P023 The unprecedented peroxidase-like activity by Nitrophorin-2, the NO carrying heme protein from *Rhodnius prolixus*

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We have characterized the NO carrier protein Nitrophorin-2 (NP2) and its variants from *Rhodnius prolixus* for their reaction with H_2O_2 and peroxyacetic acid (PAA). The enzyme demonstrated substantial peroxidase activity with a pH optimum of 6.8 using ABTS and o-dianisidine. The K_M for ABTS (500 μM) is comparable to that reported for some catalase-peroxidases (KatGs). The K_M for H_2O_2 (1.1 mM) was much higher than K_M for PAA (32 μM) but comparable to the values reported for some KatGs. Tyr38Ala variant showed lower peroxidase activity and very high K_M values for both H_2O_2 and PAA. The stopped flow analysis of the wild type and the variants was consistent with the formation of Compound I ([Fe(IV)=O Por$^{\bullet+}$]) but with different rates. The 9 GHz-EPR spectra showed the formation of two different [Fe(IV)=O Por$^{\bullet+}$] species, one weakly ferromagnetically coupled signal (typical of peroxidases) at basic pH, and a novel strongly ferromagnetically coupled signal at neutral pH, exclusively observed in model heme complexes. Moreover, we also identified an [FeIV=O Tyr$^{\bullet}$] species formed by intra-molecular electron transfer. Characterization of NP2 variants indicated Tyr85 being the site for the protein radical. Our results suggest that NP2 can perform not only the "heme-edge" oxidation but can also use alternative protein-based radical intermediates as shown in the case of KatGs.