Unique biradical intermediate in the mechanism of the heme enzyme chlorite dismutase discovered using microsecond timescale freeze hyperquench

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Chlorite dismutase is a unique heme b dependent enzyme that catalyzes the conversion of chlorite (ClO_2^-) to molecular oxygen (O_2) and chloride (Cl^-). This reaction involves O-O bond formation, which is rare in nature. The enzyme catalyzes a single turnover in less than a millisecond, which makes it technically challenging to study the pre-steady state kinetics of this enzyme. The catalytic mechanism of chlorite dismutase was investigated using microsecond timescale mixing techniques and the natural substrate chlorite.[1] Two different in-house developed ultrafast kinetic techniques were used: Nanospec, ultrafast continuous flow UV-vis spectrophotometry; MHQ, microsecond timescale rapid freeze hyperquenching. The dead times of these instruments are 100X shorter than commercially available devices. These techniques allowed us to observe transient intermediates of Cld during a single turnover with its natural substrate chlorite. The UV-visible, EPR and RR spectra of these intermediates were obtained. Distinct intermediates were found that are not observed with the artificial substrate peracetic acid. Most notably a triplet state EPR signal that we attribute to two weakly coupled amino acid based cation radicals, 'compound T', was transiently formed. The formation of compound T is direct evidence of a two electron transfer process which means that the Cl-O bond break is heterolytic, unlike the most recent proposed mechanism for this enzyme. To our knowledge such a triplet state has never been identified in any heme enzyme.

[1] J. Püschmann, D. Mahor, D.C. de Geus, M.J.F. Strampraad, B. Srour, W.R. Hagen, S. Todorovic and P.-L. Hagedoorn, *ACS Catalysis*, **2021**, *11*, 14533-14544