High-Throughput MS Detection of Histone H3 Demethylation by Lysine-Specific Demethylase 1 (LSD1)

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 methyl or 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 products. The LSD1 protein, for example, can serially demethylate multiply-modified lysine residues on histone H3. As such, high-throughput bioassays that enable the direct quantification of multiple modification states are beneficial.[1, 2]

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Using RapidFire/MS to Analyze Histone H3 Demethylation

Mass spectrometry is particularly well-suited for the measuring epigenetic modifications because it enables the concurrent analysis of multiple species and is highly sensitive. However, because MS requires sample purification prior to injection and the throughput of MS-based detection has historically been limited. The Agilent RapidFire platform relieves this bottleneck by performing online sample preparation with an approximately 7 second cycle time. As a result, RapidFire/MS systems provide the advantages of MS-based detection with high-throughput speeds, facilitating the collection of superior data for epigenetic targets.

In this application note, we illustrate the results obtained using the RapidFire/MS system to analyze the serial demethylation of histone H3 by LSD1. Figure 1 shows the sites of sequential demethylation (from K4me2 to K4me1 and K4me0) by LSD1.

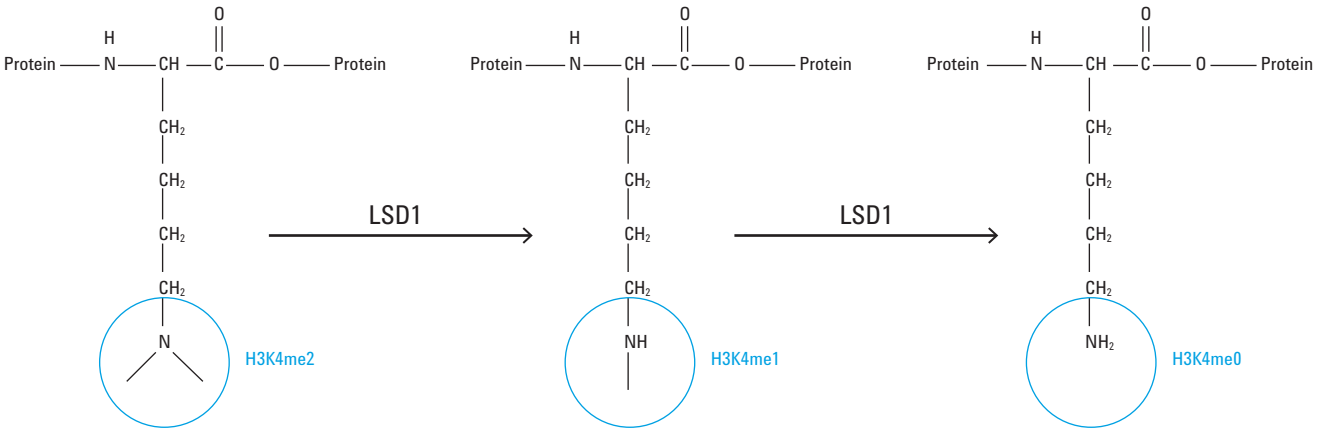

Figure 1. LSD1 removes methyl groups consecutively from specific methylated lysine residues in proteins.

Fast and Direct Analysis of Multiple Reaction Products

The RapidFire-MS platform enables the direct concurrent detection of native analytes in high-throughput mode. Following typical enzymatic reactions, RapidFire/MS was used to monitor each possible methylation state of a histone peptide. In Figure 2, the sequential demethylation of Histone H3 is demonstrated over time. Direct substrate-to-product conversion measurements allowed determination of linear conversion ranges and reaction velocities (Figure 3) and enabled the determination of the Michaelis constant. The calculated K_m value (3.5 mM) matches the literature value reported for similar reaction conditions (4.2 mM).[3]

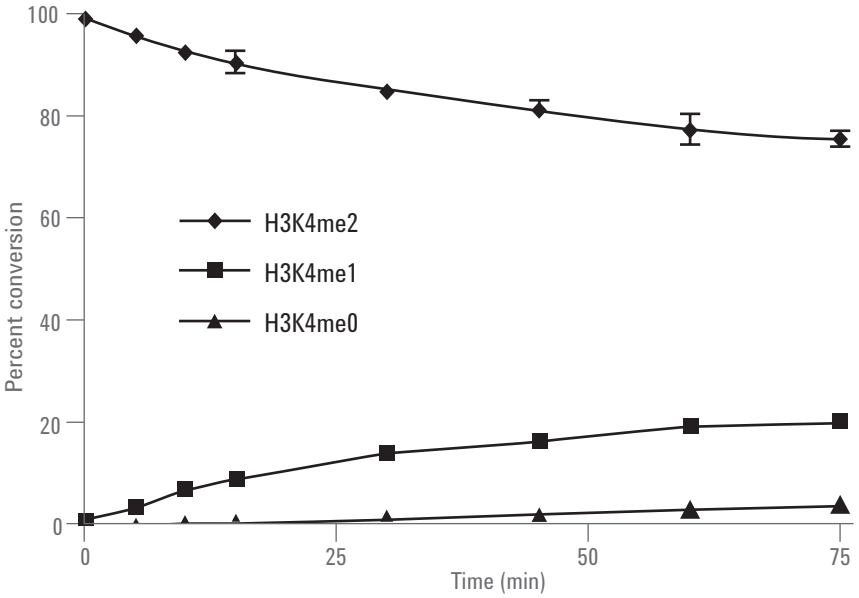

Figure 2. RapidFire/MS data displaying the sequential demethylation of a Histone H3 peptide over time.

