Specificity of copper-dimethylphenantroline assay for detection of H_2O_2 in cell-culture mixtures treated by plasma

P. Lukeš, V. Jirásek
Institute of Plasma Physics of the Czech Academy of Sciences, Prague, Czech Republic

Abstract: We evaluated H_2O_2 concentrations determined by a copper-dimethylphenanthroline assay in different organic mixtures treated by He/O_2 plasma to assess the specificity of this assay for detecting H_2O_2 in cell-culture mixtures treated by plasma. Significant interferences of this assay with organic compounds present in the cell culture media were determined that could cause a high overestimation of H_2O_2 in plasma-treated media.

1. Introduction

Atmospheric plasmas produce a variety of reactive oxygen and nitrogen species in plasma-treated liquids. These species have highly cytotoxic properties and biochemical and antibacterial activity. However, direct cause-effect correlations are still not defined. In some works, plasma-generated H_2O_2 was theorized to be a major effector of tumor cell killing. Another work reported that biological responses are not solely induced by long-lived, aqueous H_2O_2, and plasma components other than H_2O_2 contribute to cell death [1]. Undoubtedly, more processes can be involved, including plasma-modified organic compounds in the treated medium. However, one of the reasons for these incomprehensible observations could be the low specificity of analytical diagnostics used for detecting particular compounds in plasma-treated medium. The complex chemical composition in plasma-treated media increases the difficulty of unambiguously identifying the RONS possibly responsible for effects on cells or tissues and provides one of the challenges in standardizing chemical measurements related to such interactions.

Several colorimetric methods were used for H_2O_2 determination in plasma-treated solutions, including the titanium oxysulphate ($TiOSO_4$) method, the Amplex red assay, or the fluorimetric assay based on peroxidase. The copper-dimethylphenanthroline (Cu-DMP) assay was recently reported as a validated method for selective detection of H_2O_2 in cell-culture media [2]. Using this assay, stimulated H_2O_2 formation in plasma-treated media was determined in several research works [3,4], and it was suggested that plasma-induced oxidation reactions of the aromatic ring of organic compounds in the cell culture media, particularly of tyrosine, could be the origin for observed enhanced productions of H_2O_2 in plasma-treated cell culture media.

In this work, we evaluated H_2O_2 concentrations determined by Cu-DMP assay in different organic mixtures treated by He/O_2 plasma to assess the specificity and possible interferences of Cu-DMP assay of H_2O_2 in cell-culture mixtures treated by plasma.

2. Methods

The COST-Reference plasma jet was used as a plasma source to treat all liquids studied in this work [5]. A sinusoidal voltage with a 230 V root mean square was used to power electrode of the capacitively coupled 13.56 MHz RF plasma source. The plasma was operated at a helium flow rate of 1.4 slm with a 0.6% oxygen admixture. The jet was aimed at a liquid solution, with a distance of 4 mm between the jet outlet and the liquid surface. Two colorimetric methods were used to determine H_2O_2 in plasma-treated solutions: the titanyl oxysulphate method and the copper-dimethylphenantroline assay. Evaluations of H_2O_2 were made in plasma-treated phenol, tyrosine, DMEM, and RPMI culture media solutions. High-performance liquid chromatography was used to analyze organic products in plasma-treated solutions.

3. Results and Discussion

We compared H_2O_2 concentration determined by $TiOSO_4$ and Cu-DMP assay in a plasma-treated phenol solution (He/O_2 plasma, 2 min treatment, 0.5 mM and 5 mM phenol in D.I. water). Much higher concentrations of H_2O_2 were determined by Cu-DMP (6 and 15 times more compared to those determined by $TiOSO_4$ assay). Differences in H_2O_2 concentrations determined by these assays were even more significant in plasma-treated tyrosine solutions. From subsequent experiments and analysis of plasma-treated liquids studied in this work, it was determined that certain aromatic compounds modified by plasma treatment react directly with Cu-DMP and give false responses to the Cu-DMP colorimetric assay of H_2O_2. Possible reasons for these reactions were determined.

4. Conclusion

Significant interferences of colorimetric Cu-DMP assay with organic compounds present in cell culture media, including amino acids, but even more with hydroxylated aromatics, were determined that could cause a high overestimation of H_2O_2 produced in plasma-treated media.

References

[1] S. Bekeschus, et al., Plasma Chem. Plasma Process. **38**, 1–12 (2018).
[2] V. Veronico, et al., Plasma Proc. Polym. **18**, e2100062 (2021).
[3] V. Veronico, et al., Plasma Proc. Polym., **19**, e2100158 (2022).
[4] B. Myers, et al., J. Phys. D: Appl. Phys. **54**, 145202 (2021).
[5] M. M. Hefny, et al., J. Phys. D.: Appl. Phys., **49**, 404002 (2016).