J|A|C|S
COMMUNICATIONS

Published on Web 03/16/2006

A Mechanism-Based Inactivator for Histone Demethylase LSD1

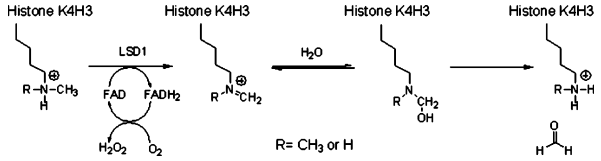
Jeffrey C. Culhane,† Lawrence M. Szewczuk,† Xin Liu,‡ Guoping Da,‡ Ronen Marmorstein,‡ and Philip A. Cole*,†

Department of Pharmacology and Molecular Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, and The Wistar Institute and the Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received January 13, 2006; E-mail: pcole@jhmi.edu

Histone modification has emerged as a major mechanism of regulation in gene expression, replication, and repair.[1] Such histone modifications are thought to affect histone interactions with DNA and other proteins.[2] Among the post-translational histone modifications, methylation of lysine residues has emerged as critical in blocking histone acetylation and mediating protein−protein interactions that can activate or repress transcription.[3] Lysine methylation was viewed as a permanent histone mark until recently when the first histone lysine demethylase, LSD1 (also called BHC110), was discovered.[4] LSD1 belongs to the amine oxidase family[5] which are flavin-dependent enzymes that utilize O_2 and generate H_2O_2 and formaldehyde as byproducts (Scheme 1). LSD1 has been shown to be specific for Lys-4 of histone H3 and can oxidatively demethylate the dimethyl or monomethyl Lys-containing substrates.[4]

Scheme 1. Proposed Mechanism of LSD1 Catalysis

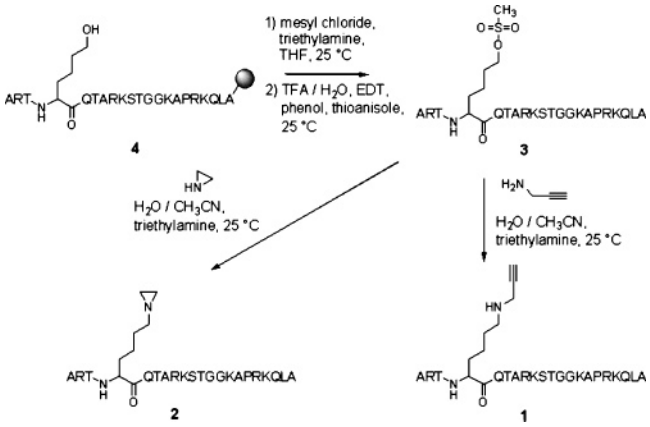


LSD1 functions as a transcriptional repressor,[4] and synthetic inhibitors of LSD1 would likely serve to activate a subset of genes and might play a role in therapeutics. Pargyline, a clinically useful small molecule monoamine oxidase inhibitor, has recently been proposed to block LSD1,[6] but this has not been reproduced with an in vitro system.[7] Here we report that a propargyl-Lys-derivatized peptide **1** functions as a potent and selective time-dependent inactivator of LSD1.

We targeted two lysine derivatives (**1** and **2**) as potential LSD1 inhibitors based on structural considerations and in analogy to known strategies for blocking amine oxidases.[8] We pursued a post-assembly modification synthetic strategy[9] particularly because of the instability of the aziridine function. Thus, a side-chain-protected peptide containing an oxa-analogue of lysine at the fourth position of a 21 amino acid N-terminal histone H3 tail (**4**) was constructed on solid support. After mesylation, the protected peptide was cleaved from resin and deblocked allowing for HPLC purification of the mesylate peptide (**3**). Displacement of the mesylate with either propargylamine or ethyleneimine afforded the desired compounds **1** and **2** (Scheme 2). While compound **1** was found to be stable, compound **2** was found to decompose when lyophilized to dryness. However, both compounds could be stored indefinitely in dilute acidic solution at −80 °C.

Compounds **1** and **2** were assayed against recombinant LSD1 using a H_2O_2 detection assay as recently described.[7] While

Scheme 2. Synthesis of LSD1 Inhibitors



compound **2** showed moderately potent inhibitory action against LSD1 with an $IC_{50} = 15.6 \pm 1.7$ μM, there was no evidence of time-dependent inhibition (Figure S1). It is likely acting as a reversible competitive inhibitor[10] without generating an enzyme inactivating species. In contrast, compound **1** showed clear time-dependent inhibition of LSD1.[11,12] The rate of enzyme inactivation was dependent on the concentration of **1**, as shown in Figure 1A. Further evidence for time-dependent enzyme inactivation came from preincubation studies. As shown in Figure 1D, allowing compound **1** to incubate with enzyme prior to addition of substrate (inactivated) gave more pronounced inhibition than simultaneous addition of substrate and inhibitor (1.5 μM **1**) to enzyme. From the progress curves in Figure 1A, the rate of inactivation (k_{obs}) could be calculated from a nonlinear curve fit of the data at each inhibitory concentration. A replot of these data fit nicely to a rectangular hyperbola according to the Kitz−Wilson equation.[13] In this way, a $K_{i(inact)} = 16.6 \pm 3.4$ μM and $k_{inact} = 0.258 \pm 0.030$ min^{-1} were determined. The k_{cat}/k_{inact} is 5.4, which suggests that the rate of inactivation is in the same range as the rate of substrate dealkylation.

