22nd International Symposium on Plasma Chemistry
July 5-10, 2015; Antwerp, Belgium

FTIR spectroscopy of cysteine as a ready-to-use model for plasma-induced chemical modifications

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Abstract: Cysteine is an amino acid known for its structural relevance in proteins. Due to its chemical composition, it is also suited as a model for the investigation of plasma-induced modifications at three groups highly abundant in biological macromolecules. FTIR spectroscopy is a fast way to assess the influence of plasma treatment on this chemical group and compare different treatment conditions or plasma sources with each other.

Keywords: cysteine, model, chemical modifications, FTIR spectroscopy

1. Introduction

Interactions between plasmas and biological samples rather often occur in liquid or semi-liquid environments. This is true for the treatment of wounds and other clinical applications, as water is the main compound of most living organisms. It also holds true for the investigation of basic mechanism with model substrates such as proteins or DNA, as these macromolecules typically occur in liquid solutions. Plasma treatment of liquid solutions quickly results in the generation of a high abundancy of various highly reactive species, such as hydroxyl radicals and nitric oxide [1], making investigations of plasma-treated biomolecules in solution rather complex. The specific radical species generated as well as their corresponding ratios not only depend on the employed plasma source but also on the discharge and treatment conditions. A fast and easy-to-use model to investigate the impact of plasma treatment on biologically relevant groups would be ideally suited as a rapid method to compare various treatment conditions as well as different plasma sources with each other. We developed a rapid screening method using cysteine as a readily-available biological model substrate, which can be easily investigated by FTIR spectroscopy to shed light on different reaction mechanisms occurring during plasma treatment of biological samples. Cysteine is an amino acid featuring, as all amino acids, an amino ($R\text{-}NH_2$) and a carboxyl (R-COOH) group. These chemical groups can be found in nearly all biologically relevant macro molecules, ranging from DNA to proteins and lipids. Furthermore, cysteine has a unique thiol (R-SH) group, which has already been shown to be highly susceptible to plasma-induced oxidation [2].

Takai *et al.* showed that sulfur-containing amino acids (methionine and cysteine) are modified in different ways by plasma treatment, though the investigation and annotation by mass spectrometry is highly challenging for numerous reasons. Our approach employs FTIR spectroscopy, which should be present in most physical labs, to track changes in the chemical composition of the cysteine substrate. Here, we present the impact of a dielectric barrier discharge (DBD) on the cysteine model substrate.

2. Methods

The DBD employed for the experiments is described in detail elsewhere [3], though a brief description is given here: The distance between the driven AlO_2-covered copper electrode and the sample was 1 mm. The plasma was ignited in this 1 mm gap using ambient air as the process gas and voltage pulse with a maximum amplitude of -13.5 kV and a trigger frequency of 300 Hz. For follow-up experiments, the frequency was varied between 150 Hz and 600 Hz. Cysteine solution was treated with the DBD for up to 5 minutes. Afterwards, samples were dried on silicon wafers and desiccated. Dried wafers were analyzed by FTIR spectroscopy and resulting spectra recorded between 750 cm^{-1} and 4000 cm^{-1} with a spectral resolution of 2 cm^{-1}. For each spectrum, 64 single spectra were recorded and normalized to one final spectrum. Background spectra of untreated samples were recorded after each plasma-treated sample to compensate possible alterations of water and carbon dioxide content in the air due to the ambient measurement conditions. Resulting transmission spectra were converted into absorption spectra. Spectra were normalized after base line correction by applying the Euclidean norm. Feature annotation was performed following [4]. All experiments were performed in triplicates and standard errors under consideration of Student's t-test shown as shadows for each spectrum.

3. Results

FTIR spectra of DBD-treated cysteine samples showed significant changes in several bands compared to the untreated controls. Some examples are indicated in Fig. 1

as arrows. Annotation of these bands indicate that the thiol (νS-H) signal around 2600 cm^{-1} loses intensity in a treatment time-dependent way. Furthermore, a band at ~1040 cm^{-1} is formed during treatment, which is annotated as an oxidized sulfur signal (νS=O) [5].

