A fluorescence-based assay of the epigenetic enzyme histone deacetylase 1 (HDAC1)

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- **Histone modifications caused by HDAC are strongly related to epigenetic regulation of transcription**
- **HDAC inhibitors (HDIs) are a possible strategy in cancer therapy**
- **Fluorescent HDAC activity and inhibitor assay was performed on a microplate reader from BMG LABTECH**

Introduction

Post-translational histone modifications like acetylation play a pivotal role in the epigenetic regulation of transcription. Catalyzing the latter reaction HDACs affect various cellular processes especially cancerogenesis. Although the mechanism of starting cancerogenesis by epigenetic events is not clearly explained inhibition of HDACs has highlighted as a viable principle in cancer therapy. Inhibition of HDACs results in histone overacetylation that in turn can lead to a controlled cell death (apoptosis). Several HDAC inhibitors (HDIs) are in phase I or II clinical trials, for example suberoylanilide hydroxamic acid (SAHA; ZOLINZA®, Merck). In the last years research focused on the development of selective HDIs. To determine the inhibitory effect fluorogenic assays with recombinant proteins could offer a valuable performance. In this application note, a fluorescence microplate reader from BMG LABTECH was used to determine the inhibitor effect of SAHA against HDAC1.

Assay Principle

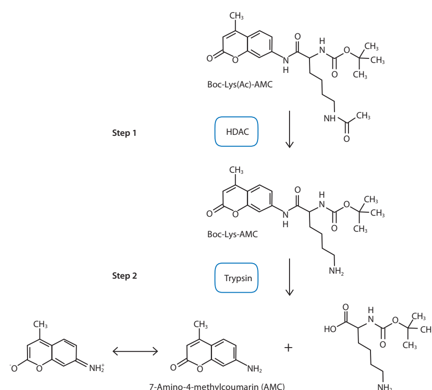

Fig. 1: Assay principle for HDAC activity evaluation.

Determination of HDAC1 activity was perfomed by a two-step enzyme assay. The principle bases on the ε-acetylated lysine moieties deacetylation of the substrate Boc-Lys(Ac)-AMC caused by HDAC. In a second step the deacetylated substrate is cleaved by trypsin resulting in the release of fluorogenic AMC (7-Amino-4-methylcoumarin; Fig.1).

Materials & Methods

- Microplate reader (BMG LABTECH, Ortenberg, Germany)
- Black 96-well half area plates (Greiner Bio-One)
- HDAC1 (BPS Bioscience)
- Boc-Lys(Ac)-AMC (Bachem)
- Trypsin from bovine pancreas (Sigma)
- SAHA (Cayman Chemical Company)
- FB188 buffer (15 mM Tris-HCl pH 8.0, 50 mM KH_2PO_4/K_2HPO_4, 250 mM NaCl, 250 µM EDTA and 0.001 % Pluronic F-68)

Evaluation of the K_M value of HDAC1

To determine the K_M value measurements using different substrate concentrations were carried out in FB188 buffer. In a first step HDAC1 (4.5 nM final concentration) was incubated with a 1:2 serial dilution of the substrate Boc-Lys(Ac)-AMC (initial substrate concentration was 512 µM). After a 1 hour incubation at 30°C trypsin (1.7 mg/mL) and SAHA (5 µM) were added in order to stop the reaction and to release the fluorogenic AMC (excitation filter: 340/10, emission filter: 460/10).

Evaluation of the IC_{50} value with SAHA versus HDAC1

Performing the assay as described above HDAC1 was incubated with SAHA for 15 min at room temperature after performing a 1:3 serial dilution (initial value 35 µM). Substrate was added in the second step with a final concentration of 20 µM followed by a incubation of 1 h at 30°C. Trypsin (1.7 mg/mL) and SAHA (5 µM) finished the reaction and AMC was measured directly.

Results & Discussion

The K_M value of HDAC1 was determined by using different substrate concentrations (Fig. 2).

Each data point corresponds to an extra kinetic measurement. The RFU (relative fluorescence unit) values are the average of the last 5 data points after the equilibrium of the enzymatic reaction is reached. The resulting K_M value was determined to be 58.89 µM.

Subsequent to the K_M evaluation the assay was performed in presence of a known HDAC1 inihbitor (SAHA). While the substrate concentration was strict at 10 µM the inhibitor were used in a range between 35 µM and 0.002 µM (Fig. 3).

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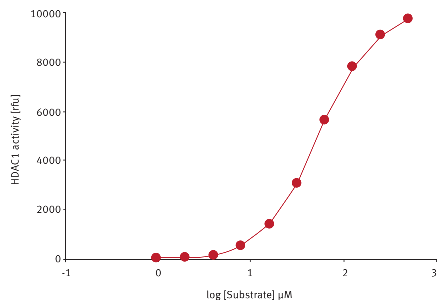

Fig. 2: K_M value of Boc-Lys(Ac)-AMC with HDAC1. HDAC1 (4.5 nM) was incubated with different concentrations of the substrate Boc-Lys(Ac)-AMC after performing a two-step activity assay by measuring the release of AMC (excitation filter: 340/10 nm; emission filter: 460/10 nm).

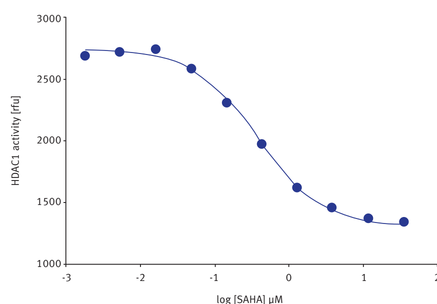

Fig. 3: IC_{50} value of HDAC1 with SAHA. HDAC1 (4.5 nM) was incubated with 1:3 serial dilution of SAHA and 20 µM Boc-Lys(Ac)-AMC after performing a two-step activity assay by measuring the release of AMC (excitation filter: 340/10 nm; emission filter: 460/10 nm).

The two-step activity assay using SAHA as inhibitor, results in a IC_{50} value of 374 nM.

Conclusion

Determining the activity of recombinant HDAC1 with a fluorogenic substrate using a microplate reader from BMG LABTECH offers high precision and performance. Using Boc-Lys(Ac)-AMC it has been shown that a wide range of substrate concentration results in a stable detectable signal. Furthermore, the assay allows for the determination of inhibitory effects against HDAC isoforms 1,2,3 and 6 as well as bacterial histone deacetylase-like amidohydrolase HDAH from *Bordetella/Alcaligenes* strain FB188.

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