Poster Presentation

MS78.P04

Structural insights of mediated electron transfer by P450 BM3 monooxygenase

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P450 BM3 is a 119-kDa water-soluble heme monooxygenase originating from Bacillus megaterium. P450 BM3 and variants are known to oxidize structurally diverse substrates. However, the requirement for the natural cofactor, NADPH, limits cell-free applications of P450 BM3 in drug synthesis, fuelling efforts to establish alternative cofactor system. Hence, P450 BM3 variants have been generated which circumvent the requirement for NADPH, and enabled P450 BM3 to be driven with alternative electron sources. In this study, crystal structures of the P450 BM3 M7 heme domain variant (F87A, V281G, M354S) with and without cobalt (III) sepulchrate are reported. Cobalt (III) sepulchrate acts as an electron shuttle in an alternative cofactor system employing zinc dust as the electron source. The crystal structure shows a binding site for the mediator cobalt (III) sepulchrate at the entrance of the substrate access channel. The mediator occupies a position which is far from the active site and distinct from the binding of the natural redox partner (FAD/NADPH binding domain). The unusual binding position suggests that the mediator shuttles electrons to the heme-centre through new routes. Electron transfer could occur by a 'through-protein' or a 'substrate-relayed' pathway. The latter seems more plausible since it would ensure efficient use of electrons only in the presence of a substrate in the active site. The structural evidence also indicates that the use of a positively charged mediator is important to effectively reduce the catalytic heme domain. Understanding the mediator-monooxygenase interface opens new avenues for tailoring P450 BM3 to match application demands. Structural and molecular understanding of mediated electron transfer enables a paradigm shift from a mediator acceptance screening to a rational mediator design which considers only stability and electron transfer performance parameters.

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Keywords: P450 BM3 monooxygenase, cobalt (III) sepulchrate, NADPH