Glycine Oligomerization by Pulsed Discharge Plasma over Aqueous Solution under Atmospheric Pressure

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Abstract: Chemical reactions of amino acids induced by discharge plasma are important for understanding the mechanism of biological effects of discharge plasma in biomedical applications. In this study, we generated a nano-second pulsed discharge plasma under atmospheric pressure over an aqueous solution containing glycine. The reaction products after the pulsed discharge plasma treatments were analyzed by high-performance liquid chromatography and matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy. The oligomerization reaction of glycine was induced in aqueous solution and produced glycine oligomers at the beginning of the discharge plasma. However, the glycine oligomers were decomposed into products with low molecular weight by excessive pulsed discharge plasma. According to comparative experiments, physical force of the plasma is believed to induce the glycine reaction. Moreover, the reactions depended on the pH, but not the conductivity, of the glycine solution. Glycine in aqueous solution was reacted by the discharge plasma only at neutral pH because the reaction proceeded only when glycine ions were in the zwitterionic state. Anions and cations of glycine reacted very little under the discharge plasma.

Keywords: amino acids; pulsed discharge plasma; oligomerization; glycine; protein

1. Introduction

Discharge plasma in contact with an aqueous solution has great potential for various applications because it induces chemical reactions in the solution [1–4]. When discharge plasma is generated through a metal electrode in the gas phase over an aqueous solution, active species are produced near the surface of the water and affect the solution. Oxidation reactions are usually induced when a discharge plasma interacts with water [5–7] because oxidation species such as OH radicals and hydrogen peroxide, which have strong oxidizing power [8–10], are produced by dissociation of water molecules by electric discharge. The active species include hydrogen, and oxygen, along with other oxidative species such as hydroperoxyl radicals and reductive species such as hydrogen radicals, superoxide radical anions, and aqueous electrons were also generated. When these active species formed by discharge plasma in the gas phase, they may dissolve into the water solution and form hydrogen peroxide in the solution. Next, these active species formed at the gas-liquid interface assisted the rapid decomposition of the organic material [5–10].

Recently, plasma irradiation for biological materials by discharge plasma has recently been actively investigated for applications in medical science [11–13]. They reported that discharge plasma caused inactivation of bacteria [14], blood coagulation [15], and DNA damage [16]. On the micro level, biological materials are composed mostly of proteins. Proteins are made up of 20 amino acids that
combine by peptide bonds. Because proteins and amino acids strongly influence biological reactions, an understanding of chemical reactions of amino acids under a discharge plasma is important for determining the mechanism of the biological effects of the discharge plasma. Discharge plasma reportedly induces chemical modifications of peptides and amino acids such as phenylalanine [17], methionine [18], and glutathione [19]. However, little is known about the reactions of amino acids with low molecular weight under a discharge plasma.

Reactions related to amino acids are also studied in the field of prebiotic synthesis. Several amino acids could be produced by electric discharge plasma in a mixed gas [20–22]. It has also been confirmed that amino acids were produced from cyanide by a discharge plasma [23,24]. Such studies implied that oligomerization of amino acids was induced by electric discharge plasma, but it has not been examined in previous works. On the other hand, proton irradiation, X-ray radiation, or UV irradiation produced glycine oligomers [25–27]. In my opinion, electric discharge plasma also induces oligomerization of amino acids.

We focused on the reactions of glycine (Gly), which is the simplest amino acid, in water under an electric discharge plasma. A nano-second pulsed discharge plasma was generated over a glycine solution under atmospheric argon and induced reactions of glycine in aqueous solution. The nano-second pulsed power provides fast pulse rise time and short pulse width, leading to non-thermal plasma with high energy efficiency [28,29]. The products after the discharge plasma treatment were analyzed and examined to clarify the reactions of glycine induced by the discharge plasma.

2. Materials and Methods

2.1. Materials

Glycine (C\(_2\)H\(_5\)NO\(_2\), 98.5%) as a starting material was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. It was dissolved in distilled water which obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Pentadecafluorooctanic acid (C\(_8\)HF\(_{15}\)O\(_2\), 98.0%) from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan was used as an analytical solvent. Other used chemicals such as 2,5-dihydroxybenzoic acid (C\(_7\)H\(_6\)O\(_4\), 99.5%), hydrochloric acid (HCl, 35.0–37.0%), sodium chloride (NaCl, 99.5%) and sodium hydroxide (NaOH, 97.0%) were bought from Wako Pure Chemical Industries, Ltd., Osaka, Japan. All chemicals were used without further purification.

