

Ectoine protects biomolecules from ionizing radiation: Molecular mechanisms

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Ectoine properties

- Produced by halophilic bacteria to survive in saline environments
- Zwitterionic structure (fig. 1)
- Exerts kosmotropic effect on the local water structure[6]
- Influences protein functions[5]
- Effective cell protectant against UV-A (315-400 nm) radiation[1]

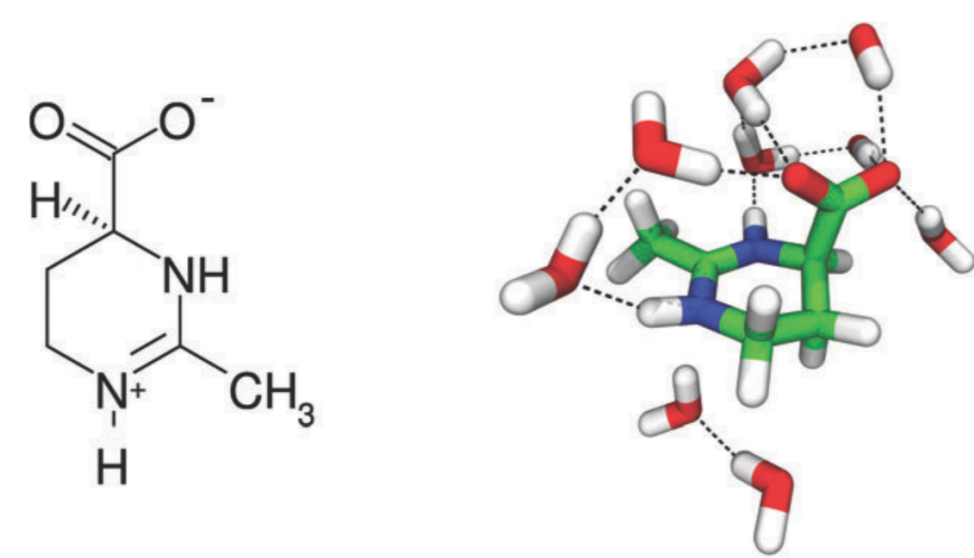


Figure 1: Chemical structure of Ectoine and its first hydration shell. Source: [6]

