generated from amino acids in high concentration (0.1–0.3 M).[5] These kinds of organic aggregates or microsphere are expected to have functions such as catalysts[2] to accelerate chemical evolution in primitive ocean. Further work is warranted to study the structure and functions of these organic aggregates.

Table 2. Amounts of glycine (nmol) in the filtrate and the residue of CNW after being heated at various temperatures for 2 min in SCWFR

	Heat temp/°C			
	r.t.[a]	250	300	350
Filtrate (F)	189.8	68.3	22.5	15.5
Residue (R)	n.d.[b]	3.3	4.3	4.5
(R/F)/%		4.8	18.9	29.0

[a]r.t.: room temperature. [b]n.d.: not detected.

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