2.2. Experimental Setup and Procedure

A schematic diagram of the experimental apparatus is shown in Figure 1. Experiments were conducted using a batch-type reactor made of stainless steel (SUS-316, AKICO Co., Tokyo, Japan). A cylindrical copper electrode 1.00 mm in diameter was set 3 mm above the aqueous solution surface. A positive nano-second pulsed voltage was applied to the copper electrode by a pulsed power supply (MPC2000S, Suematsu Electronics Co. Ltd., Kumamoto, Japan) shown in Figure 2. The pulse rise and duration time was 66 and 100 nanoseconds, respectively. Four pulsed voltage per second was provided to the center of the copper electrode, which was covered by polyether ether ketone (PEEK). The aqueous solution (7.0 mL) with 10 g/L of dissolved glycine was placed in a reactor. It should be noted that the various initial concentration of glycine as a starting material was not subjected during the course of an experiment. The reactor was grounded, and its interior was purged by argon gas at atmospheric pressure. Discharge plasma was generated between the copper electrode and the aqueous solution surface. The discharge voltage and current were measured using a high-voltage probe (EP50K, Pulse Electronic Engineering Co. Ltd., Chiba, Japan) and a current transformer (4997, Pearson Electronics Inc., Palo Alto, CA, USA), which were connected to a digital oscilloscope (TDS2024C, Tektronix Inc., Beaverton, OR, USA).
The glycine concentration after treatment by the discharge plasma was examined by high-performance liquid chromatography with a UV detector (HPLC, JASCO Co., Tokyo, Japan) under the following conditions: column, Cosmosil ODS-C18 (Nakalai Inc., Tokyo, Japan); eluent, 0.5 mM pentadecafluorooctanic acid; flow rate, 1.0 mL/min; temperature, 38 °C; measurement wavelength 210 nm [30,31]. Additionally, products in the aqueous solution were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF-MS, Ultraflex III, Bruker Daltonics Inc., Bremen, Germany) with 2,5-dihydroxybenzoic acid as the matrix [32]. The conductivity and pH of the glycine solution were adjusted by the addition of HCl, NaCl, and NaOH to the aqueous solution. Liquid products after the discharge plasma treatment were neutralized at pH 7.0, and processed desalting before the discharge plasma for avoiding the effect of sodium ion on analysis results.

3. Results and Discussion

Figure 3 shows the conversion of glycine as analyzed by HPLC after treatment by the pulsed discharge plasma at applied voltages of 12.4, 15.5 and 18.6 kV. Pulsed discharge plasma was generated repeatedly over a glycine solution under atmospheric argon. This pulsed discharge plasma seemed to have ability to convert glycine in the aqueous solution. Conversion of glycine increased immediately in the initial stage of the pulsed discharge plasma. Subsequently, it decreased after 1000 discharge pulses at any voltage. Then, it increased gradually with increasing pulse discharges. Glycine in aqueous solution reacted easily under the discharge plasma at a high voltage. It is expected that acetic acid and ammonia are produced by decomposing of glycine molecule. However, according to chromatogram of the product by the treatment of 1000 discharge plasma pulses at 18.6 kV shown in Figure 4, acetic acid
did not detected. Namely, glycine monomer did not decomposed directly by the discharge plasma, but reacted with each other.

Figure 3. Conversion of glycine after treatment by pulsed discharge plasma at voltages of 12.4, 15.5 and 18.6 kV.

Figure 4. Chromatogram of the product by the treatment of 1000 discharge plasma pulses at 18.6 kV.

To identify the reaction products after the pulsed discharge plasma treatment, the products in the aqueous solution were analyzed by MALDI-TOF-MS, which yielded their mass spectra. Mass spectra of the raw material (glycine) and the products after pulsed discharge plasma treatment at 18.6 kV are shown in Figure 5. The molecular weight of glycine is 75, but glycine was detected m/z 38 in the mass spectrum (Figure 5a). The mass spectra of divalent ions generally indicate half of the molecular weight [33]. Thus, glycine is believed to be detected as a divalent ion because it had not only an amino group acting as a cation but also a carboxylic group acting as an anion.