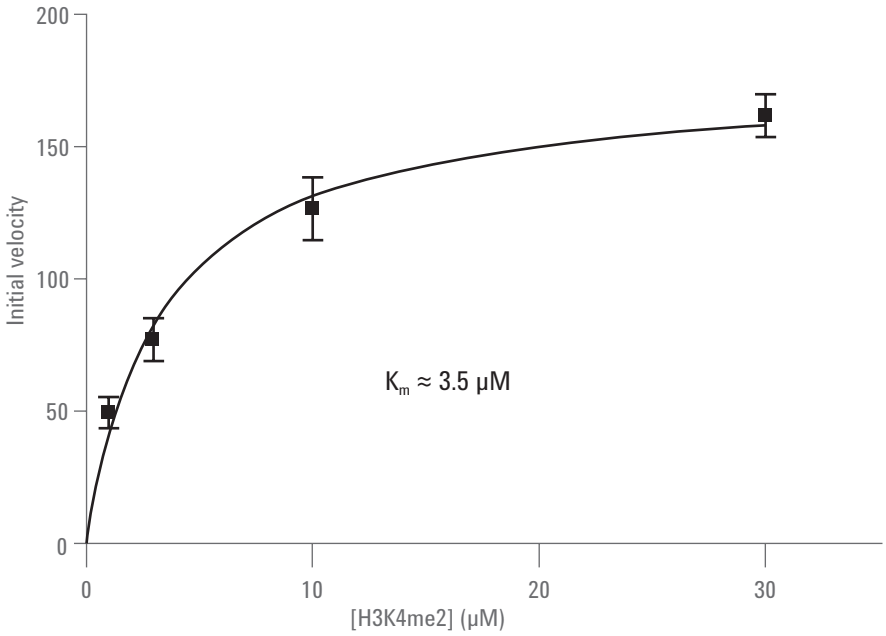

Figure 3. Plot of initial reaction velocity as a function of substrate concentration enables the Michaelis constant to be determined as 3.5 µM.

In addition, the RapidFire/MS technique was used to measure the inhibition of LSD1 by tranylcypromine (Figure 4). In this experiment, an IC_{50} value of 54 µM was obtained. This IC_{50} value is in good agreement with one previously reported for similar reaction conditions.[4]

Conclusions

RapidFire/MS was used to analyze LSD1 reactions at a sustained rate of ~7 seconds per sample. The label-free method circumvented the need for non-native surrogate substrates and radioactive methodologies. RapidFire/MS analysis of LSD1 reactions established kinetic parameters suitable for determining Km and IC50 values that agreed well with those previously reported. Because RapidFire/MS can measure multiple analytes from each sample quickly and directly, the system is particularly applicable for studying epigenetic changes which involve sequential modifications, and provides unparalleled data for lead discovery.

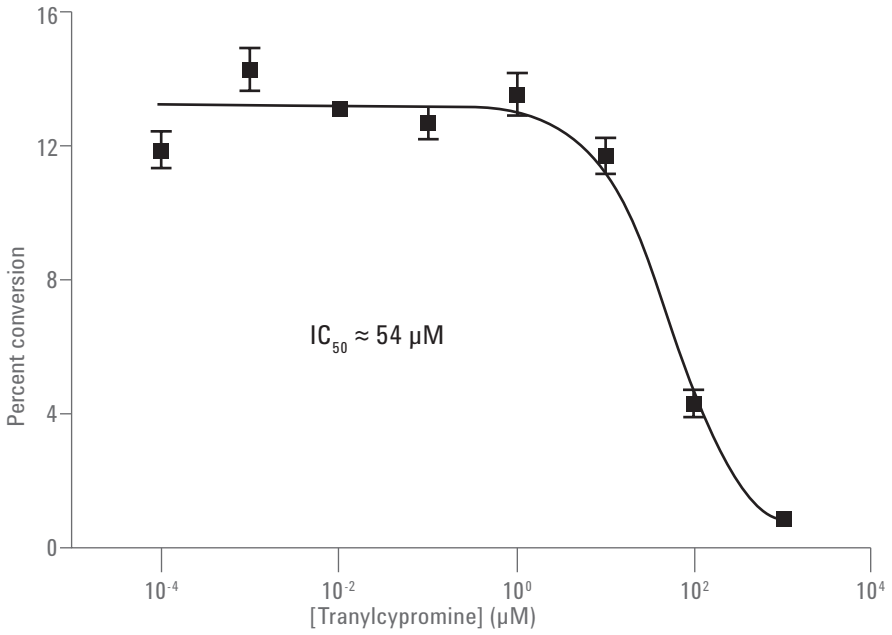

Figure 4. Inhibition of LSD1 by tranylcypromine displays an IC_{50} value of 54 µM.

References

1. Rye PT, *et al.* Advances in Label-Free Screening Approaches for Studying Sirtuin-Mediated Deacetylation. *J Biomol Screen*., **2011**, Sep 12. [Epub ahead of print].
2. Rye PT, *et al*. Advances in Label-Free Screening Approaches for Studying Histone Acetyltransferases. *J Biomol Screen*. **2011**, Sep 9. [Epub ahead of print].
3. Forneris, F. *et al*. Human Histone Demethylase LSD1 Reads the Histone Code. *J Biol Chem*., **2005**, 280(50):41360-41365.
4. LabChip Assay: Histone H3 Demethylase LSD1. *Caliper Life Sciences Application Note #302*.

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