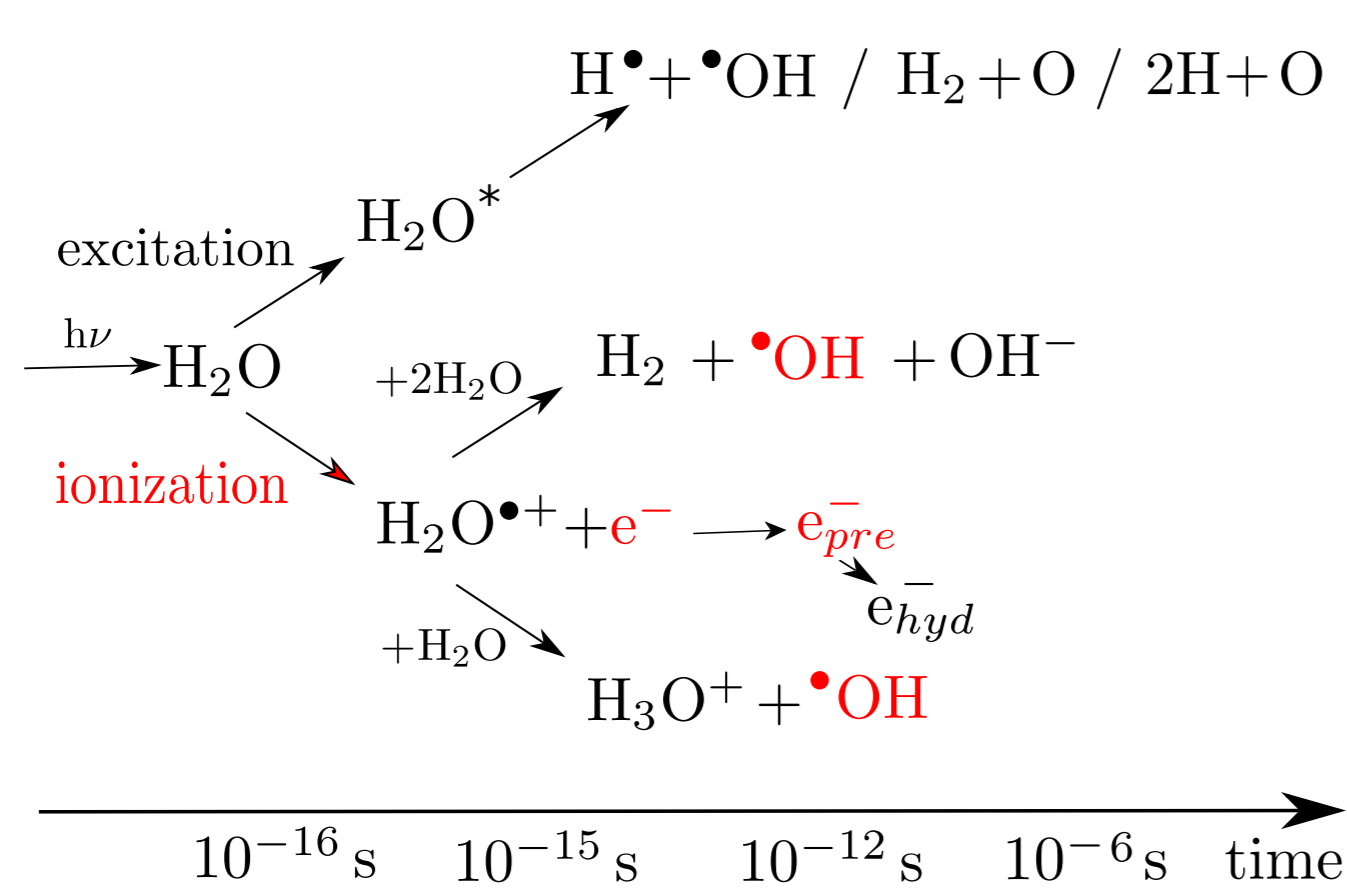


Figure 2: Physico-chemical stage of radiation water interaction & production of secondary damaging species.

Electron irradiation of DNA in solution

- Irradiation of solution by a scanning electron microscope through a nanomembrane (fig. 3)[4]
- Solutions: Plasmid DNA with cosolutes (0-1 M ectoine, 0.5 M NaCl, 1 M ectoine+0.5 M NaCl)
- DNA strand-break detection by gel electrophoresis

