Low-temperature plasma-induced regulated dimerization of (-)-epigallocatechin gallate (EGCG) in methanolic solution

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Abstract: (−)-Epigallocatechin gallate (EGCG), which is the one of natural polyphenols found in the leaves of green tea (Camellia sinesis), is generally unstable under oxidative conditions. The regulated dimerization of EGCG induced by low-temperature plasma treatment in MeOH solution was firstly reported without changes in its stereochemistry. In this study, we report the influence and correlation of plasma treatment for efficient dimerization of EGCG, and mechanism of dimerization induced by plasma is described using a plausible pathway.

Keywords: Low-temperature plasma, DBD, EGCG, dimerization, reactive species.

1. Introduction

Epigallocatechin gallate (EGCG) is known to have potent pharmacological effects responsible, especially antiangiogenic, antibacterial, antidiabetic, and anti-aging, hypocholesterolemic properties. Many studies on the effect of its structural transformation have been conducted using various treatment methods because ECGC is very unstable under oxidative conditions. Plasma technology has been widely used in various fields including plasma processing in the semiconductor and display industries, surface modification, nanoparticle synthesis, gasification, and catalysis. Recently, research on the use of plasma in the bio field is being actively conducted. In this study, low-temperature plasma technology has been attempted for structural modification of natural compound, and EGCG dimerization was successfully achieved without changes in stereochemistry as shown in Fig. 1 [1, 2].

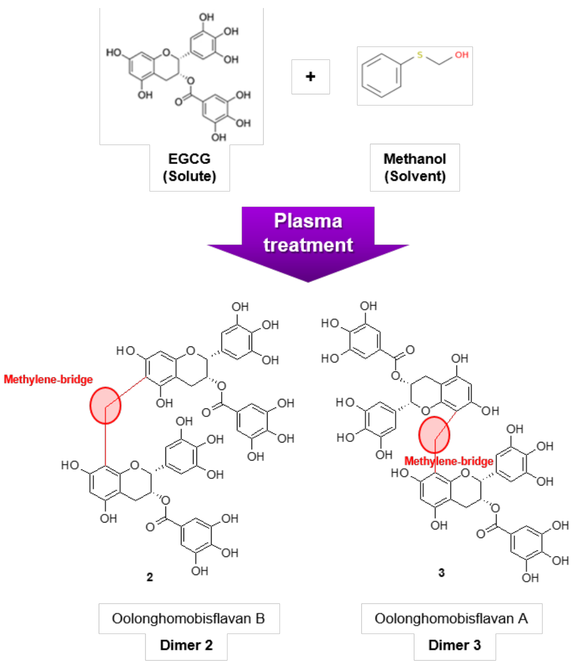

Fig. 1. Chemical structures of new products generated from EGCG by the plasma treatment.

2. Methods

For the treatment of the natural product, Low-temperature atmospheric pressure plasma was generated at four surface dielectric barrier discharge (sDBD) electrodes. Each electrode One electrode consists of two metal sheets attached to both sides of am alumina plate (100 × 100 ×0.7 mm^3), and one metal sheet has a rounded square open pattern, and the other has no open pattern. Both sheets are composed of a nickel-chromium alloy to prevent oxidation by the highly reactive oxidizing species used during the plasma operation. The processing chamber made of Teflon to ensure low chemical reactivity. The ambient air was used as the discharge gas, and the gas inside the processing chamber was circulated in a closed state during processing.

Various measuring instruments, such as high voltage prove, current probe, capacitor, oscilloscope, gas analyser, temperature sensor, have been used to check the operation status during the plasma treatment. Figure 1 shows the schematic drawing of the plasma treatment setup. Plasma treatment at atmospheric pressure can affect the sample by chemical effects by reactive species and by physical effects by UV, electric field, and heat. Structural transformation of the compound was confirmed through HPLC analysis under the three treatment conditions of physical effect, chemical effect, and physiochemical effect. To study the correlation between plasma and structural transformation of a high molecular compound, the production amount of new compounds for each treatment condition was compared.

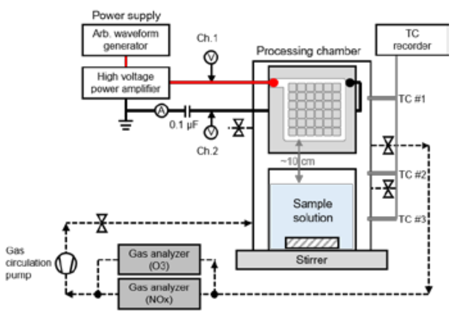

Fig. 2. Schematic drawing of the test setup for the plasma treatment.

