Different modes of protein inactivation by atmospheric pressure plasmas

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Atmospheric pressure plasmas are known for their fast inactivation of microorganisms, but the specific mechanisms are not well understood. We investigated different biological macromolecules regarding their susceptibility to different plasma components to deepen our understanding of plasma-induced damage inside a cell. We employed a specific atmospheric pressure plasma jet configuration, called X-Jet [1], to split the effluent into photons and particles. Furthermore, all experiments were performed in a He atmosphere to minimize secondary effects of ambient air as well as allowing VUV radiation emitted by the plasma to reach the surface.

Proteins are key players in life. Information on their composition is stored in genes on the DNA level, these are transcribed into RNA, which in turn serves as translational template for protein synthesis. Nearly all cellular processes are based on protein catalyzed reactions and protein interactions. Reflecting the diversity of their functions, proteins exist in a multitude of forms, as well as featuring diverse levels of sensitivity towards different kinds of damage. Therefore an array of different model proteins should be used to study plasma-induced protein damage.

We present the effects of plasma treatment on three different proteins. The protein GapDH is an essential part in the central energy metabolism. Its active form consists of four subunits linked via thiol groups, which are prone to oxidation. Enzyme activity can be measured spectroscopically *in vitro* via the reduction of its energy donor NAD^+ to NADH. It was shown that plasma emitted (V)UV radiation inactivates GapDH very slowly. Treatment with emitted particles is more effective, whereas treatment with the undivided effluent proved to be most efficient for inactivation. As a second model protein, RNase A was used. RNases in general are among the most stable proteins known. Activity of RNase A was measured by monitoring spectroscopically the degradation of its model substrate cCMP. None of the plasma treatments with effluents (photons, particles or combined) had any significant effect. The same experiments performed with an atmospheric DBD plasma source [2] showed that direct contact with plasma inactivates RNase A samples within 5 minutes. The third employed protein is mCherryRED, which consists of a very stable β-barrel structure sheltering a single chromophore. When excited with light at 562 nm mCherry emits red light with a maximum at 607 nm. Relative fluorescence was measured during a time-course experiment with the untreated control set to 100% fluorescence. Interestingly, all jet configurations were effective in protein decoloration, but the protein regained its fluorescence after 18h of incubation. Only after long treatment times no regeneration was observed.

References

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