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Substrate-specific Effect on Sirtuin Conformation and Oligomerization

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ABSTRACT

Human sirtuins are a family of nicotinamide adenine dinucleotide (NAD^+)-dependent enzymes that are responsible for removing acyl modifications from lysine residues. Sirtuins are involved in the formation and proliferation of cancers and are thought to regulate the progression of neurodegenerative diseases. Although sirtuins can be pharmacologically targeted by small molecules, it is not easy to modulate the substrate selectivity of sirtuins despite the chemical diversity of their substrates. Here, we report substrate-specific effects on sirtuin conformation and oligomerization that regulate enzyme deacylase activity. We used fluorescent acyl peptide probes to study substrate interactions with two sirtuin isoforms: SIRT2 and SIRT6. We observed that some of the fluorescent acyl peptides bind sirtuins and change their conformation, whereas other probes bind sirtuins without causing such structural changes. Our fluorescent probes also revealed that SIRT2 forms a dimer at relevant cellular concentrations (~100 nM) in contrast to SIRT6, which is exclusively monomeric. SIRT2 undergoes a conformational transition from dimer to monomer when bound to myristoyl-substrate which slows its demyristoylase reaction, but SIRT2 remains dimeric when performing its deacetylase reaction. Our fluorescent peptide probes will continue to be used to examine substrate specific effects on sirtuin structure and function in order to understand how to pharmacologically modulate sirtuin substrate selectivity.

INTRODUCTION

- Sirtuins are a family of nicotinamide adenine dinucleotide (NAD^+)-dependent enzymes that are responsible for removing acyl modifications from lysine residues [1].
- Humans have seven sirtuin proteins, SIRT1-7, and all of them share an evolutionarily conserved central catalytic domain [1-2].
- Each Sirtuin prefers to remove specific acyl modifications from substrates [3].
- Sirtuins are implicated in various cellular processes including transcription, DNA repair, glucose homeostasis, and cell proliferation [4-7].
- Sirtuins are involved in a variety of diseases including cancers and neurodegenerative diseases [8-12].
- Sirtuins can be pharmacologically targeted by small molecules [13-14]. However, it is not easy to modulate their substrate selectivity despite the chemical diversity of their substrates.

METHODS

- Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)
- High-performance liquid chromatography (HPLC)
- Size exclusion chromatography (SEC)
- Electrophoretic mobility shift assay (EMSA)
- Cuvette-based binding assays
- Partial Sirtuin proteolysis

RESULTS

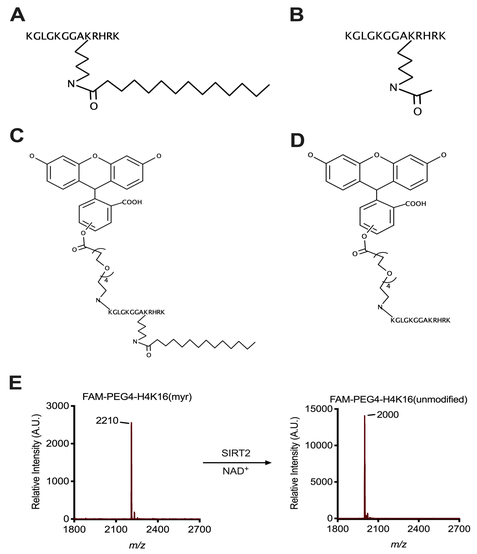

Figure 1. Sirtuin substrate structures. (A) Myristoylated H4K16 peptide; the amino acids sequence of histone H4 is shown. (B) Acetylated H4K16 peptide. (C) Structure of FAM-PEG4-H4K16(myr) peptide. (D) Structure of FAM-PEG4-H4K16(unmodified) peptide. (E) SIRT2 removed the myristoyl modification from FAM-PEG4-H4K16(myr) peptide in the presence of NAD^+. High-performance liquid chromatography was then used to purify the product from this reaction.