3. Results and discussion

Figure 3 shows the content (sum of dimers 2 and 3) of the newly formed compounds produced by the synergy of physical and chemical effects based on direct plasma treatment, chemical effect based on the indirect plasma treatment using only reactive species, physical effect based on UV light under identical operating conditions.

For the synergy of physical and chemical effects, the EGCG was directly treated with plasma, and the content of newly generated compounds was highest at about 172 mg/g. When the sample was treated with only RONS for the chemical effect, the newly formed compound amounted to approximately 136 mg/g. In the case of physical effect based on UV light, no major compound was generated when the samples were treated while excluding RONS. The concentrations of ozone according to the plasma treatment methods were measured and found to be 39 ppm, 30 ppm, and 307 ppm, respectively. However, the concentrations of NO_x did not measure. During the discharge, the maximum gas temperatures located next to the sample were 25.3 °C, 22.8 °C, and 23.7 °C, respectively, and the measured power consumption in all three cases was approximately 20 W. The major factors of the plasma treatment for regulated dimerization were identified as reactive oxygen and nitrogen species, especially ozone.

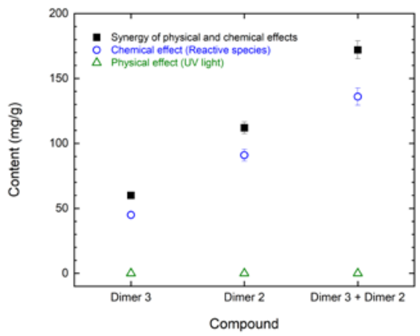

Fig. 3. Contents of individual components generated by the plasma treatment.

To investigate the mechanism of EGCG dimerization induced by plasma, the literature on the relationship between EGCG and oolonghomobisflavans A and B was studied based on the present work. According to findings by literature, flavan 3-ols analogue is generated as oligomers through a methylene-bridge by formaldehyde, and ozone and methanol reacted to form formaldehyde. We attempted to identify whether formaldehyde was generated in the sample treated by the plasma, and confirmed using GC–MS/MS (Thermo Scientific TRACE 1300 Series) that formaldehyde was indeed generated.

Based on these results, a plausible pathway of the EGCG dimerization induced by the plasma in a methanol was deduced as shown in Fig. 4. The mechanism of EGCG dimerization by plasma as follows: ozone generated by plasma reacts with methanol to form formaldehyde, after which EGCG is generated as oolonghomobisflavans A and B through a methylene-bridge stemming from the formaldehyde reaction.

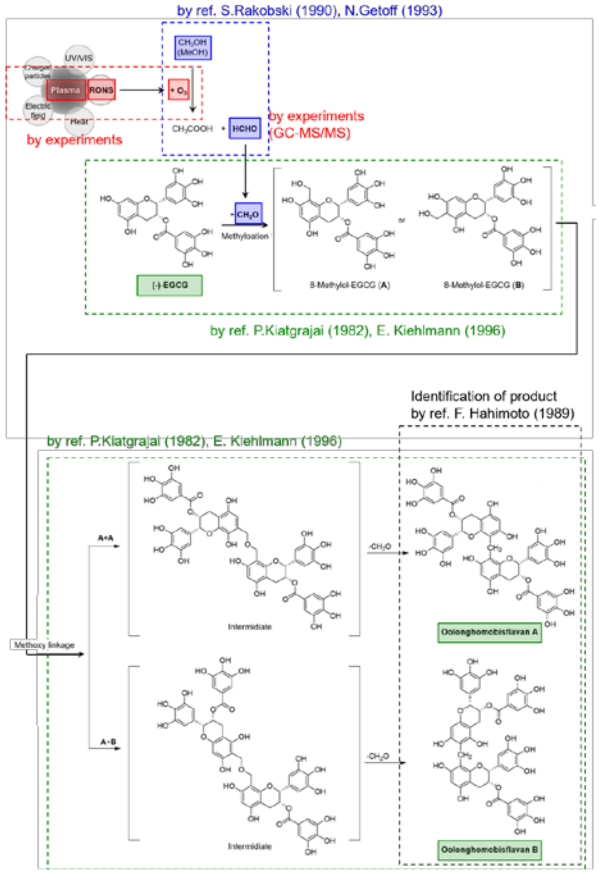

Fig. 4. A plausible pathway of the EGCG dimerization induced by plasma in a methanol.

4. Conclusion

The main factors of plasma were studied to understand the plasma effects on regulated dimerization of EGCG by varying the plasma treatment method. Ozone is the major factor in plasma-induced dimerization, and there is an operation window of ozone concentration according to the operating conditions for efficient dimerization. Based on these results and literature, a plausible pathway of EGCG dimerization was deduced, and the mechanism of EGCG dimerization induced by plasma was proposed using this pathway.

5. References

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6. Acknowledgements

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