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Pressure Effects on the Abiotic Polymerization of Glycine

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Abstract Polymerization experiments were performed using dry glycine under various pressures of 5–100 MPa at 150°C for 1–32 days. The series of experiments was carried out under the assumption that the pore space of deep sediments was adequate for dehydration polymerization of pre-biotic molecules. The products show various colors ranging from dark brown to light yellow, depending on the pressure. Visible and infrared spectroscopy reveal that the coloring is the result of formation of melanoidins at lower pressures. High-performance liquid chromatography and mass spectrometry analyses of the products show that: (1) glycine in all the experimental runs oligomerizes from 2-mer to 10-mer; (2) the yields are dependent on pressure up to 25 MPa and decrease slightly thereafter; and (3) polymerization progressed for the first 8 days, while the amounts of oligomers remained constant for longer-duration runs of up to 32 days. These results suggest that pressure inhibits the decomposition of amino acids and encourages polymerization in the absence of a catalyst. Our results further imply that abiotic polymerization could have occurred during diagenesis in deep sediments rather than in oceans.

Keywords Deep sediment · Diagenesis · Glycine · High pressure · LC/MS · Peptide · Polymerization · Pre-biotic molecular evolution

Introduction

Peptide formation is an essential process in chemical evolution (e.g., Bernal 1951; Rode 1999). It is still an open question, however, as to what event in the early history of the Earth caused polymerization of organic molecules. Submarine hydrothermal vents on the

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primitive Earth have been proposed recently as a likely environment for the pre-biotic synthesis of organic molecules (Corliss et al. 1979; Edomond et al. 1982; Ferris 1992; Shock 1996; Russell and Hall 1997). Several experiments have already attempted to simulate submarine hydrothermal environments (Matsuno 1997; Imai et al. 1999; Ogata et al. 2000). Other oceanic environments have also been implicated as "the realms of Archaean life" (Nisbet 2000). However, in principle, those places are chemically and thermodynamically inadequate for peptide formation, i.e. dehydration polymerization, as indicated by many studies (Miller and Bada 1988; Shock 1992; Qian et al. 1993).

The grain-boundary spaces of deep sediments, during diagenesis in the early history of the Earth, have been proposed recently as a chemically and geologically viable place for abiotic polymerization (Nakazawa et al. 1993; Nakazawa 2006). Amino acids would undergo dehydration because of the elevated temperature and pressure if the mixture were buried in sediments. Deep sedimentary environments are more ideal for dehydration reactions of amino acids because free water is easily consumed by formation of clay and pore spaces are essentially under-saturated with free water. Although this might seem geologically reasonable, no experimental study has verified the influence of pressure on peptide formation.

Powdered glycine was encapsulated and treated under various pressures at 150°C, assuming the deep sediment conditions. The experimental process might be too simple for simulating the entire process of sedimentation and dehydration of organic molecules, but the occurrence of polymerization of amino acids in deep sediments can be investigated as a first approximation.

Experimental

High-pressure experiments were carried out in a test-tube-type autoclave using gold (99.95 purity) tubes of 25 mm length and 5.5 mm diameter. The gold tubes were cleaned by 10% HNO_3 solution, washed with ultra-pure water and annealed at 900°C for 5 h before use. Powdered glycine (100 mg, 99.9%; Wako Pure Chemical Industries Ltd.) was encapsulated within the gold tube and treated under pressures of 5–100 MPa at 150°C for 1–32 days. The pressure was applied first and the temperature was then raised to 150°C. Accuracies in temperature and pressure measurements were respectively within ±0.5°C and ±0.5 MPa. After the experiment was completed, the gold tube was quenched in water.

After treatment, the experimental products were removed from the gold tubes and stored in a refrigerator for analyses. The products showed variation in their color from dark brown to light yellow, depending on the applied pressure.

A portion of the sample (10 mg) was dissolved in 1 ml ultra-pure water and centrifuged at 15,000 rpm for 30 min. The sample was analyzed using a visible spectrometer (VIS, PD-303; APEL) at wavelengths of 380–780 nm. The solution was dried, mixed with KBr, pressed to form a thin pellet, and analyzed using a Fourier transform infrared spectrometer (FTIR, MFT-2000; Jasco Inc.).