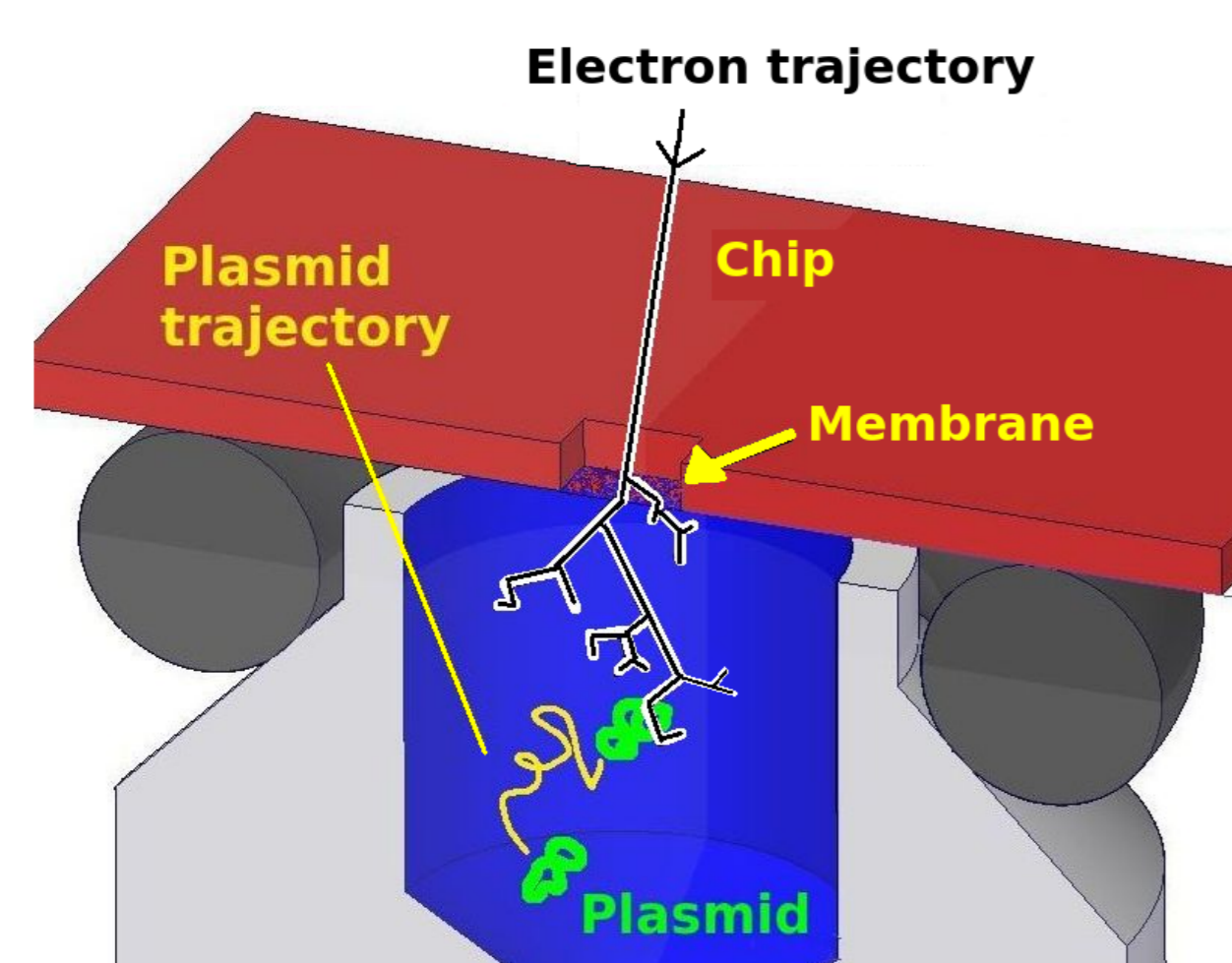


Figure 3: Electron irradiation of plasmid DNA in liquid. The plasmids (green) diffuse on a random trajectory (yellow) through water (blue). They cross the trajectory of the electrons (white/black). Source: [2]

Irradiation results

- Ectoine protects DNA efficiently from ionizing radiation
- The protection is concentration dependent
- The influence of NaCl is small

