Investigation of the binding mechanism of dansyl hydroxamates to a histone deacetylase-like amidohydrolase by stopped-flow kinetics combined with global fit analysis

h_da
HOCHSCHULE DARMSTADT
UNIVERSITY OF APPLIED SCIENCES

C. Meyners, M. Zimmermann, S. A. Kalman, F.-J. Meyer-Almes

Department of Chemical Engineering and Biotechnology, University of Applied Sciences Darmstadt, Darmstadt, Germany

INTRODUCTION

The Investigation of binding mechanisms and the determination of kinetic rate constants provides useful information for the selection of promising compounds in the early phase of drug development. The simplest way to describe a binding reaction is a one step association and dissociation mechanism. In practice, binding mechanisms are often more complex and involve multiple binding sites or modes, cooperativity and induced fit or conformational selection steps. The assignment of a certain binding mechanism on the basis of observed rate constants is often hard and usually suffers from ambiguity. An approach to largely overcome these limitations is a global fit analysis. Thereby, all kinetic traces of a kinetic concentration dependence study are fitted simultaneously to a binding model[1]. As an example, the binding of a dansyl hydroxamate to a histone deacetylase-like amidohydrolase (HDAH) from *Bordetella/Alcaligenes FB188* was investigated by combining stopped-flow experiments with global fit analysis using Gepasi[2]. HDAH is a member of the therapeutically important histone deacetylase family. In solution HDAH forms a homodimer with two active sites which is consistent with published crystal structures (Fig. 1).

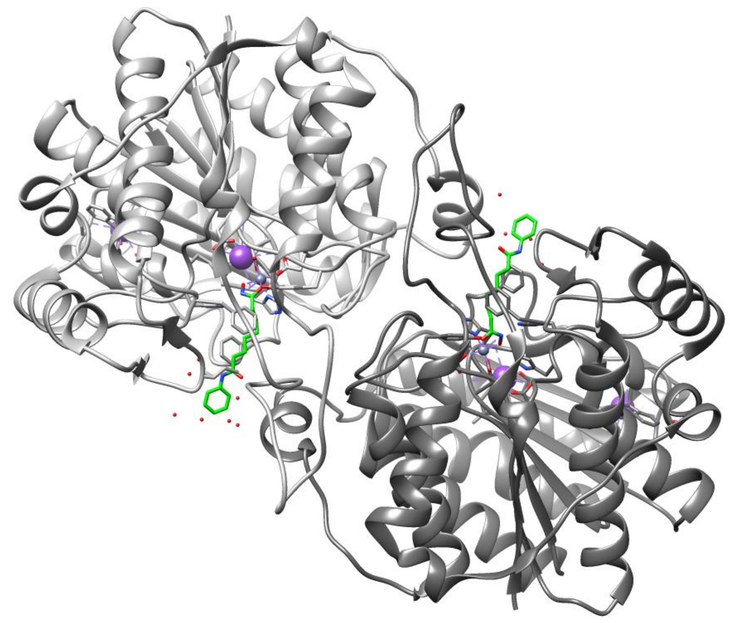

Fig. 1 Crystal structure of HDAH bound with SAHA (PDB ID 1zz1).

RESULTS

Binding of the dansyl-hydroxamate (Fig. 2) to HDAH resulted in a binding dependent förster resonance energy transfer from the intrinsic tryptophane residues to the dansyl moiety of the ligand (Fig. 2). This signal was exploited to determine the concentration dependency of the association kinetics by using a stopped-flow system. The observed kinetics had to be fitted to a two exponential function (Fig. 4). The observed rate constants for the fast and the slow reaction showed both a saturable concentration dependency (Fig. 5). For the determination of the binding mechanism all kinetic traces were implemented in the program Gepasi and subjected to a global fit analysis. From a total number of 20 different binding mechanisms, only ? one mechanism fitted well to the data (Tab. 1 and Fig. 4). The identified mechanism described a sequential binding reaction, which involved an induced fit and a positive cooperativity for the binding of the dansyl hydroxamate to the second binding site.

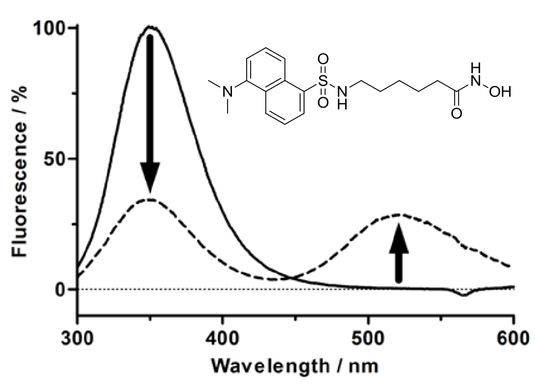

Fig. 2 FRET-Signal upon binding of the dansyl hydroxamate. Fluorescence spectra of 100 nM HDAH without (solid line) and with (dashed line) 12.8 µM of the dansyl hydroxamate (structure shown in the inlet).