Another portion of the sample (10 mg) was dissolved in 1 ml aqueous solution of HCl (0.02 N). Its glycine concentration was determined using a high performance liquid chromatograph (HPLC, L-7100 system with a L-7485 fluorometric detector; Hitachi Ltd.) equipped with a post-column of *o*-phthalaldehyde (OPA) derivative. The experimental conditions were as follows: the column was of a cation exchange type (3 μm; 4.0×150 mm, #2619 PH; Hitachi Ltd.); the injection volume was 10 μl; gradient eluents were A (0.07 M sodium citrate perchloric acids, pH 3.2 with 7% ethanol) and B (0.2 M sodium citrate boric acid-NaOH, pH 10); the flow rate was 0.3 ml/min; the column temperature was 60°C; and

the detector was a fluorometric detector (excited wavelength, 355 nm and emission wavelength, 435 nm).

For analyses of oligo-glycines smaller than the 6-mer, another portion of the experimental product (10 mg) was dissolved in 1 ml eluent and analyzed by reverse-phase HPLC (L-7100 system with a L-7455 photograph diode array detector; Hitachi Ltd.). The column was a reverse-phase type (5 μm; 4.6×250 mm, Hydrosphere ODS; YMC Co. Ltd.). The injection volume was 20 μl. The eluent was 10 mM $C_6H_{13}SO_3Na$ solution adjusted to pH 2.5 using H_3PO_4 (Bujdák and Rode 1999). The flow rate was 1.0 ml/min. The column temperature was 25°C, and the detector was a diode array detector at 200 nm. All chromatogram fractions were confirmed through comparison with standard oligoglycines from a 2-mer to 6-mer (99%; Sigma Chemical Co.).

For the investigation of longer oligoglycines than the 7-mer, another portion of the product (10 mg) was dissolved in 1 ml eluent and analyzed using a liquid chromatograph/mass spectrometer, LC/MS (JMS-LCmate; JEOL). The experimental conditions were the following: the column was reversed-phase type (3 μm; 2.0×200 mm; Hydrosphere ODS; YMC Co. Ltd.); the injection volume was 20 μl; the eluent was 5 mM $C_5FC_{11}OOH$ solution (Pearson and McCroskey 1996); the flow rate was 0.2 ml/min; and the column temperature was 25°C. Oligoglycines were detected in the selected ion recording (SIR) mode.

Results

Formation of Melanoidins

Spectrometric analyses on the products dissolved in water were carried out. The results showed continuous absorption at shorter wavelengths (from blue to violet) and negligible absorption at longer wavelengths (>500 nm: Fig. 1). Those results suggest the formation of melanoidins, which result from the reaction between amino acids and aldehydes via a Maillard reaction (Browning reaction). To confirm the melanoidin formation, the supernatant was dried and analyzed using FTIR, which showed absorption bands at 3,080 cm^{-1} (stretching vibration

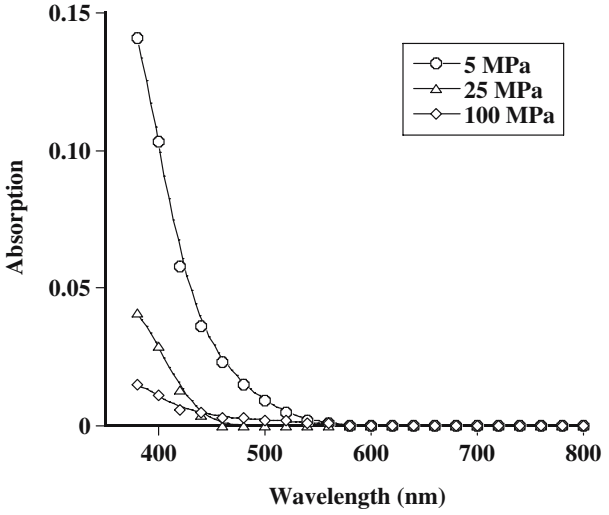

Fig. 1 VIS absorption spectra of products treated at pressures of 5–100 MPa at 150°C for 8 days

