

A Fluorescent Real Time Assay for Inhibition of Sirtuin Deacetylase Activity

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Sirtuins (Sir2) are a family of NAD⁺-dependent protein deacetylases that have roles in regulation of transcription, metabolism, cellular stress response, gene silencing and DNA repair in eukaryotes, archaea and bacteria. These enzymes use NAD⁺ as a cofactor to hydrolyze the acetyl moiety of the target protein and produce lysine, 2'-O-acetyl-ADP-ribose and nicotinamide. Sirtuins, like all enzymes, increase the velocity of a specific chemical reaction.

The main focus of this project was expression and purification of CobB, measurement of CobB activity to determine its kinetic properties, and enzymatic inhibition analysis. After propagation of sirtuin expression vector and transformation of an *E.coli* expression strain with the vector, CobB was expressed and purified using immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC), and the purified proteins were analysed using denaturing gel electrophoresis (SDS-PAGE) and Coomassie staining.

CobB enzymatic activity was established using a real time fluorometric assay SIRT1 and the CLARIOstar multiplate reader, and kinetic values K_m , K_{cat} and V_{max} for CobB were determined. The K_m value (Michaelis-Menten constant) determines the concentration of substrate at $\frac{1}{2} V_{max}$. V_{max} is maximum velocity of a reaction and the enzyme is saturated with substrate at this point. The K_{cat} value measures the number of substrate turn over per enzyme per time unit. All kinetic values were measured using fluorometric methods. Finally, the two sirtuin inhibitors EX527 and Salermide were applied to investigate their effects on the CobB enzyme. Both inhibitors were weak inhibitors for CobB. EX527, which is a novel sirtuin inhibitor, was selected for determination of IC_{50} and K_i values.

The results showed that EX527 is a weak inhibitor of CobB and the K_i value of this inhibitor is higher than K_m . The IC_{50} value for EX527 with CobB was larger than for human sirtuin like SIRT1, SIRT2 and SIRT3. The IC_{50} value is the concentration of an inhibitor corresponding to 50% inhibition of an enzyme. Pharmacologically useful inhibitors generally have low K_i values in comparison to than K_m but the K_i value of the CobB is larger than K_m .

The results from this project were published in an application note for the CLARIOstar multiplate reader (BMG Labtech application note number 292).