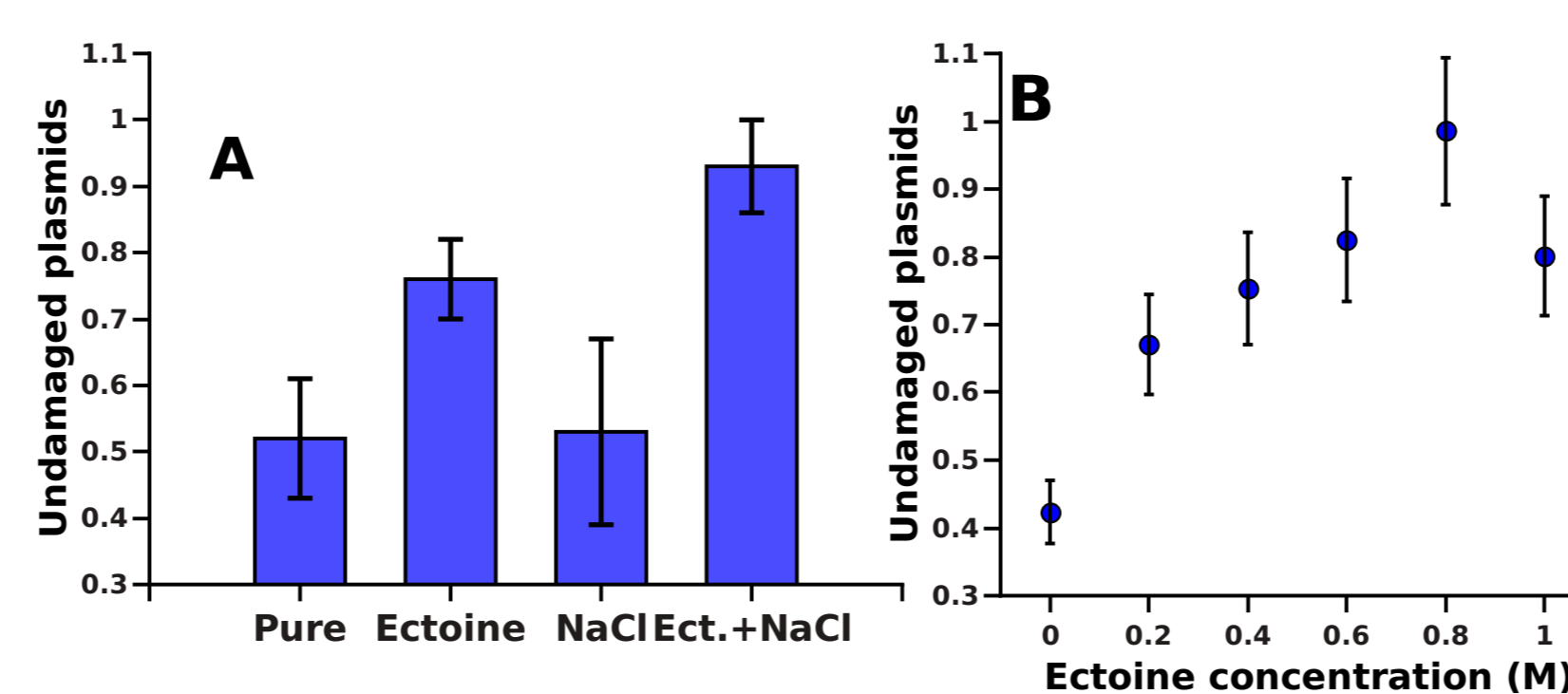


Figure 4: A) Undamaged plasmids in PBS after irradiation without cosolute (pure), with 1 M ectoine, 0.5 M NaCl, and 1 M ectoine + 0.5 M NaCl. B) Undamaged plasmids after irradiation in dependence of the ectoine concentration. Source: [3]

Monte-Carlo simulations to characterize the ionizing radiation

- Ionizing radiation leads to water radiolysis (fig. 1)
- Radiolysis produces secondary damaging species ($\cdot OH$, e_{LEE}^-)
- Amount, production ratio & location depend on kinetic energy spectrum of the radiation and location of scattering events
- Determined by Monte-Carlo simulations (Geant4)