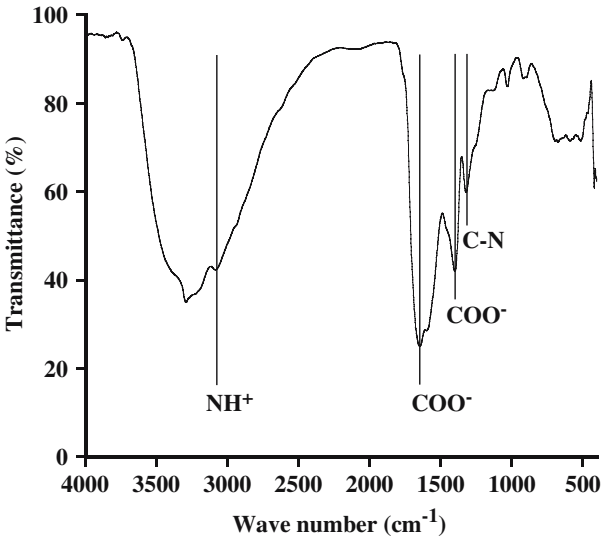

Fig. 2 FTIR spectrum of the product treated at 5 MPa at 150°C for 8 days

of N–H bond), 1,400 cm^{-1} (asymmetric stretching vibration of O–C–O group), and at 1,250 cm^{-1} (stretching vibration of C–N bond, Fig. 2). This IR spectrum corresponds well with those reported from previous studies (Rubinsztain et al. 1986). The formation of melanoidins indicates the partial oxidation and dehydrogenation of glycine.

The pressure-dependence of the formation of melanoidins was investigated using spectrometric measurements at 380 nm (Fig. 3). The sample was treated at 5–100 MPa at 150°C for 8 days. The absorbance intensities of Fig. 3 corresponded to the amount of melanoidins. Correlation between pressure and amount of melanoidins is clear, indicating that higher pressure inhibits further formation of melanoidins.

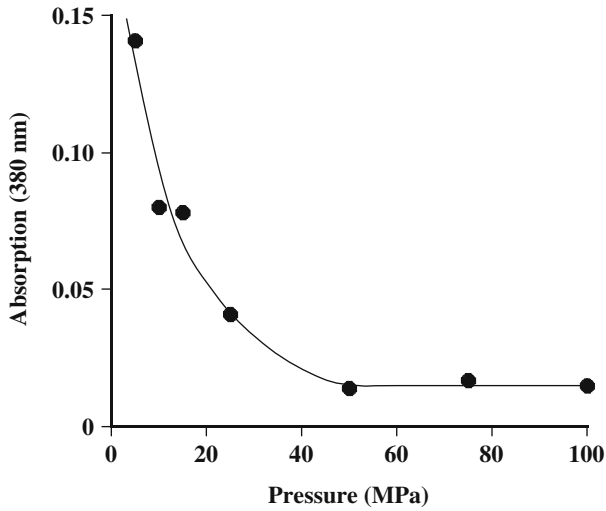

Fig. 3 Pressure-dependence of relative concentration of melanoidins, as indicated by visible light absorbance at 380 nm. Samples were prepared at various pressures at 150°C for 8 days

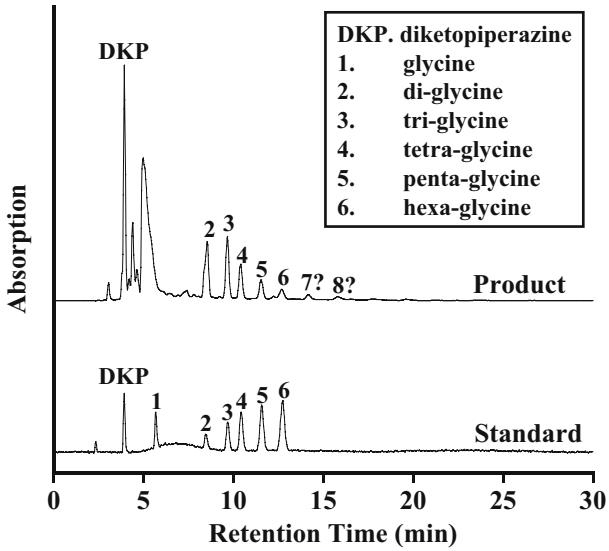

Fig. 4 HPLC chromatogram of the products treated at 100 MPa at 150°C for 8 days

Formation of Glycine Oligomers

Figure 4 shows an example of a HPLC chromatogram of the product treated under conditions of 100 MPa at 150°C for 8 days. Oligoglycines from 2-mer to 6-mer were identified clearly along with diketopiperazine (DKP). Longer oligomers up to 9-mer and possibly 10-mer were also detected using LC/MS in the same product, indicating that the glycine polymerizes simultaneously and directly into such oligomers of different lengths (Fig. 5).

