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Nitrogen Radicals in Aminoacid Processing in Different Plasma Circumstances
種々のプラズマ条件下のアミノ酸プロセスにおける窒素ラジカルの役割

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This study aims to reveal the role of nitrogen radicals in aminoacid processing. Low pressure nitrogen plasma was produced in a parallelepipedic stainless steel chamber using 2 kW microwave power at different operating pressures. A grounded grid interposed between plasma and samples blocked the charged radicals to reach the samples to investigate the effect of neutrals. Neutral molecular species seem to enhance the physical sputtering while N_2^+ ions increase the nitrogen content of aminoacids. The processing proves to be dependent on the structure of the biomolecule.

1. Introduction

Recently, the field of plasma medicine applications increased considerable. Despite these great outcomes of plasma medical applications (such as facial rejuvenation and fast wound healing after nitrogen plasma skin treatment [1]), there is a lack of information on the insights of how and why it is possible to obtain such effects, and, of course, improve the methods for better results. It is also important to develop novel technologies for the inactivation of biohazardous molecules (e.g. prion). Thus the necessity of revealing the interactions between plasma and such molecules arises, proteins being an important component of human body.

Reactive plasmas bring interesting features to plasma bio-processing because the abundance of radicals but UV radiation as well, easily favoring chemical reactions with the samples. This study aims to reveal the role of nitrogen radicals in aminoacid processing.

2. Experiment

Low pressure nitrogen plasma was produced in a parallelepipedic stainless steel chamber using 2 kW microwave power at different operating pressures from 1 to 7 Pa. A grounded grid interposed between plasma and samples blocked the charged radicals to reach the samples to investigate the effect of neutrals. A schematic of the setup used in these experiments is presented in Fig.1. The relative concentrations of ions and neutrals were evaluated from emission measurements and ion detection by quadrupole mass spectrometer.

In the conditions presented above, several aminoacid samples were processed by plasma with and without grounded grid, respectively, and then the results were compared and discussed in relation with plasma parameters.

3. Results and Discussions

Several conditions were studied, the discharge being ignited in the same conditions except the gas pressure. This was varied from 1 to 7 Pa in order to study the effects of different species with different concentrations on aminoacid modification. Fig.2 (a) and (b) show the results of plasma species charged and uncharged, respectively. The same trend was found in the maximum of the emitted lines for these plasmas [2]. When plasma is blocked by the grounded grid, the main species reaching the aminoacid samples are plasma neutrals and some VUV/UV radiation according to the optical transparency of the grid.

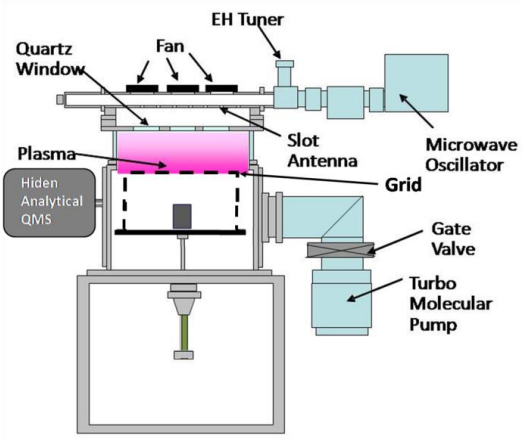

Fig.1. Schematic of the experimental setup

In all the processing conditions the temperature near the sample place didn't exceed 75°C for direct plasma treatment, and 50°C for plasma neutral irradiation, therefore we can consider that the effects were not caused by heating but by plasma species.

Both plasma and only neutral exposure caused the increase of the nitrogen content of the samples, though the increase was higher in the case of direct plasma treatment, as can be deduced form XPS measurements in

Fig.3 where the values for Lysine ($C_6H_{14}N_2O_2$, molecular mass 146.19 g/mol) are compared for plasma and neutral exposure for 1 and 3 Pa discharge pressure, respectively.

