Chemical modification of tyrosine by atmospheric pressure plasma exposure

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Abstract: Chemical modifications of tyrosine by He+H_2O plasma plume and dry air plasma effluent exposure were investigated using absorption spectroscopy. Unlike He+H_2O plasma, observed absorption spectrum of tyrosine solution after the air plasma effluent treatment has a well-defined peak around 475 nm, in agreement with previously-reported spectrum of dopachrome. In addition, 0.82 µmol/min of its production rate was comparable to the transfer rate (1.6 µmol/min) of N_2O_{5gas} estimated from FT-IR analysis, indicating that N_2O_{5gas} may be one of key species in the interesting chemical modification.
Keywords: plasma medicine, dinitrogen pentoxide, tyrosine, gas–liquid interface

1. Introduction

Non-equilibrium atmospheric-pressure plasma (APP), which has a higher electron temperature (~several eV) than gas (ion) temperature, has been of great interest and has been extensively investigated. This allows for a non-equilibrium chemical reaction of O_2 and N_2 in ambient air and uniquely produces multiple reactive products, such as reactive oxygen nitrogen species (OH_{gas}, NO_{gas}, O_{3gas}, N_2O_{5gas}, …). The plasma-induced reactions in the gas phase and on the gas–liquid interface often accompany unexpected chemical processes. Despite many recent reports of innovative applications of non-equilibrium APP [1-7], key species and action mechanism remain unclear in most cases. One of the reasons seems to be due to a lack of fundamental experiment on the interaction of plasma-generated reactive species with biomolecules such as proteins (peptide, amino acids), phospholipids, and enzymes.

The non-equilibrium APP treatment of biomolecules can be achieved by exposing biological fluid solutions containing biomolecules to (a) plasma plume and (b) plasma effluent gas as shown in Fig. 1. In the former case, the solution is exposed to a complex of ultraviolet (UV) light, charged particles, and reactive species, which is likely to generate more reactive products in the liquid phase.

In this study, we have examined the interaction of APP with a tyrosine, which is one of the 20 standard amino acids and reportedly plays important roles in many cellular processes such as phosphorylation. Then, He+H_2O plasma and dry air plasma are employed for the plasma plume and the plasma effluent gas exposure, respectively.

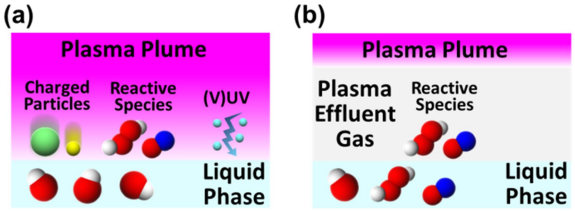


Fig. 1. Treatment methods with non-equilibrium APP. (a) Plasma plume and (b) plasma effluent gas exposure.

2. Experimental Setup and Methods

Figure 2 shows experimental apparatus of DBD plasma jet using (a) He+H_2O and (b) dry air as working gases. Both plasma jets consist of a DBD section, a gas supply, and a high voltage AC power supply.

The He+H_2O plasma jet has an improved affinity for biological fluid solution owing to the increased humidity in the gas phase. Dry He (3 L/min) gas is supplied through a gas washing bottle bubbler, after which the helium can be assumed to be saturated by water vapor (2.6%) at room temperature (295 K). A 200 µL sample of 1 mM Tyrosine / 20 mM phosphate buffer (pH 7.2) was prepared and treated by the He+H_2O plasma plume exposure for a controlled time t_i. Immediately after the exposure, the absorption spectrum of the exposed solution was measured.

In case of air plasma effluent gas exposure, the air plasma effluent gas (16 L/min) is collected at 10 cm downstream with a device that circulate the solution to accumulate the dissolved species from the gas phase (named circulatory reactive species trap (CRT) system) [8]. The circulating water solution in the CRT partly flows through quartz cells for the optical absorption spectroscopy. A 38 mL sample of 1 mM Tyrosine / 20

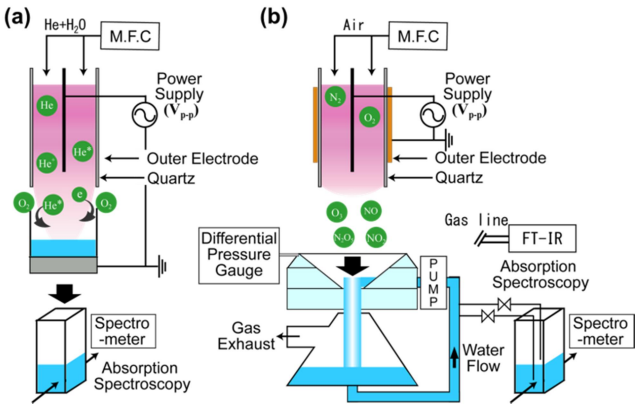
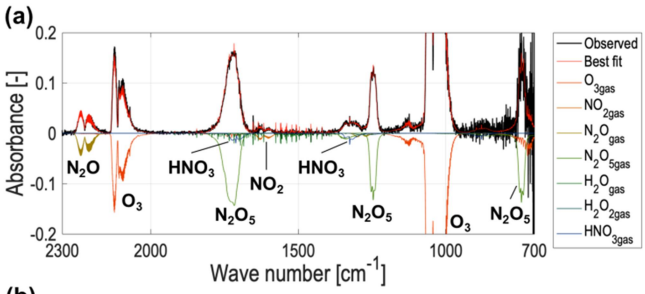


Fig. 2. Experimental apparatus of (a) He+H_2O plasma jet and (b) air plasma jet with a CRT system combining absorption spectroscopy.