Time-Dependence of the Yields of Oligoglycines

The time-dependence of yields of oligoglycines is shown in Fig. 6 for those products that were treated at 150°C and under 100 MPa for durations of 1–32 days. The yield is defined as the molar ratio (%) of the oligoglycine to that of initial mole of glycine. The contents of the residual glycine monomer are also plotted (%) in Fig. 6. It is readily apparent that (1)

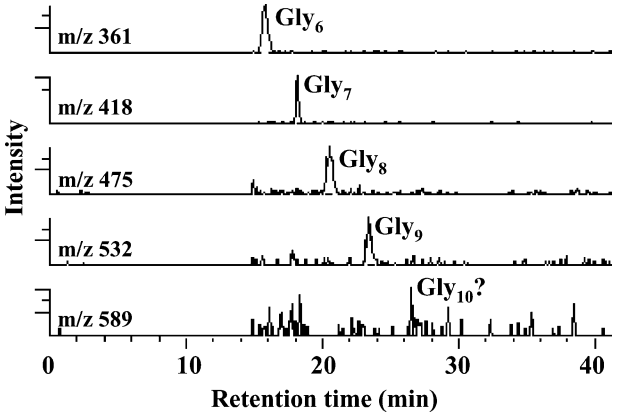

Fig. 5 Selected ion recording (SIR) chromatograms of glycine oligomers in those products treated at 100 MPa and 150°C for 8 days. Gly_i denotes oligoglycines

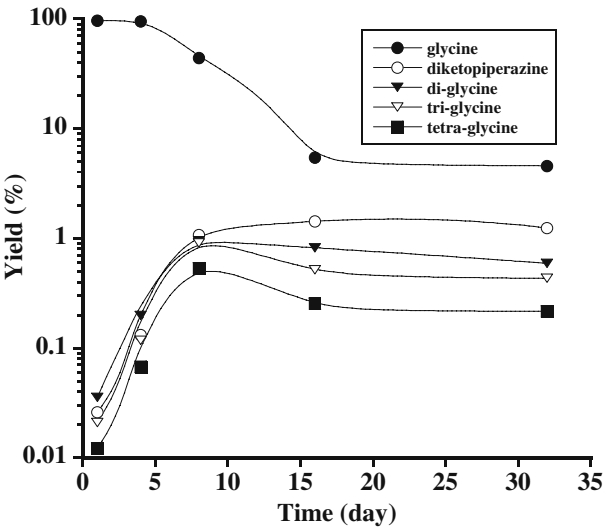

Fig. 6 Yields of glycine oligomers produced at 150°C and 100 MPa with various duration times of 1–32 days. Yields of penta-glycine and hexa-glycine are not shown here because of their inaccurate quantification

the yields for oligomers were in the order of oligomerization from 2-mer to 6-mer for all runs and (2) that the oligomerization progressed for the first 8 days and showed no substantial change afterwards.

Pressure-Dependence of Oligomerization

Pressure-dependence of the yields of oligoglycines is shown in Fig. 7 for those products treated at 150°C at various pressures of 5–100 MPa for 8 days. The figure shows that (1) the yields of the oligomers are inversely related to molecular length from 2-mer to 6-mer for all runs under various pressures, and (2) the yields increase with pressure up to 25 MPa and

