High-Throughput Analysis of Epigenetic Targets with Agilent RapidFire/MS Systems: Sirtuin (SIRT) Enzymes

Application Note

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Introduction

Histones and other proteins are subject to a variety of posttranslational modifications that regulate a host of biological processes. One such modification is the deacetylation of specific lysine residues by sirtuins, whose activity has been associated with inflammatory, cardiovascular, proliferative, neurodegenerative, and metabolic disorders.[1] High-throughput bioassays designed to identify sirtuin modulators are therefore of interest, particularly when coupled to the highly-sensitive and specific detection available with mass spectrometry (MS).

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Fast and Direct Measurement of Multiple Reaction Products

Studying sirtuins by RapidFire/MS offers numerous advantages over other existing assay formats.[2] First, the system circumvents the costs and special handling procedures required when using radioactive substrates. Second, RapidFire/MS based assays do not involve secondary or coupled reactions that can complicate data interpretation. Third, because the peptide species are measured directly by MS, there is no need for fluorescent tags which can cause data artifacts. Finally, in situations where multiple products can be formed on a single peptide (which is common for sirtuins and other epigenetic targets), MS detection enables every modification state to be measured discretely and concurrently.[2, 3] Figure 1 shows a triply-acetylated (3ac) p53 peptide, which is a good candidate for RapidFire/MS analysis because it has three possible deacetylation products (2ac, 1ac, and 0ac).

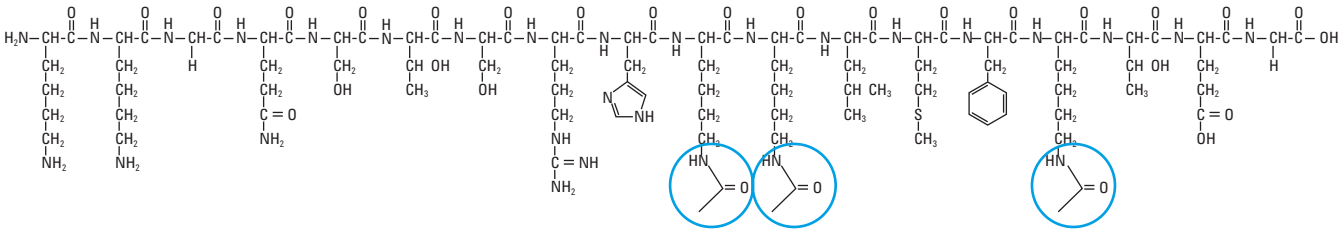

Figure 1. The p53 peptide spanning amino acids 372 to 389 contains multiple lysine residues (including 381, 382, and 386), which are known deacetylation sites.[4]

Fast and Direct Measurement of Multiple Reaction Products

The RapidFire platform enables mass spectrometric analysis of native molecules by performing high-throughput online desalting. Following enzymatic reactions between SIRT1 enzyme and a multiply-modified substrate, RapidFire/MS/MS was used to monitor every possible acetylation state of the peptide with a sustained throughput of seven seconds per sample. Analysis by RapidFire/MS/MS enabled the fast and direct measurement of multiple acetylated species (Figure 2).

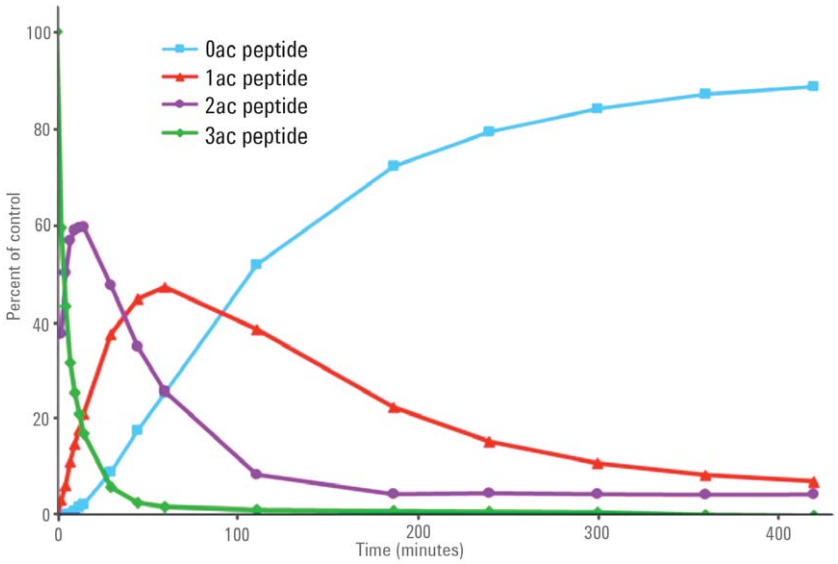

Figure 2. Sequential modification of a triply-acetylated p53 peptide by SIRT1 over time.

Label-Free Assays with Meaningful Data

The RapidFire/MS system measures native molecules directly, thereby obviating the need for fluorescently-tagged materials or secondary reactions which can be susceptible to compound interference. Label-free analysis enables substrate-to-product conversion measurements to be made directly, facilitating the determination of accurate kinetic parameters for hit identification. Figure 3 shows SIRT1, SIRT2, and SIRT3 inhibition curves with IC_{50} values that concur with those from previous reports.[5,6]

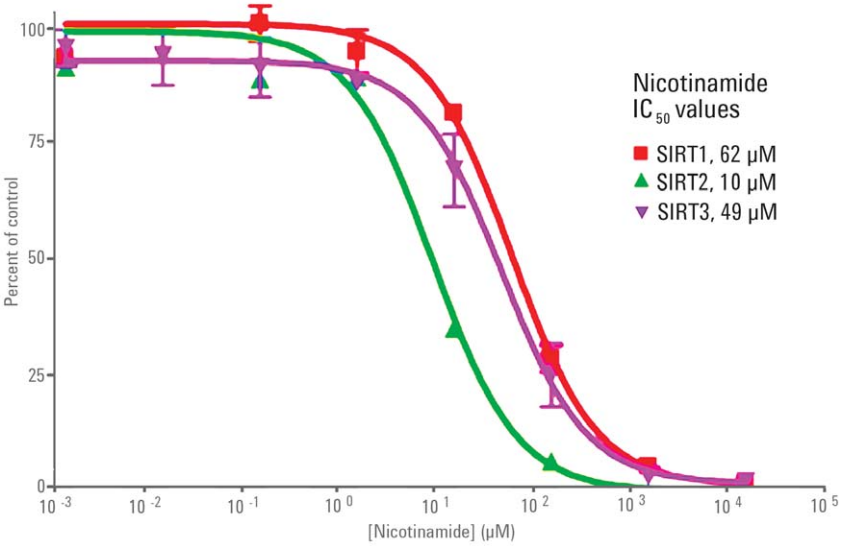

Figure 3. SIRT1, SIRT2, and SIRT3 inhibition curves using the small molecule nicotinamide.

Conclusions

RapidFire/MS/MS was used to analyze SIRT1, SIRT2, and SIRT3 reactions at a sustained rate of approximately seven seconds per sample. The label-free method circumvented the need for non-native surrogate substrates and radioactive methodologies. RapidFire/MS/MS enabled direct detection of peptide reactants and facilitated the identification of kinetic parameters for accurate IC_{50} determinations. Because RapidFire/MS can measure multiple analytes from each sample quickly and directly, the system is particularly well-suited for studying epigenetic changes which involve sequential modifications.

References

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Published in the USA, November 21, 2011
5990-9345EN

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