

P008 Mutation induces multiple reactive configurations in an enzyme-coenzyme complex which inter-convert at high-pressure

Pudney CR., Hay S., Lafite P., Leys D. and Scrutton NS.
University of Manchester

A typical reaction cycle involves (i) substrate capture (binding), (ii) chemical transformation (reaction), and (iii) product release, each with their associated rate constants. Monitoring an intrinsic rate constant, free from complexity is usually very difficult. Kinetic isotope effect analysis has been of particular use, allowing the occurrence of kinetic complexity to be identified and intrinsic rate constants to be extracted. Kinetic complexity often arises when the observed rate constant is not for a single chemical step. However, given the stochastic nature of enzymatic catalysis, an additional contributor to kinetic complexity may be the formation of a number of productive, observable catalytic substates. In the complex formed between the morphinone reductase active site mutant, N189A MR and the reductive substrate, NADH, such sub-states are observed. Using single-turnover, fast-reaction kinetic techniques we show that the catalytic substates, so called multiple reactive configurations (MRCs), can be monitored, with individual reactive configurations being kinetically observable. We further show that high-pressure (and to a lesser extent temperature) modulates the population of the MRCs. Such studies, combined with the X-ray crystal structure of the N189A MR-NADH₄ complex, allow the origin of MRCs in this system to be inferred.