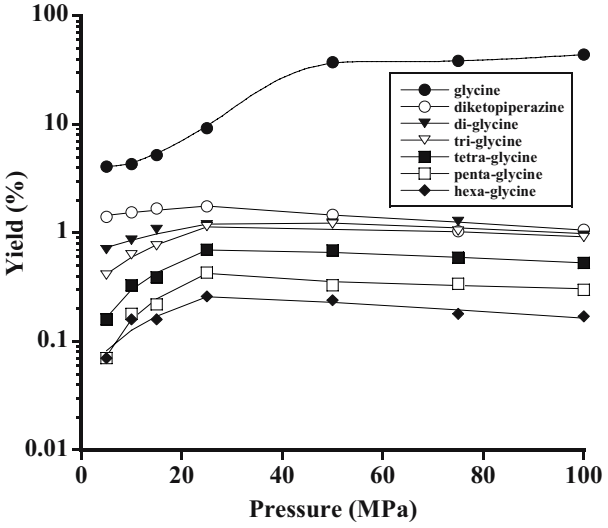

Fig. 7 Yields of glycine oligomers produced at 5–100 MPa and 150°C for 8 days

show a slight decrease with pressure thereafter. The contents of residual glycine are also plotted in the same figure, showing pressure-dependence.

Discussion

Formation of Oligomers Under Non-Aqueous Conditions and Pressure Effects

Results of recent studies suggest that polymerization of at least 10 amino acids is necessary to form the simplest protein (Honda et al. 2004). On the other hand, the synthesis of such long polymers has not been successful in aqueous solutions that resemble oceanic environments. In principle, oceanic environments are chemically and thermodynamically inadequate for peptide formation because the peptide formation reaction is a dehydration reaction. The present experiment demonstrates that peptide oligomers longer than 6-mer can be formed easily in a simple system under non-aqueous conditions, even in the absence of a catalyst.

In addition to aquatic conditions, temperatures greater than 100°C are believed to be unsuitable for peptide formation because of the increase of molar volume of peptide with temperature: increase of the molar volume results in a thermodynamically unfavorable reaction, thereby inhibiting peptide formation. Our experiments indicate that high-pressure conditions, which reduce the partial volume of peptide, overcome the temperature effect on peptide formation.

Another effect of temperature is often the formation of melanoidins: peptide and/or amino acid are converted to melanoidins under high-temperature conditions during reaction with other substrates. Both visible and infrared spectroscopic analyses indicate that pressure inhibits the formation of melanoidins. Consequently, the residual glycine polymerizes to form various oligomers (Figs. 3 and 7).

These results are the first to demonstrate that pressure and dryness are the keys for polymerization of amino acids. Previous investigators reported that the pressure greatly expands the temperature stability of many biomolecules and the pressure appears to favor certain reactions (Ulmer 2000; Benito et al. 1999; Allen and Bartlett 2000). The results of the present study are consistent with those published results.

Previous investigators have also proposed that peptides can be elongated easily up to 11-mer (Maurel and Orgel 2000) or 4,000 Da (Ito et al. 1990; Yanagawa et al. 1990), especially under heated and anhydrous conditions. Although our experiments are conceptually similar to previous ones, the major difference is in terms of the starting materials used. Our experiments are based on the polymerization of free amino acids rather than the polymerization of amides (Ito et al. 1990; Yanagawa et al. 1990) or activated amino acids (Maurel and Orgel 2000). Selection of amino-acid amides or activated amino acids as starting materials for the synthesis experiments provide a relatively easier path towards the formation of oligopeptides, and has been favored by previous investigators. But direct formation of polypeptides from free amino acids has remained a challenge. Our new data demonstrate that the activation of amino acids, or the use of catalysts are not mandatory for the formation of peptides, when taking into account the pressure effects under dry conditions.

Implication of Pre-Biotic Molecular Evolution in Deep Sediments

Experimental conditions of the present study correspond to several-kilometer depths of marine sediments or late diagenetic conditions on a geological scale.

The present results further support the hypothesis that the pre-biotic molecular evolution would more likely have occurred in deep sediments rather than in an oceanic environment (Nakazawa et al. 1993; Nakazawa 2006). This hypothesis is consistent with the thermodynamic calculations of Shock (1992), suggesting that peptides are actually less stable at elevated pressures under aqueous, thus oceanic, conditions and might also agree with the discovery of microbial communities in various crustal environments (Parkes and Maxwell 1993; Wellsbury et al. 1997; Chapelle 1987; Lovely 1990; Krumholtz 1997; Pedersen 1997; Stevens and McKinley 1995).

The relative yields for oligomers decrease in the order of the molecular length from 2-mer to 6-mer (Figs. 6 and 7). The measured yield, e.g., of about 2.5% for the products at 150°C under 100 MPa for 8 days, might be sufficiently high for further evolution, when the rate is extrapolated over a longer time scale.

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