Structure-function relationships in the case of plasma modified proteins

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Plasma applications in medicine and biology gained an increased attention in the last years. In the plasma medicine community, lots of efforts are concentrated to understand the physical and chemical basis for the clinical results of plasma in use. In this paper we present results related to protein structure and function after exposure to helium dielectric barrier plasma jet. Pepsin and bovine serum albumin powders were exposed to helium plasma jet and then spectroscopic investigations were carried out in order to probe structural modifications induced during the action of plasma components. Function of plasma modified pepsin was tested using an enzymatic assay.

Atmospheric pressure plasma contact with biological materials is nowadays an intriguing research field. Due to development of plasma medicine in the last years, plasma applications in medicine and biology were implemented with lot of enthusiasm. Results from cell studies to animal and even human clinical trials, all indicate that atmospheric pressure non-thermal plasmas technologies can have benefic effects, there where all conventional medical methods failed. Physicists, side by side with biologists and medical doctors, search for new therapeutic solutions involving plasmas at atmospheric pressure: healing of severe infected wounds, tooth and root canal treatment, apoptosis of cancerous cells, blood coagulation [1].

Taking into account these rapidly advancing clinical results of plasmas in use, it is necessary to start investigations on the plasma exposure effects on the biomolecular environment. Moreover, plasma technologies entered also in food industry, here being applied for surface decontamination or polymerization of protective layers. Excepting the fact that high power plasmas can destroy proteins and nucleic acids, we know very few aspects on the mechanisms of plasma action on biomolecules at low powers [2].

We present in this paper results related to structure-function relationships in the case of plasma modified proteins. Bovine serum albumin and pepsin powders (Sigma Aldrich products) were exposed in microtiter plate to a helium plasma jet for 1 min (Fig. 1). The plasma is produced in dielectric barrier discharge configuration, using a quartz tube and external aluminum electrodes separated by 10 mm gap. Voltage and current traces are monitored and stored using a digital scope [3].

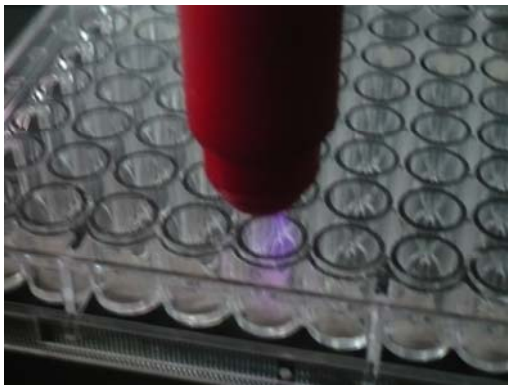

Fig. 1: Exposure of protein powders to the action of helium plasma jet.

After plasma exposure a part of the protein powder was used for electron microscopy investigations and the rest was transferred into distilled water, in order to obtain a solution of 1 mg/mL concentration. Then spectroscopic investigations were carried out in order to obtain information on protein structure and function. Intrinsic fluorescence spectroscopy was used to study thermal denaturation / renaturation of plasma exposed proteins. Extrinsic fluorescence spectroscopy was used to study the protein hydrophobic domains and the unfolding events due to plasma exposure. Circular

dichroism and ATR-FTIR spectroscopy were used to determine the α-helix, the β-sheet and β-turns content of plasma treated proteins. The function of plasma modified pepsin was tested using an enzymatic assay, using both native and plasma treated albumin as substrate.

Scanning electron microscopy images of the plasma treated proteins show no visible effects (Fig. 2). From experiments with thermal plasmas we know that on materials we can find marks of local etching, melting and redeposition. At all used magnifications (from 300 x to 40k x) no marks of plasma damage were observed on protein powder.

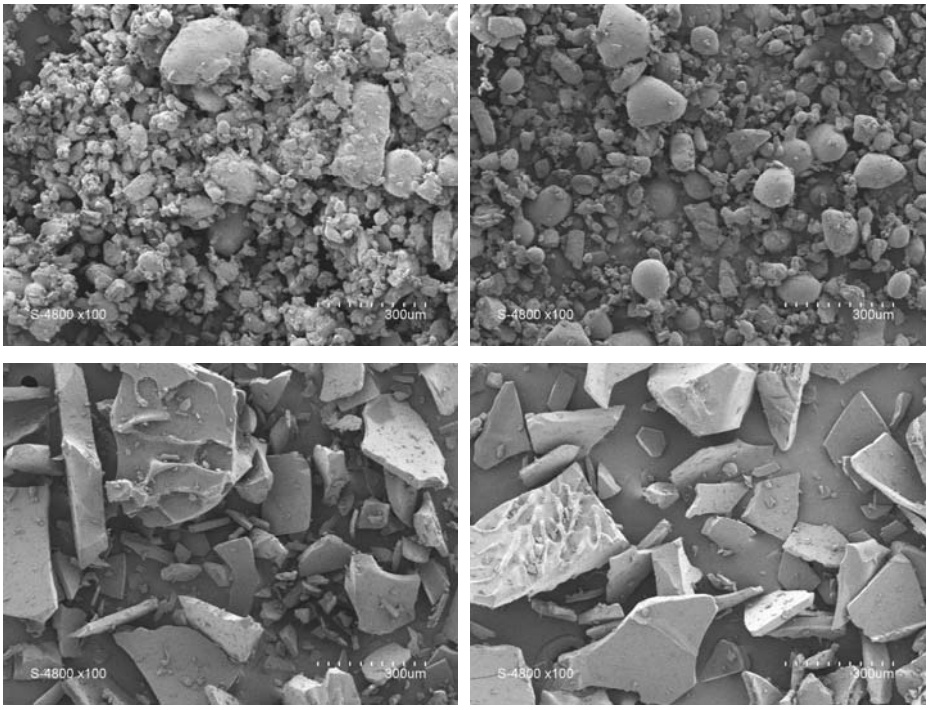

Fig. 2: SEM images protein powders: pepsin, top line and albumin, bottom line; native state, left column and plasma modified, right column.

The intrinsic fluorescence signals from native proteins are always more intense than the ones of plasma treated proteins. This can be related to destruction of protein molecules during plasma treatment. The behavior was maintained for all temperature values in the range tested for proteins thermal denaturation, and differences of around 3°C were found for both denaturation midpoint temperature and renaturation midpoint temperature.

Extrinsic fluorescence spectroscopy of bovine serum albumin marked with 1,8-ANS revealed increased signal for plasma treated proteins, this being associated with changes in tertiary and quaternary structure and exposure of hydrophobic domains.

Results from the enzymatic assay related to the capacity of plasma treated pepsin to digest albumin offered no spectacular results. The enzyme is still able to perform its role, but in comparison with untreated proteins its activity is lowered. This can be correlated with destruction of enzyme molecules during plasma treatment, but also with changes in the molecular structure (e.g. plasma oxidation), as enzymes are known to have high specificity for their substrate.

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References

[1] Z. Machala, K. Hensel, Y. Akishev (Editors), *Plasma for bio-decontamination, medicine and food security,* Heidelberg: Springer Publishing (2012) 1-479.

[2] F. Rossi, O. Kylián, "Sterilization and decontamination of surgical instruments by low pressure plasma discharges," in *Biomaterials and Plasma Processing*, N. Dumitrascu and I. Topala (Editors), Iasi: Alexandru Ioan Cuza University Press (2010) 47-67.

[3] A. Nastuta, I. Topala, C. Grigoras, V. Pohoata, G. Popa, *J. Phys. D: Appl. Phys.* **44** (2011) 105204.