(b)

$Species_{gas}$	O_3	NO	NO_2
Density (cm^{-3})	2.4×10^{16}	< LOD (< 6.0×10^{14})	5.4×10^{13}
$Species_{gas}$	N_2O	N_2O_5	HNO_3
Density (cm^{-3})	1.8×10^{14}	4.7×10^{14}	9.6×10^{13}

Fig. 3. (a) FTIR absorbance spectrum of the air plasma effluent. The absorbance spectra of individual species are shown as negative absorbance spectra. (b) Density table of reactive species in the air plasma effluent by our FTIR measurement.

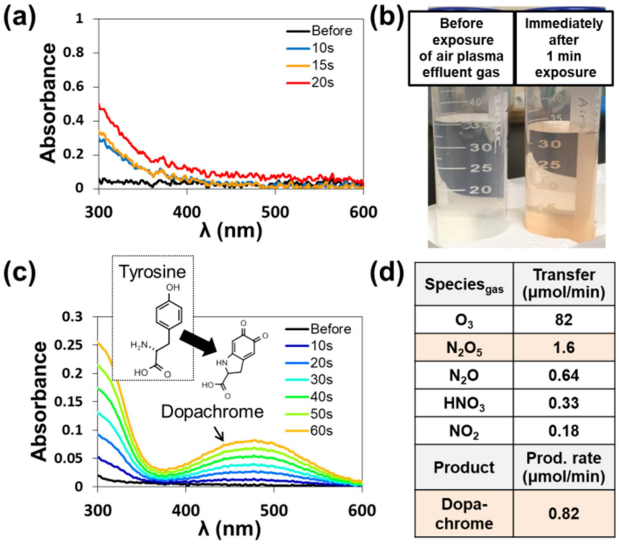
mM phosphate buffer (pH 7.2) was exposed to the air plasma effluent gas in the CRT at a time whose absorption spectra were concurrently measured during the exposure.

The gas phase species in the air plasma effluent has been analyzed with Fourier-transform infrared spectroscopy (FT-IR) as shown in Fig. 3. O_{3gas}, NO_{gas}, NO_{2gas}, N_2O_{gas}, N_2O_{5gas}, HNO_{3gas}, HNO_{4gas}, H_2O_{gas}, H_2O_{2gas}, CO_{gas}, CO_{2gas} were considered for the spectra fitting. The detectable major species were O_{3gas}, NO_{2gas}, N_2O_{gas}, N_2O_{5gas}, HNO_{3gas}.

3. Results and Discussion

Figure 4(a) shows the absorption spectra of tyrosine solution after He+H_2O plasma plume exposure. While pristine tyrosine has relatively sharp rise of the absorption around 275 nm (data not shown), absorption, broadly ranged to 600 nm, increases with the plasma exposure time. Because of the negligible absorbance increase for phosphate buffer solution without tyrosine, the absorbance increase indicates that tyrosine-derived products can be generated by the plasma plume exposure. As previously noted, solution in contact with plasma plume is exposed to a complex of UV light, charged particles, and reactive species. Aqueous reactive species such OH radical generated by these factors are considered to be key factors in the reaction.

On the other hand, the absorption spectrum of tyrosine solution treated with the air plasma effluent [Fig. 4 (c)] differs from that exposed to the He+H_2O plasma plume exposure [Fig. 4 (a)]. As shown in Fig. 4(b), color of the solution was obviously changed by only 1 min treatment of the air plasma effluent. Therefore, a relatively major gaseous species seemed to play key roles in the chemical modification. In addition, the observed absorption spectrum around 475 nm is in good agreement with that of reported dopachrome, an unstable intermediate in



(d)

$Species_{gas}$	Transfer (μmol/min)
O_3	82
N_2O_5	1.6
N_2O	0.64
HNO_3	0.33
NO_2	0.18
Product	Prod. rate (μmol/min)
Dopa-chrome	0.82

Fig. 4. (a) Absorption spectra of tyrosine solution after He+H_2O plasma plume exposure at varying the exposure time. (b) Picture of tyrosine solution before and after the air plasma effluent exposure. (c) Absorption spectra of tyrosine solution measured by a CRT system combining absorption spectroscopy. The time indicates elapsed time after the start of exposure. (d) Estimated transfer rate of gaseous species into the solution and calculated production rate of dopachrome.

tyrosine metabolism. This dopachrome has relatively high molar extinction coefficient of approximately 3.7 $mM^{-1} \cdot cm^{-1}$ at 475 nm [9]. Thus, the production rate of dopachrome was calculated to be approximately 0.82 μmol/min, which was comparable to the transfer rate of N_2O_{5gas} estimated from density by FT-IR analysis with considering dilution in a round free jet stream. Therefore, the observed chemical conversion of tyrosine to dopachrome, which was hardly induced by He+H_2O plasma plume exposure, may require N_2O_{5gas} at least. Thus, chemical modification of biomolecules could potentially be controlled by exposure method and composition of gaseous species.

4. References

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