

P025 Intramolecular Electron Transfer vs. Substrate Oxidation in Lactoperoxidase: Investigation of the Radical Intermediates by Stopped-flow Absorption Spectrophotometry and EPR Spectroscopy
Alistair J. Fielding[‡], Rahul Singh[‡] and Barbara Boscolo[§], Sun Un[‡], Elena Ghibaudi[§] and Anabella Ivancich[‡]

[‡]Service de Bioénergétique, Biologie Structurale et Mécanismes. URA 2096 CNRS and iBiTec-S, CEA Saclay, 91191 Gif-sur-Yvette, France.

[§]Dipartimento di Chimica I.F.M., Università di Torino, Italy.

We have characterized the intermediates formed by the reaction of lactoperoxidase with hydrogen peroxide and their reactivity with selected substrates. The enzyme reaction with hydrogen peroxide was investigated using Electron Paramagnetic Resonance (EPR) spectroscopy. The complementary information obtained from stopped-flow UV-Vis spectrophotometry on the enzyme reaction allowed us to better understand the sequential formation of the intermediates. In the absence of substrates, two protein-based radical intermediates were formed. Advantageous resolution of the g-tensor of the radicals measured at higher field/frequencies (10 T/285 GHz) allowed us to identify a tyrosyl and a tryptophanyl radical. Moreover, two chemically different Tyr radicals with distinct g-values and proton hyperfine coupling were detected as a function of pH. Comparison of the enzyme reactivity of typical peroxidase substrates such as o-dianisidine, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS), and benzohydroxamic acid (BHA) with o-anisidine and mitoxantrone, two clinically relevant molecules, revealed not only important differences in reaction times of the $[\text{Fe}(\text{IV})=\text{O} \text{Por}^{*+}]$ intermediate but also competition between intramolecular electron transfer to the protein-based radical intermediates and substrate oxidation.