

Differential Inhibition of Cytochrome P450 Oxidoreductase (CYPOR) Crystal Kadakia

Three recombinant mutant cytochrome P450 oxidoreductase (CYPOR) enzymes, associated with human polymorphisms, were tested for differential effects of uniquely synthesized flavoprotein inhibitors. By testing the effects of various inhibitors with these mutant enzymes, it may be possible to discern how a specific inhibitor interacts with CYPOR. Different compounds are used in order to compare inhibition differences between wild type and known mutant enzymes, which might provide insight into the structure and function of these enzyme-inhibitor interactions. All enzymes used in these studies were recombinantly expressed in bacteria and were purified by affinity chromatography. Three Antley-Bixler syndrome (ABS) mutant CYPOR enzymes were pre-incubated in the presence of three inhibitors, DPI, KK-7, and MMB-2, under turnover conditions. The activity was then measured as the enzyme reduced a non-physiological electron acceptor, cytochrome *c*, using a wavelength corresponding to its maximal absorbance. The uninhibited activity of the mutant enzymes was compared to the uninhibited activity of wild-type human CYPOR. The mutant A287P had the highest uninhibited activity in comparison to wild type human CYPOR. V492E activity was greatly decreased to approximately 10% in comparison to wild type human CYPOR. The activity of the enzymes was also compared to a control, which contained the enzyme, but no inhibitor. DPI had the greatest inhibition efficiency with almost 0% activity detected with all enzymes. KK-7 was also an efficient inhibitor with an average of 3% residual activity. The mutant R457H was the most affected by KK-7 inhibition. MMB-2 had almost no inhibitory effect with most enzymes retaining approximately 100% activity. The activity of the mutant A287P was the most affected by MMB-2, being decreased by 20%. The mutant V492E showed a slight, but significant increase in activity in the presence of MMB-2. The three ABS mutant CYPORs showed evidence of differential inhibition and increased susceptibility to inhibition in comparison to wild type CYPOR.