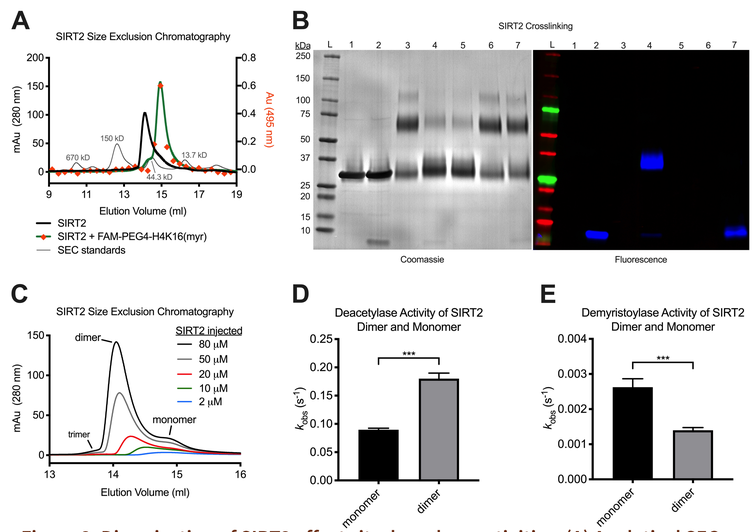

Figure 2. Dimerization of SIRT2 affects its deacylase activities. (A) Analytical SEC chromatograms for SIRT2, the SIRT2—FAM-PEG4-H4K16(myristoyl) peptide complex, and SEC standards. (B) SIRT2 crosslinking experiment with Bis-(NHS)-PEG5 as visualized by SDS-PAGE. Lane1: SIRT2 only; lane 2: SIRT2 + FAM-PEG4-H4K16(myr) peptide; lane 3: SIRT2 + crosslinker; lane 4: SIRT2 + FAM-PEG4-H4K16(myr) peptide + crosslinker; lane 5: SIRT2 + unlabeled H4K16(myr) peptide + crosslinker; lane 6: SIRT2 + unlabeled H4K16(ace) peptide + crosslinker; lane 7: SIRT2 + FAM-PEG4-H4K16(unmodified) peptide + crosslinker. The fluorescence image (right panel) was taken before staining by Coomassie blue. (C) Analytical SEC chromatograms collected while analyzing multiple SIRT2 concentrations. (D) The deacetylase activity of primarily dimeric or monomeric populations of SIRT2. (E) The demyristoylase activity of primarily dimeric or monomeric populations of SIRT2. *** denotes $p < 0.001$.

Proposed SIRT2 Deacylase Activity Mechanisms

(A) E + AcylR ⇌ E*AcylR + NAD^+ ⇌ E*AcylR*NAD^+ ⟹

(B) E*E + AcylR ⇌ E*E*AcylR ⇌ E + E*AcylR + NAD^+ ⇌ E*AcylR*NAD^+ ⟹

(C) E*E ⇌ E + E + AcylR ⇌ E + E*AcylR + NAD^+ ⇌ E*AcylR*NAD^+ ⟹

(D) E*E + AcylR ⇌ E*E*AcylR + NAD^+ ⇌ E*E*AcylR*NAD^+ ⟹

Figure 3. Proposed SIRT2 reaction mechanisms. (A) Substrate binds to SIRT2 monomer and forms a monomer-substrate complex, then upon binding the co-factor NAD^+, the reaction proceeds forward. (B) Substrate first binds to SIRT2 dimer and forms a dimer-substrate complex, then one SIRT2 molecule dissociates from the complex to create a complex that binds to NAD^+. (C) Dimerized SIRT2 dissociates into monomer, which can then form a monomer-substrate complex. Again, binding to NAD^+ allows the reaction to proceed. (D) SIRT2 forms a dimer and forms a dimer-substrate complex that can bind NAD^+. E: enzyme (SIRT2); AcylR: acylated substrate.

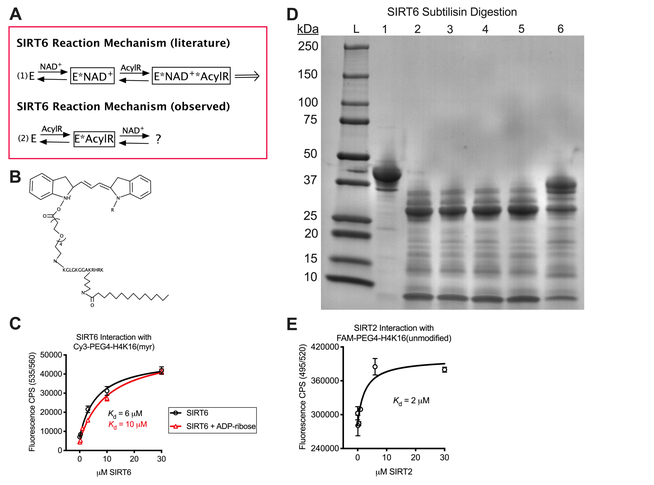

Figure 4. Non-productive interaction between Sirtuins and substrate. (A) Probable SIRT6 deacylase activity mechanisms. (1) SIRT6 binds to NAD^+ and forms a complex, then upon binding of substrate, the reaction proceeds. (2) SIRT6 binds to substrate first before NAD^+. E: enzyme (SIRT6); AcylR: acylated substrate. (B) Structure of Cy3-PEG4-H4K16(myr) peptide. (C) The affinity of SIRT6 for Cy3-PEG4-H4K16(myr) peptide. (D) Partial digestion of SIRT6 with subtilisin as observed on a Coomassie-stained SDS-PAGE gel. Lane 1: SIRT6 only; lane 2: SIRT6 + subtilisin; lane 3: SIRT6 + FAM-PEG4-H4K16(myr) peptide + subtilisin; lane 4: SIRT6 + Cy3-PEG4-H4K16(myr) peptide + subtilisin; lane 5: SIRT6 + unlabeled H4K16(myr) peptide + subtilisin; lane 6: 300μM ADP-ribose. (E) The affinity of SIRT2 for FAM-PEG4-H4K16(unmodified) peptide.

CONCLUSIONS

- Myristoyl peptides, but not acetyl peptides, change the conformation and oligomerization of SIRT2.
- Oligomeric transitions of SIRT2 regulate deacylase activities.
- Some fluorescent probes form non-productive complexes with Sirtuins.

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