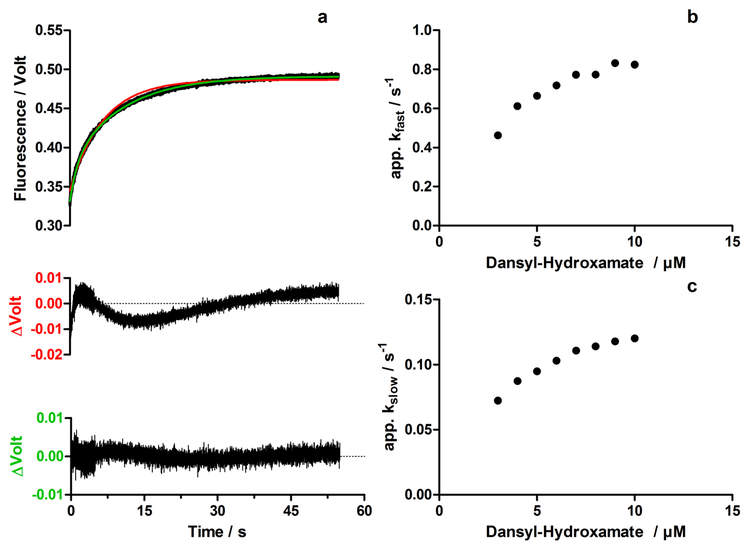

Fig. 3 Stopped-flow kinetics of the binding of the dansyl hydroxamte to 100 nM HDAH. a. association kinetics of 5 µM of the dansyl hydroxamate. The smooth lines represent a single (red) and a two (green) exponetial fit model. **b** and **c.** concentration dependence of the fast and the slow apparent rate constants.

Tab. 1 Top three mechanisms obtained by the global fit analyses. Mechanism **1** is a sequential binding mechanism, where *E* denotes the concentration of the dimer. For mechanism **2** and **3** *E* denotes the concentration of the monomers as the binding occurs simultaneously.

Nr.	Mechanism	Sum of squares
1	$E + L \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EL \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} EL^* \underset{-L,\, k_{-3}}{\overset{+L,\, k_3}{\rightleftarrows}} EL_2$	0.1696
2	$E_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} E_2 \underset{-L,\, k_{-2}}{\overset{+L,\, k_2}{\rightleftarrows}} EL \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} EL^* \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} EL^{**}$	0.2153
3	$E_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} E_2 \underset{-L,\, k_{-2}}{\overset{+L,\, k_2}{\rightleftarrows}} EL \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} EL^*$	0.2174

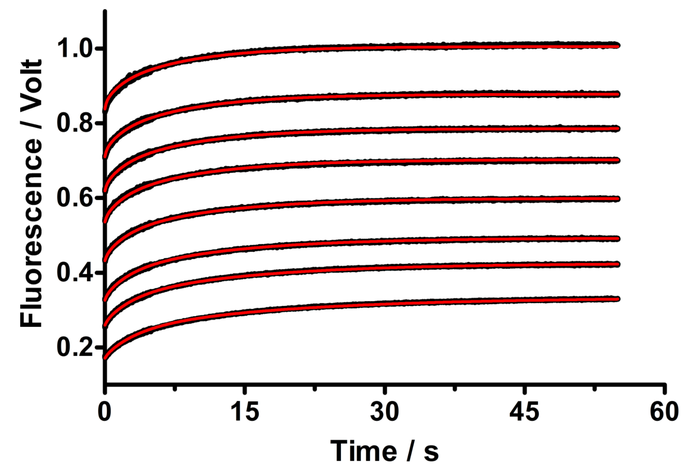

Fig. 4 Global fit analysis using mechanism 1 for the binding of increasing concentrations of the dansyl hydroxamate to HDAH. The smooth red lines represent the fitted curves.

DETERMINED MECHANISM

$$E + L \underset{1.48s^{-1}}{\overset{0.42\times10^6 M^{-1}s^{-1}}{\rightleftarrows}} EL \underset{0.099s^{-1}}{\overset{0.21s^{-1}}{\rightleftarrows}} EL^* \underset{-L,\, 0.012\ s^{-1}}{\overset{+L,\, 0.051\times10^6 M^{-1}s^{-1}}{\rightleftarrows}} EL_2$$

SUMMARY AND OUTLOOK

Investigation of the binding mechanism of a dansyl hydroxamate to HDAH revealed a rather complex binding reaction. Just on the bases of the apparent rate constants a determination of the binding reaction would have been difficult. Through the use of a global fit analysis 19 mechanism, which were in principal able to result in two saturable apparent rate constants, could be excluded. Currently**,** investigations are ongoing to confirm the proposed binding mechanism and to determine the molecular determinants by site directed mutagenesis. In our ~~point of~~ view the application of global fit analysis to the analysis of sets of high-density kinetic data is a valuable tool for the discrimination between possible binding **models, especially**, when the binding reactions are complex.

REFERENCES

[1] C. Meyners, M. G. J. Baud, M. J. Fuchter, F.-J. Meyer-Almes, *Anal. Biochem.* **2014,** *460*, 39-46.

[2] P. Mendes and D. B. Kell, *Bioinformatics* **1998,** *10*, 869-883.