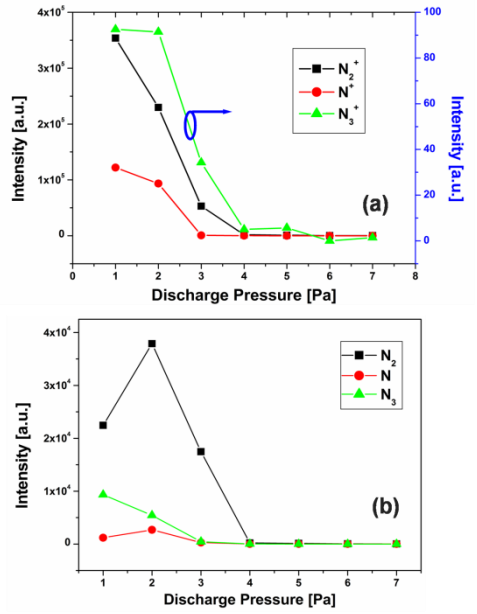

Fig.2. Plasma species (a) ionic, and (b) neutrals, respectively, vs. discharge pressure.

In the same time, high performance liquid chromatography (HPLC) results indicated the presence of aminoacid fragments after processing. The chromatogram for the untreated aminoacid has one peak corresponding to a retention time of 35 minutes, all the chromatograms for the processed Lysine present some peaks at lower retention periods. This means that there is a competition between bond cleavage and the formation of new bonds with nitrogen (as discussed based on XPS results). The cleavage seems to be preferential since only specific aminoacids are found in the processed samples, the same fragments in all treatment conditions.

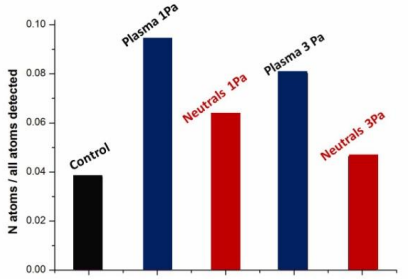

Fig.3. Relative atomic composition of Lysine samples (from XPS measurements)

For a discharge pressure of 1 Pa the nitrogen addition proved to be stronger than for example for 3 Pa. The differences between the two situations can be analyzed from Fig.2: while neutral concentrations seem to be similar, there is a huge difference regarding the ionic species, which have higher concentrations for 1 Pa. Thus one can affirm that the ionic species are responsible mostly for nitrogen addition.

The processing was monitored by mass spectrometry. The main conclusion is that there is not a noticeable difference between nitrogen plasma processing and nitrogen plasma neutrals processing regarding the volatile by-products. Considering this and the fact that the common species for the two situations are the plasma neutrals and the radiation, one can assume that neutrals would be responsible for bond cleavage, with the contribution of the radiation as well.

For higher pressure the process of bond breaking is considerable reduced, probably due to the decreasing of the concentrations of all plasma species but also to the VUV/UV radiation as well, for higher pressure some low wavelength lines being absent and the whole spectrum having a lower intensity.

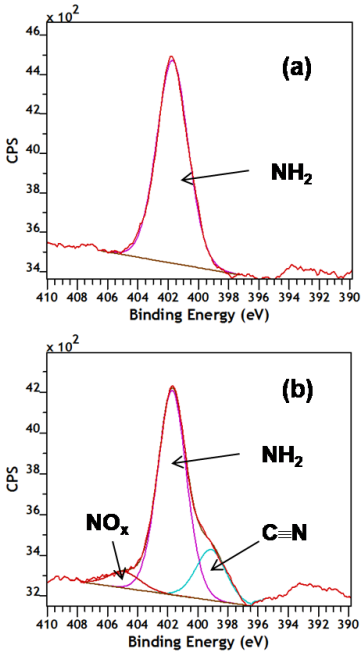

Fig.4. XPS spectra of (a) untreated Serine, and (b) exposed to nitrogen plasma produced at 3 Pa.

The processing under nitrogen plasma depends on the exposed biomolecule. In the case of another aminoacid, smaller and simpler, Serine ($C_3H_7NO_3$, 105.096 g/mol) no fragments were evidenced in the HPLC chromatograms. The same increase of nitrogen content is found as in the case of Lysine, this being a common nominator for all the studied aminoacids exposed in nitrogen plasma circumstances. The increased is shown to be the result of nitro groups formation and also C≡N as a result of aminoacid interaction with plasma species (Fig.4)

More results will be presented in the conference.

References

[1] J. D. Holcomb, K. J. Kent, D. E. Rousso, Arch. Facial Plast. Surg. **11** (2009), pp. 184.

[2] I.Motrescu, A.Ogino, S.Tanaka, T.Fujiwara, S.Kodani, H.Kawagishi, G.Popa, M.Nagatsu, Jap. J. Appl. Phys. **50** (2011) 08JF07.