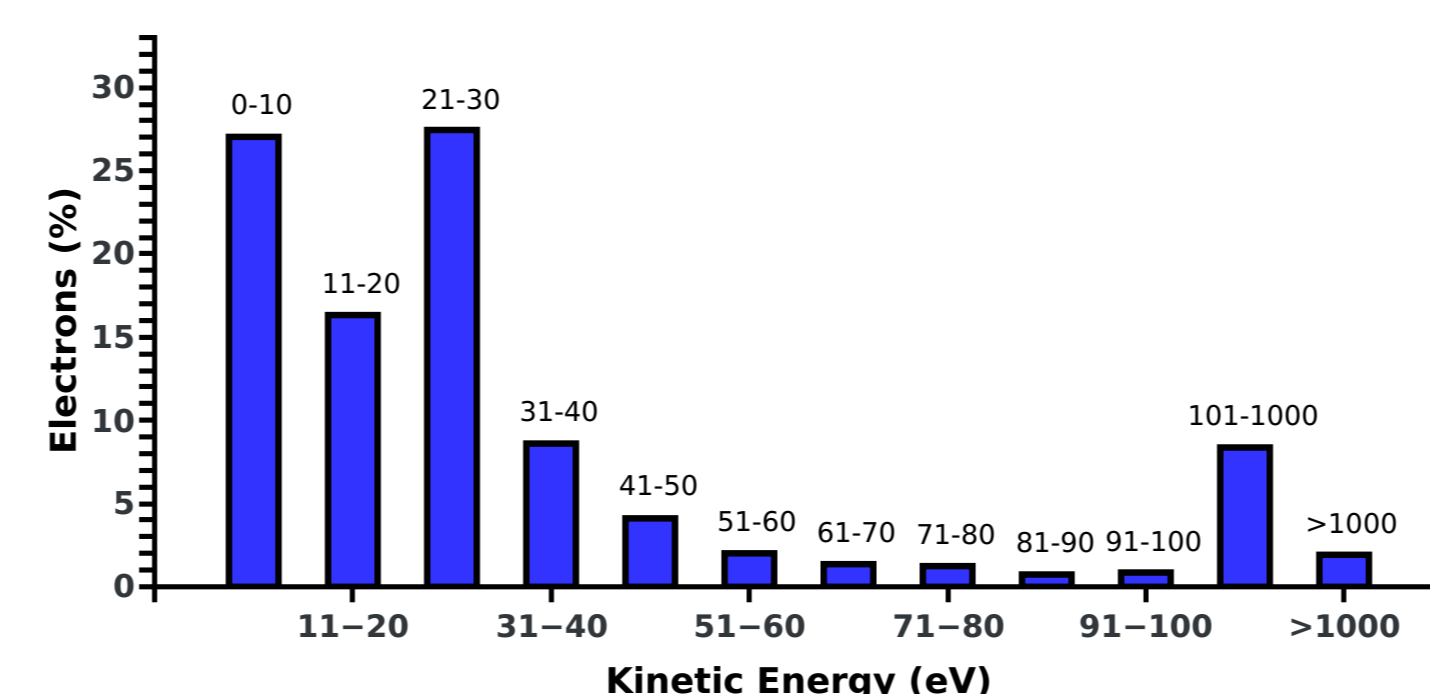


Figure 5: Histogram of the electron kinetic energy distribution at 500 μm water depth for 30 keV primary particles. Source: [3]

- Dominance of e_{LEE}^- in the kinetic energy spectrum (fig. 5)
- Ionization dominates $\Rightarrow \cdot OH : e_{LEE}^- = 1:1$ [2]

Mechanism I: Increase in low energy electron scattering

- Large increase in the intensity of the acoustic water modes upon addition of ectoine was found (fig. 6)

