

The Effects of Motif A on the Enzymatic Activity of SIRT1

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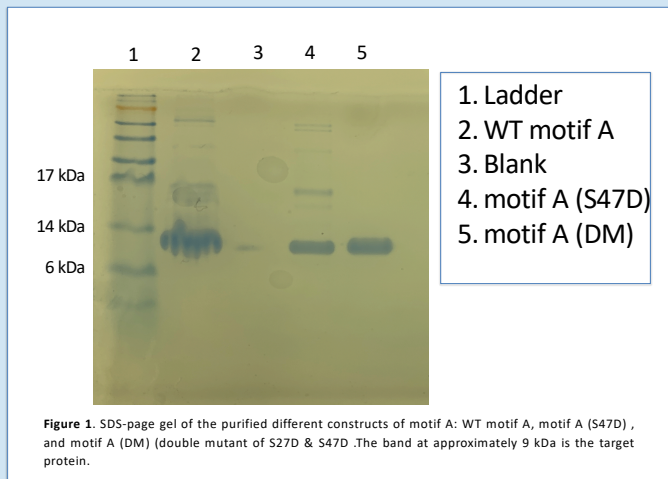
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Abstract

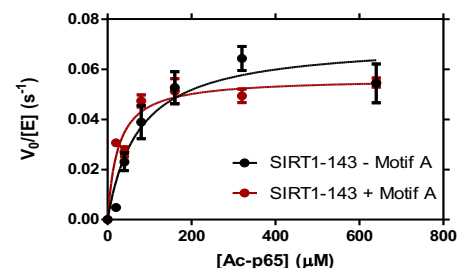
SIRT1 is an NAD⁺-dependent deacetylase that regulates many cellular pathways and have been shown to alleviate symptoms of Alzheimer's upon activation¹. We are examining the effects of motif A, a disordered region within the N-terminal region on the enzymatic efficiency of SIRT1. Previous studies have shown overexpression of the N-terminal region of SIRT1 can increase its catalytic efficiency *in situ*². Unfortunately those studies were unable to be replicated *in vitro*. Studies have shown that phosphorylation of motif A upregulates SIRT1 activity^{3,4}. We will use enzyme-coupled assays to compare motif A's effect on SIRT1 activity with and without phosphomimic mutants. To this end, we have mutated the two serine residues to aspartates which mimic the structure of phosphorylated serine.

Project Activities or Findings

- Mutated the two serine residues to aspartates using site-directed mutagenesis. (S27D, S47D single mutants and S27D S47D double mutant)
- Expressed and purified WT motif A, motif A (S47D), and motif A (DM) (double mutant of S27D & S47D)
- SIRT1 enzymatic activity *in vitro* against Ac-p65 is upregulated upon addition of WT motif A, and the main difference is seen in K_M values.



WT Motif A Increases SIRT1 Activity Against Ac-p65



SIRT1 activity	- Motif A	+ Motif A
k_{cat} (s^{-1})	0.071 ± 0.0072	0.056 ± 0.0030
K_M (μM)	74 ± 22.9	25 ± 6.2
k_{cat} / K_M ($M^{-1}s^{-1}$)	960 ± 312	2240 ± 568

Figure 2. Michaelis-Menten graph and table comparing SIRT1-143 (truncated version of SIRT1) enzymatic activity on the substrate p65 with and without 4 μM Motif A⁵. There is a significant decrease in K_M values.

Research Questions

- Will WT motif A have an effect on SIRT1 activity? Would this effect be motif A-concentration dependent?
- Will the *in vitro* phosphomimic of motif A have different effects on SIRT1 activity compared to WT motif A?

Citations

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4. Sasaki, T., Maier, B., Koclega, K. D., Chruszcz, M., Gluba, W., Stukenberg, P. T., ... Scrabble, H. (2008). Phosphorylation Regulates SIRT1 Function. *PLoS ONE*, 3(12), e4020.
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