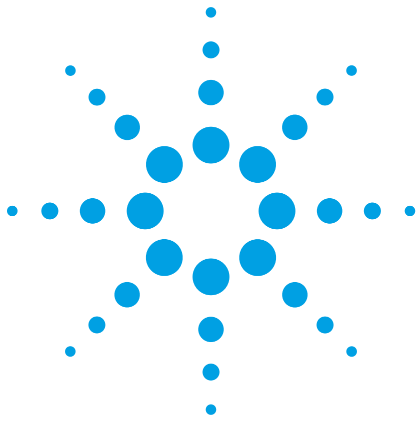

High-Throughput Analysis of Epigenetic Targets with Agilent RapidFire/MS Systems: p300 Histone Acetyltransferase (HAT)

Application Note

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Introduction

The field of epigenetics focuses on the investigation of enzymes that alter gene expression through modification of their target substrates, frequently by adding or removing acetyl groups. Historically, studying the activity of such enzymes in a high-throughput manner has proved challenging due to the relatively small molecular alterations as well as the possibility of sequential modifications leading to multiple analytes of interest. The p300 protein, for example, can serially acetylate multiple lysine residues on the C-terminus of the p53 protein. As such, high-throughput bioassays that allow the direct, concurrent quantification of multiple modification states are advantageous.[1, 2]

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Fast and Direct Analysis of Multiple Reaction Products

The RapidFire platform enables high-throughput mass spectrometric analysis of native molecules from *in vitro* reactions by performing online desalting in seconds. The system has traditionally been used in conjunction with triple quadrupole mass spectrometers to measure multiple peptide species simultaneously by MS/MS. However, recent developments in high-throughput MS have taken advantage of time-of-flight (TOF) mass spectrometers, and facilitated the fast and direct measurement of epigenetic changes to whole protein substrates.

In this application note, we analyze the p300 mediated acetylation of a p53 peptide by RapidFire/MS/MS and whole histone proteins by RapidFire/TOF. Figure 1 shows a p53 peptide with four known lysine acetylation sites, making it a good candidate for RapidFire/MS analysis.

Preferred acetylation sites for p300

Figure 1. The peptide spanning amino acids 368 to 386 of the p53 protein contains four known lysine acetylation sites[3] (residues 372, 373, 381, and 382) and is therefore a good candidate reactant for RapidFire/MS detection.

Rapid, Direct Measurement of Multiple Reaction Products

In Figure 2, we demonstrate the p300-mediated sequential acetylation of a p53 peptide (Amino acids 368-386) by RapidFire/MS/MS. Analysis by RapidFire/MS/MS enabled the fast and direct measurement of multiple acetylated species concurrently.

In addition, we analyzed whole histone proteins by RapidFire/TOF to illustrate a range of options for measuring HAT activity by high-throughput mass spectrometry. Figure 3 shows the inhibition of p300 by garcinol using whole histones as the substrate. Deconvoluted RapidFire/TOF data were acquired for 18 reactions (endpoint IC_{50} for 6 concentrations in triplicate) and indicated increasing acetylation with decreasing inhibitor concentration.

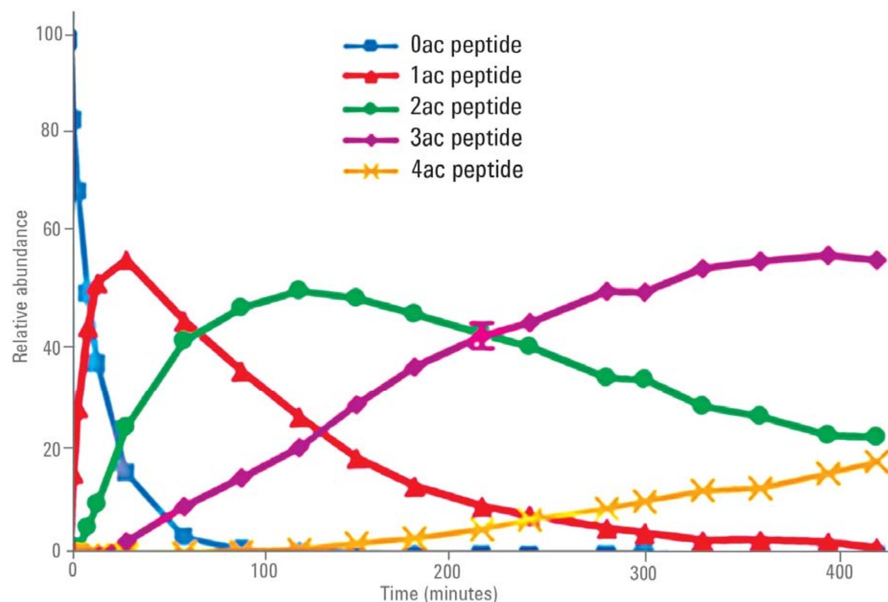

Figure 2. Sequential acetylation of a p53 (368-386) peptide by p300 over time.