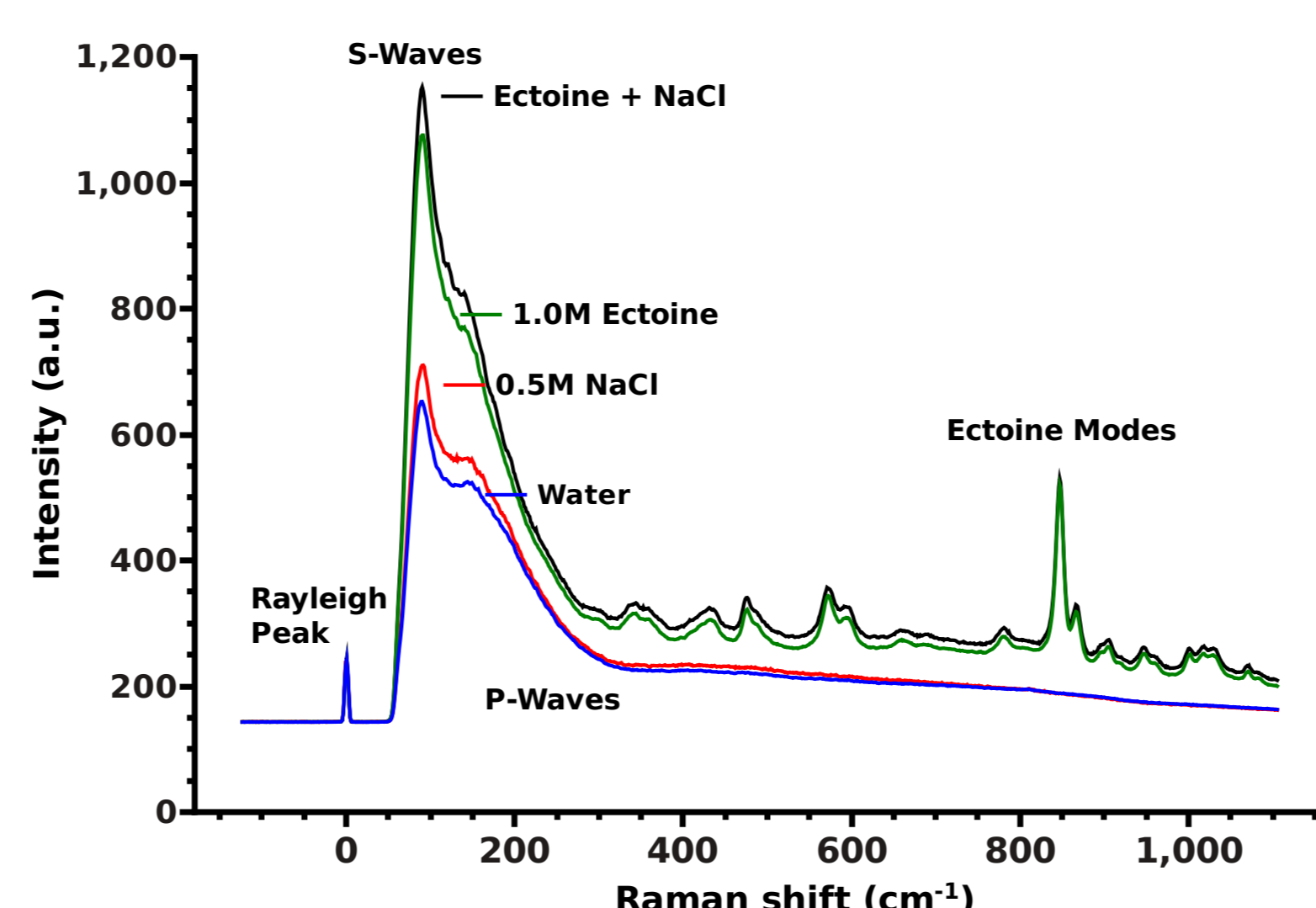


Figure 6: Raman spectra of the acoustic water modes. Pure water (blue), 0.5 M NaCl (red), 1 M ectoine (green), & ectoine+NaCl (black). Source: [3]

- Electron scattering cross-sections correspond to the Raman spectra

- Ectoine leads to an increase in the inelastic scattering probabilities of LEE *via* energy-losses by the excitation of phonons
- Results in decrease of the total energy deposit by ionization and the total number of secondary damaging species

Mechanism II: Action as radical scavenger

- EPR measurements to test scavenging capabilities ($\cdot OH$ -radicals)
- Comparison of ectoine, glycine betaine (negative control) and isopropanol(positive control)
- Ectoine scavenges $\cdot OH$ -radical (fig. 7)