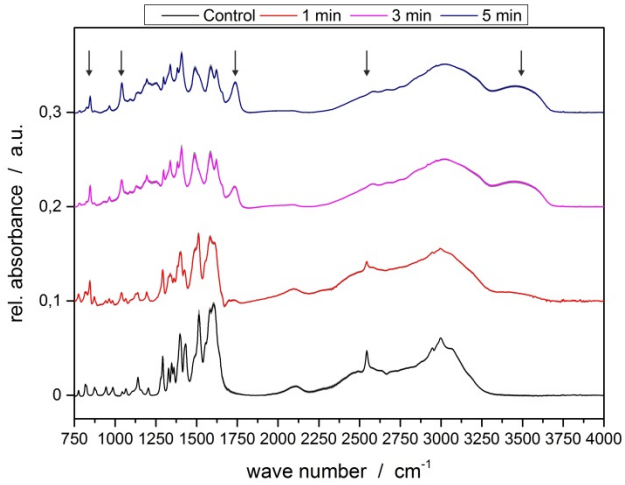

Fig. 1. Time-dependent spectral changes after DBD treatment of cysteine. For better visibility, graphs are stacked in 0.1 steps.

Beside these changes, several other changes could be observed after plasma treatment, such as the occurrence of a new band around 1735 cm^{-1}, which can be annotated as νC=O (ester or aldehyde) or the appearance of the band at 850 cm^{-1}, which can be annotated as νN-O [5]. To quantify the increase and decrease of sulfur and thiol relative intensities of both signals were plotted against the treatment time (Fig. 2).

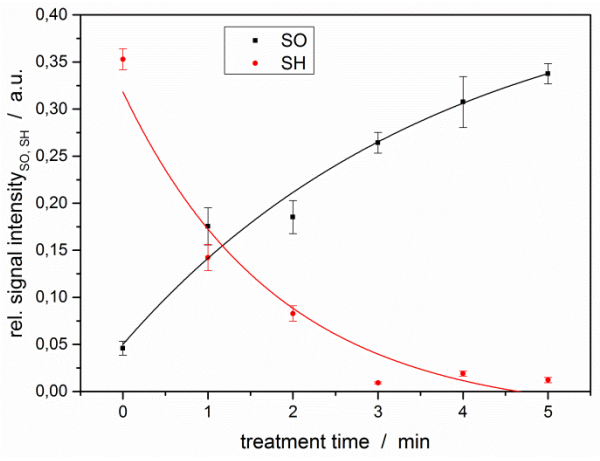

Fig. 2. Changes in band intensities during DBD treatment

The shifts in intensity from the νS-H to the νS-O band are in good time-dependent order with each other.

As this method can be used to easily compare influence of different treatment parameters on biochemical groups, the frequency of the employed DBD plasma was modified and resulting spectra were compared (data not shown). Here, the loss of the SH signal around 2600 cm^{-1} was stronger when a higher DBD frequency was used for treatment, as was the increase of the SO signal.

4. Discussion

After DBD treatment, several changes in the FTIR spectra could be observed. The loss of the thiol signal and the parallel increase of the oxidized sulfur band indicate that the free thiol group of cysteine is oxidized by DBD treatment, which was also shown by [2, 6]. Furthermore, several other changes were observed, e.g. the feature around 1750 cm^{-1}, indicating that not only the thiol but also formations of new aldehydes or esters. Moreover, changes in the plasma frequency during treatment results in a faster generation of oxidized sulfur from free thiols, indicating that reactive species capable of oxidizing thiols are generated to a higher degree or are kept active during shorter plasma cycles.

Our results demonstrate that FTIR spectroscopy of a suitable model substrate is a fast and convenient method to investigate the impact of a plasma source on biologically relevant chemical groups. We present cysteine as a model substrate for three chemical groups found in all organisms. The normalization by applying the Euclidean norm allows comparison between unrelated spectra and quantification of occurring band shifts and intensity changes.

5. Acknowledgements

This work was funded by the German Research Foundation (DFG) with two packet grants (PAK728 & PAK816).

6. References

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