After the discharge plasma treatment, high-molecular-weight products were produced from glycine, as shown in Figure 5b–e. For the products observed after 1000 discharge plasma pulses in Figure 5c, the peak intervals were m/z 28. Considering reactions between two glycine molecules, the ammonium ion (–NH$_3^+$) of one glycine molecule could react with the carboxylic ion (–COO$^-$) of the other glycine molecule and form glycine dimer (diglycine). In this case, the molecular weight of diglycine is 132.
Figure 5. Mass spectra of (a) raw material and products after (b) 500, (c) 1000, (d) 3000, and (e) 5000 discharge plasma pulses at 18.6 kV.

Thus, the peak interval of the mass spectrum is increased by m/z 28 by every combination of glycine molecules when glycine oligomers are detected as divalent ions. In other words, the peak intervals in Figure 5c coincide with the molecular weight of glycine. Therefore, the mass spectrum in Figure 5c implies that the pulsed discharge plasma induced glycine oligomerization, and the products after the pulsed discharge plasma were oligomers of glycine. Because the synthesis of glycine oligomers is a dehydration reaction, oligomer synthesis from amino acids in water is a difficult reaction thermodynamically and kinetically [34,35]. Researchers have indicated that oligomerization of glycine occurred under sudden changes in the conditions, such as quenching [36] or the application of high pressure [37]. In our experiments, the momentary non-equilibrium state after a pulsed discharge
plasma was thought to enable the dehydration reaction in water, leading to advance of the glycine oligomerization rapidly.

In contrast, the products detected after 3000 and 5000 discharge plasma pulses (Figure 5d,e) had lower molecular weight than those observed after 1000 discharge plasma pulses (Figure 5c). The peak of glycine monomer (m/z 28) increased after 3000 discharge plasma pulses compared to 1000 discharge plasma pulses. It indicates that oligomers were hydrolyzed into glycine monomers. This depressed the glycine conversion after 3000 discharge plasma pulses, as shown in Figure 3. Focused on mass spectra of glycine oligomers, intensity of m/z 368 diminished by the additional discharge plasma pulses over 1000 discharge plasma pulses. Additionally, a lot of peaks were observed in a range between m/z 200 and 400 after 5000 discharge plasma pulses in Figure 5e, but the peak intervals did not coincide with the molecular weight of glycine. It meant that glycine oligomers decomposed into low-molecular-weight products by excessive discharge plasma. Thus, the dominant reaction varied depending on the treatment time. Oligomerization of glycine was induced at the beginning of the discharge plasma, but decomposition of oligomer was the dominant reaction with repeated discharge plasma pulses.

Discharge plasma generates not only active species, but also UV light and physical force. To determine which factors contributed glycine reactions, comparative experiments were carried out. Table 1 indicates the conversions of glycine treated by several comparative experiments. Glycine was hardly reacted by the treatments of heating the solution at 80 °C and irradiation of UV light (PU-21, TOPCON Tech. Co., Tokyo, Japan). OH radicals, which was produced by Fenton reaction using FeSO₄ and H₂O₂, converted glycine slightly, but it was not a main factor of glycine reactions by discharge plasma. Therefore, it is possible that reactions of glycine by discharge plasma are owing to physical force of the plasma.

Table 1. Conversions of glycine treated by several comparative experiments.

| Experiments               | Treatment Time | Conversion of Glycine |
|---------------------------|----------------|-----------------------|
| Discharge plasma (18.6 kV)| 250 s          | 73.1%                 |
| Heating (80 °C)           | 24 h           | 0.0%                  |
| UV irradiation            | 24 h           | 0.4%                  |
| OH radical (Fenton reaction)| 10 min       | 3.4%                  |

The properties of the aqueous solutions affected the reactions induced by the pulsed discharge plasma [38,39]. We examined the effects of the conductivity and pH of the aqueous solution on the reactions of glycine. The conductivity of the 10 g/L glycine solution was 0.75 µS/cm before the discharge plasma. Sodium chloride was added to the aqueous solution to change the conductivity in a range up to 2.6 mS/cm. Figure 6 shows the effect of the conductivity of the aqueous solution on glycine conversion after 1000 discharge plasma pulses at 18.6 kV. The conductivity of the solution had little effect on the glycine reactions. The aqueous solution with high conductivity meant that current flowed easily in the solution. Current values during the discharge influenced on plasma properties such as plasma emission [40]. In other words, different plasma properties should be observed when glycine solutions have different conductivities. However, these properties did not affect the glycine reactions because they did not depend on conductivity of the solution.