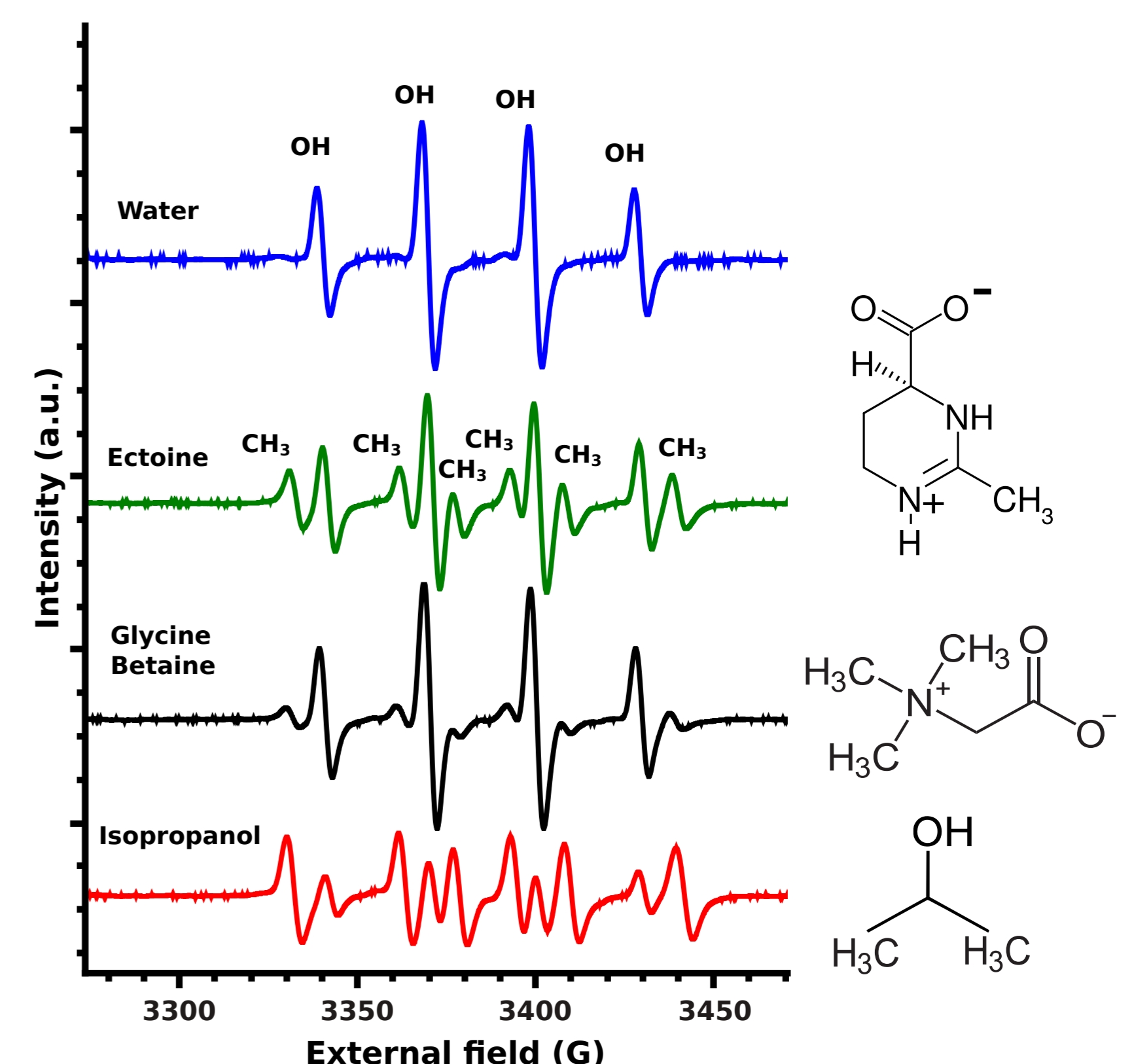


Figure 7: EPR spectra of OH-DMPO and CH₃-DMPO radicals produced by Fenton's reaction and various cosolutes. Source: [3]

Conclusion

Ectoine was found to protect DNA against ionizing radiation damage. Protection mechanisms:

- LEE energy-loss due to the scattering at vibrational water modes and the resulting decrease in secondary particle production
- $\cdot OH$ -scavenging by ectoine
- Displacement of water in the extended hydration shell of DNA

[1] Celine Botta, Carole Di Giorgio, Anne-Sophie Sabatier, and Michel De Meo. Genotoxicity of visible light (400-800 nm) and photoprotection assessment of ectoin, l-ergothioneine and mannitol and four sunscreens. *Journal of Photochemistry and Photobiology B: Biology*, 91(1):24-34, April 2008.

[2] Marc Benjamin Hahn, Susann Meyer, Hans-Jörg Kunte, Tihomir Solomun, and Heinz Sturm. Measurements and simulations of microscopic damage to DNA in water by 30 keV electrons: A general approach applicable to other radiation sources and biological targets. *Physical Review E*, 95(5):052419, May 2017.

[3] Marc Benjamin Hahn, Susann Meyer, Maria-Astrid Schröter, Hans-Jörg Kunte, Tihomir Solomun, and Heinz Sturm. DNA protection by ectoine from ionizing radiation: molecular mechanisms. *Physical Chemistry Chemical Physics*, 19(37):25717-25722, September 2017.

[4] Marc Benjamin Hahn, Susann Meyer, Maria-Astrid Schröter, Harald Seitz, Hans-Jörg Kunte, Tihomir Solomun, and Heinz Sturm. Direct electron irradiation of DNA in a fully aqueous environment. Damage determination in combination with Monte Carlo simulations. *Physical Chemistry Chemical Physics*, 19(3):1798-1805, January 2017.

[5] Marc Benjamin Hahn, Tihomir Solomun, Robert Wellhausen, Sabrina Herrmann, Harald Seitz, Susann Meyer, Hans-Jörg Kunte, Johannes Zeman, Frank Uhlig, Jens Smlatek, and Heinz Sturm. Influence of the Compatible Solute Ectoine on the Local Water Structure: Implications for the Binding of the Protein Csp to DNA. *The Journal of Physical Chemistry B*, 119(49):15212-15220, December 2015.

[6] Marc Benjamin Hahn, Frank Uhlig, Tihomir Solomun, Jens Smlatek, and Heinz Sturm. Combined influence of ectoine and salt: spectroscopic and numerical evidence for compensating effects on aqueous solutions. *Physical Chemistry Chemical Physics*, 18(41):28398-28402, October 2016.