

P022 Pseudoazurin dramatically alters the reaction between cytochrome cd_1 and nitrite

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Cytochrome cd_1 is a periplasmic nitrite reductase found in denitrifying bacteria. A previous study of nitrite reduction by this enzyme reported formation of a stable complex after mixing fully reduced cytochrome cd_1 with nitrite (in the absence of excess reductant). This was assigned as equimolar $cFe(II)d_1Fe(II)-NO^+$ and $cFe(III)d_1Fe(II)-NO$ (cd_1-X). No catalytically competent NO release was detected. In this work, we have investigated the effect of a physiological electron donor to cytochrome cd_1 , the copper protein pseudoazurin, on the mechanism of nitrite reduction by the enzyme. Our data show that initially oxidized pseudoazurin causes rapid further turnover to give a final product that we assign as all-ferric cytochrome cd_1 with nitrite bound to the d_1 heme (i.e. from which NO has dissociated). In contrast, redox inert zinc-pseudoazurin did not have this effect, indicating a crucial role for electron movement between monomers, or individual enzyme dimers, rather than simply a protein-protein interaction. Furthermore, formation of cd_1-X was, remarkably, accelerated by the presence of pseudoazurin, such that it occurred at a rate consistent with cd_1-X being an intermediate in the catalytic cycle. Is it clear that cytochrome cd_1 functions significantly differently in the presence of nitrite and electron donor protein, than in the presence of nitrite alone.