

Crystal Structures of Intermediates Occuring along the Reaction Pathway of Cytochrome P450cam

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Cytochrome P450cam from *Pseudomonas putida* catalyses the 5-exo-hydroxylation of camphor. Although many structures of different P450cam complexes have been determined by Poulos and coworkers, the structure of the biochemically important oxygen complex of p450.cam has not yet been solved as it is unstable (half-life ~3 min at 4°C) due to autooxidation. This instability requires collection of the diffraction data of the short-lived complex either very quickly (e.g., by the Laue method) or by prolonging its life time. Only the latter approach was successful for us: We used cryocrystallography to determine the structures of unstable intermediates occuring along the reaction pathway of p450cam. We chemically reduced ferric p450.camphor crystals to generate the ferrous complex. Subsequently, oxygen was diffused into the crystals to generate the ternary p450.camphor.O₂ complex; x-ray radiolysis was used to produce a state that we identify as an oxyferryl species. This intermediate can be transformed to the product 5-hydroxy-camphor by rapid thawing and refreezing of the crystals. The methods used and the structures obtained will be discussed.