Using Kinetics and Small Angle X-ray Scattering to Elucidate the Allosteric Regulation of SIRT1

SIRT1 is an NAD+ dependent deacetylase located in the nucleus and cytoplasm of the cell.\(^1\) Research has shown that SIRT1 has been implicated to be involved in many important cellular functions such as neurodegenerative diseases and aging.\(^2\) SIRT1 has also been studied with resveratrol, a polyphenol, which was shown to modulate activity.\(^3\) While the mechanism of SIRT1 has been studied extensively, exact details are still not known, specifically the allosteric regulation by the N-terminal domain. We want to see if there is a correlation between the activity of SIRT1 and the distance of the N-terminal domain relative to the catalytic core. First we will examine the kinetic parameters ($k_{cat}$ and $K_M$) of SIRT1 with peptide substrates with and without resveratrol. A recent study examined the activity of SIRT1 on peptides of different acetylation sites and found that resveratrol could either increase, decrease, or have no change on SIRT1 activity.\(^3\) We will use a continuous coupled enzyme kinetics assay, rather than an endpoint assay, to see if the main contributors to these changes were either $K_M$ or $k_{cat}$. From our studies, we find that $K_M$ is the main contributor of the changes, suggesting that resveratrol affects SIRT1 activity by a change in substrate recognition, likely by altering the conformation of SIRT1. We are also determining the overall conformation change of SIRT1 using Small Angle X-ray Scattering (SAXS) techniques. We are examining the conformation of SIRT1 complexed with different combinations of resveratrol and peptide substrates. Current SAXS profiles clearly show that resveratrol alters the overall conformation of SIRT1, however more experiments are needed to confirm whether there is a correlation between these conformational effects and resveratrol's effect on SIRT1 activity. Taken together, these studies will help us better understand the mechanism of SIRT1 activity regulation by small molecules, specifically in terms of conformational change.

\(^1\) Feldman, J.L., Dittenhafer-Reed, K.E., Denu, J.M. Sirtuin catalysis and regulation. Biol. Chem. 2012, 276(51), 42419-42427
\(^3\) Lakshminarasimhan, M., Rauh, D., Schultkowski, M., Steegborn, C. Sirt1 activation by resveratrol is substrate sequence-selective. Aging. 2013, 5(3), 151-154

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