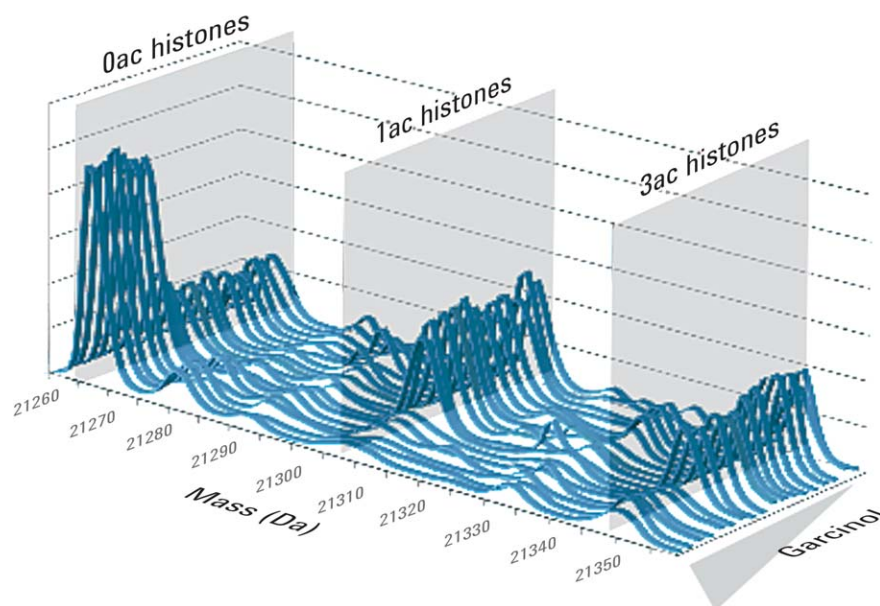

Figure 3. Inhibition of p300 by garcinol when using whole histone proteins as a substrate. Deconvoluted RapidFire/TOF data show increasing acetylation (going towards back) with decreasing inhibitor concentration.

Further evidence of the benefit of the RapidFire/MS technique is demonstrated in Figure 4. Here the data shows the inhibition of p300 acetylation by curcumin, garcinol, and anacardic acid as determined by RapidFire/MS. The calculated IC_{50} values are in good agreement with those previously reported.[4, 5]

Conclusions

The RapidFire/MS/MS and RapidFire/TOF systems were used to analyze p300 reactions at a sustained rate of ~7 seconds per sample. The label-free methods circumvented the need for non-native surrogate substrates and radioactive methodologies.

RapidFire/MS/MS enabled direct detection of peptide reactants while RapidFire/TOF enabled direct detection of whole protein species. Analysis of the reactions established kinetic parameters suitable for determining IC_{50} values that agreed well with those previously reported.[2,3]

Epigenetic changes which involve sequential modifications are an ideal application for the RapidFire System because RapidFire/MS can directly detect multiple states of peptides and proteins discretely.

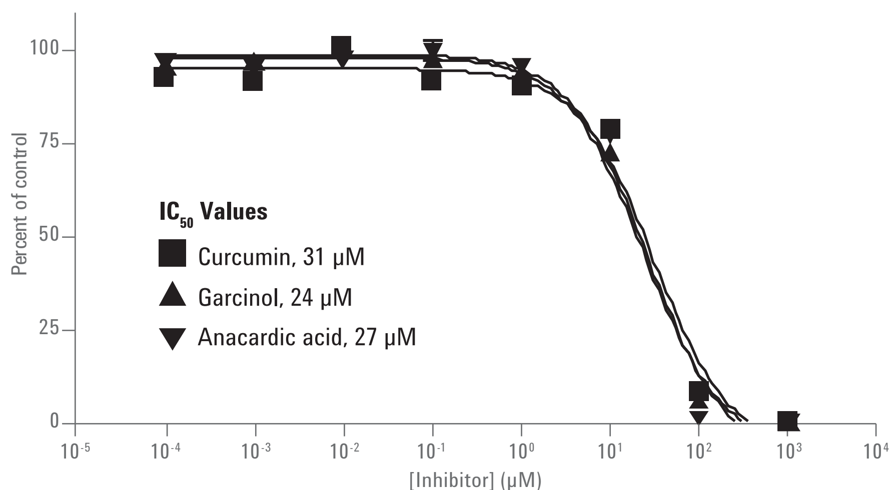

Figure 4. Inhibition of p300 by curcumin, garcinol, and anacardic acid as determined by RapidFire/MS techniques.

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