To investigate the effect of the glycine molecular state on the reactions in aqueous solution under the discharge plasma, pulsed discharge plasma was generated over glycine solutions at a wide pH range. The pH of the initial solution (10 g/L glycine) was pH 6.4, and the pH was adjusted by the addition of HCl and NaOH. The effect of the pH of the aqueous solution on glycine conversion, as analyzed by HPLC, is shown in Figure 7. Glycine in the aqueous solution was converted by the discharge plasma only at neutral pH owing to the state of glycine in water. In aqueous solution, glycine contains ammonium and carboxylate groups and has three ionic states: cationic (Gly⁺), zwitterionic (Gly±), and anionic (Gly⁻), and behave as an ammonium ion in aqueous solution at pH values below
2.4 and as a carboxylate ion at pH values above 9.8. At pH values between 2.4 and 9.8, glycine has both ammonium and carboxylate ions. The abundance ratios of glycine ions (Gly\(^+\), Gly\(^\pm\), and Gly\(^-\) ions) in aqueous solution as a function of the pH were calculated [41] and shown in Figure 8. The pH dependence of the abundance ratio of Gly\(^\pm\) shown in Figure 8 was correlated with that of glycine conversion shown in Figure 7. High glycine conversion was obtained during the discharge plasma when almost all the glycine was Gly\(^\pm\) ions in aqueous solution. In contrast, it was difficult for glycine reactions to occur in the presence of Gly\(^+\) and Gly\(^-\) ions. In other words, Gly\(^\pm\) ions of glycine reacted under the pulsed discharge plasma. Thus, it was implied that glycine molecules reacted between ammonium ion of one glycine molecule and carboxyl ion of other glycine molecule, resulted in oligomerization. In the case of anionic or cationic state, glycine molecules were difficult to react each other because either ammonium or carboxyl ion did not behave as ionic state.

![Figure 6. Effect of conductivity on glycine conversion in aqueous solution after 1000 discharge plasma pulses at 18.6 kV.](image)

![Figure 7. Effect of pH on glycine conversion in aqueous solution after 1000 discharge plasma pulses at 18.6 kV.](image)

Considering glycine conversion shown in Figure 7, anions (Gly\(^-\)) reacted slightly (about 20% conversion shown in Figure 7) at basic pH conditions over pH 9, although cations (Gly\(^+\)) hardly reacted under acidic conditions. In general, glycine ions in the anionic state have high reactivity for oligomerization [42]. Thus, the pulsed discharge plasma induced higher glycine conversion in the aqueous solution including Gly\(^-\) than in the solution including Gly\(^+\). Figure 9 shows mass spectra of the products by the discharge plasma at 18.6 kV for the glycine solution at several pH values. The oligomer produced by the discharge plasma over the glycine solution at acidic and basic condition in Figure 9b,d
had lower oligomerization degree than at neutral condition in Figure 9c. Additionally, small oligomers were produced by the discharge plasma at basic condition compared to acidic condition.

Figure 8. Abundance ratios of Gly+, Gly±, and Gly− ions as a function of the pH.

Figure 9. Mass spectra of (a) raw material and products after discharge plasma pulses at 18.6 kV for glycine solution at (b) pH 1.2, (c) pH 6.4, and (d) pH 10.4.
4. Conclusions

Pulsed discharge plasma was generated at an argon/aqueous solution interface by a DC power supply. Glycine in aqueous solution reacted under the discharge plasma. Oligomerization of glycine was induced at the beginning of the pulsed discharge plasma, and oligomers of glycine were produced. However, the dominant reaction gradually shifted to oxidation as the number of discharge plasma pulses increased. These reactions were assumed to be caused by physical force of the plasma. The oligomerization reaction of glycine was not affected by the conductivity of the aqueous solution but depended on the pH of the solution. Zwitterions of glycine (Gly±) in aqueous solution reacted under the discharge plasma and produced oligomers of glycine. In contrast, it was difficult to induce reactions of glycine anions (Gly–) and cations (Gly+) under the discharge plasma.

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