

Separating Radical-Driven and pH-Dependent Pathways in Low-Temperature Plasma-Induced DNA Damage

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Low-temperature plasma (LTP) damages DNA through reactive oxygen and nitrogen species (RONS) generated by plasma-liquid interactions, while concurrent acidification can further destabilize DNA [1,2]. Previous studies identified amino-acid-dependent protection and pH changes during plasma exposure [3,4]. Here, we used the quantification of multiple types of plasma-jet-induced DNA damage under additive-free conditions as the baseline and examined the effects of phosphate buffer and amino acids in aqueous plasmid DNA.

Plasmid DNA was irradiated using a helium atmospheric-pressure plasma jet. Single-strand breaks (SSBs), double-strand breaks (DSBs), and enzyme-sensitive sites were quantified by gel electrophoresis under additive-free conditions. In the modulation study, samples were irradiated with and without 10 mM phosphate buffer (PB) to maintain neutral pH or allow acidification, and tryptophan, tyrosine, and methionine (10–300 μ M) were added as radical scavengers. Hydroxyl-radical competition was evaluated using a coumarin assay with dimethyl sulfoxide (DMSO) as a reference scavenger.

In the additive-free system, strand breaks dominated the detected damage, while enzyme-sensitive sites were observed at lower levels and DSB signals were weak [5]. Under the 10 kV and 1 kHz condition, the solution acidified during irradiation without PB, whereas PB maintained a near-neutral pH. The SSB yield was approximately 3.8 times higher without PB than with PB, indicating that acidification contributed to the measured damage. All three amino acids increased the fraction of undamaged supercoiled DNA in a concentration-dependent manner, both with and without PB. The three amino acids showed comparable protective effects at equivalent concentrations. At μ M concentrations, the amino acids produced only minimal changes in pH. Separately, the coumarin–DMSO competition assay showed that radical scavenging reduced the hydroxyl radicals available to react with coumarin. Taken together, the results separate an acidification-dependent contribution from RONS-driven DNA damage and support radical scavenging as the main protective mechanism of amino acids at μ M concentrations.

References: 1. P. J. Bruggeman et al., *Plasma Sources Sci. Technol.* **25**, 053002 (2016). 2. S. Ptasińska et al., *Phys. Chem. Chem. Phys.* **12**, 7779 (2010). 3. A. Stypczyńska et al., *Chem. Phys. Lett.* **500**, 313 (2010). 4. C. J. G. Villavicencio et al., *Molecules* **29**, 5889 (2024). 5. H. Yu et al., *Research Square*, rs-9645581/v1 (2026).