Further kinetic analysis of the mechanism of inhibition of LSD1 involved varying the substrate concentration with a fixed inhibitor concentration. As can be seen in Figure 1C, the k_{obs} decreases with increasing substrate concentration, suggesting that the substrate can protect against time-dependent inactivation by **1**. A Dixon analysis of these data yielded a K_d apparent for substrate (diMeK4H3-21) of 80 ± 15 μM (Figure 1C), in reasonable agreement with its K_m of 200 μM in our assay conditions. This is consistent with the expected model that binding of substrate and compound **1** is mutually exclusive.

To gain further insight into the mechanism of inhibition of LSD1 by **1**, a mass spectroscopic analysis of a mixture of LSD1 incubated with compound **1** was performed. As can be seen in Figure 2A, while the starting compound **1** is still abundant, two significant

† The Johns Hopkins University School of Medicine.
‡ University of Pennsylvania.

10.1021/ja0602748 CCC: $33.50

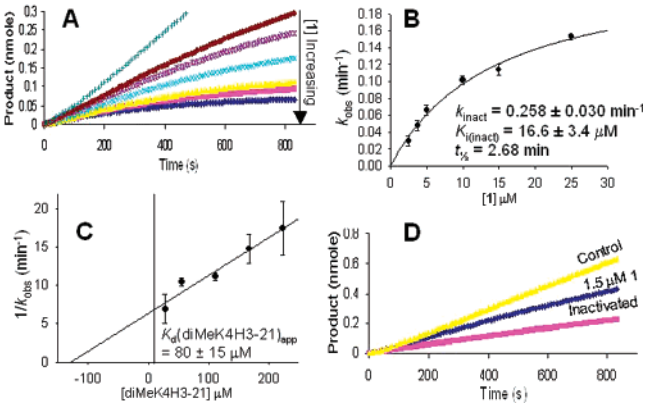


Figure 1. Time- and concentration-dependent inactivation of LSD1 by **1**. (A) Steady-state progress curves obtained for the inactivation of LSD1 by 0, 2.5, 3.75, 5, 10, 15, and 25 μM **1**. (B) Rate constants (k_{obs}) for the time-dependent inactivation of LSD1 by **1** were extracted from steady-state data by single-exponential fits and analyzed by the method of Kitz and Wilson. (C) Rate constants (k_{obs}) for the time-dependent inactivation of LSD1 by 10 μM **1** were determined in the presence of increasing concentrations of a competitive substrate (diMeK4H3-21). (D) LSD1 (11.24 μM) was preincubated with 100 μM **1** for 30 min at 25 °C then diluted 66-fold into the assay to measure remaining activity.

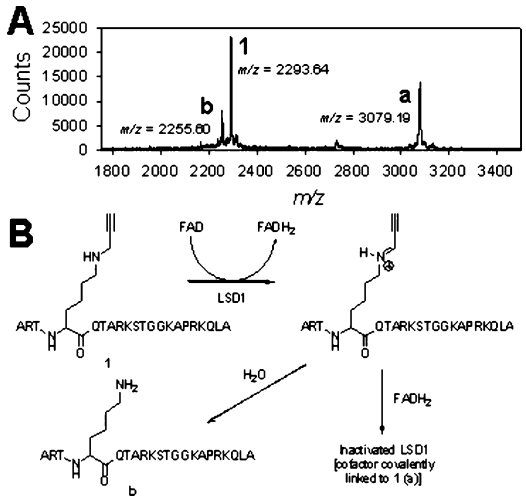


Figure 2. (A) MALDI-TOF spectrum of inhibitor−FAD conjugate. (B) Proposed scheme for inactivation of LSD1 by **1**.

peaks have appeared. The lower peak corresponds to a loss of 38.04 (**b**), which would represent dealkylation of the propargyl group. More interestingly, a peak at *m*/*z* 3079.19 (**a**) appears which corresponds to the predicted molecular weight of FAD linked to **1**. Note that no similar covalent adduct is observed by mass spectroscopy after treatment of LSD1 by **2** or the diMeK4H3-21 peptide substrate (see Figures S1 and S2). Such covalent adducts have been observed in other amine oxidases reacting with propargylamine-based inhibitors and suggest a potential chemical mechanism for inhibition.[8,14,15] It is plausible that the reduced FAD ($FADH_2$) undergoes nucleophilic attack on the propargylic imine and creates the covalent adduct observed here (Figure 2).

We were also interested in examining the specificity of compound **1** as an MAO B inhibitor and thus tested **1** as a potential inhibitor of MAO B.[7,16,17] With up to 100 μM of **1**, MAO B was not detectably inhibited. This indicates that compound **1** is at least 40-fold selective against another amine oxidase, presumably because of the markedly different substrate features.

Compound **1** thus represents a prototype for designed inhibitors of LSD1. Since it is likely to be poorly bioavailable in its current form, future work will be needed to minimize the structural features necessary for enhanced pharmacokinetic properties. However, **1** itself can presumably be useful for in vitro transcriptional analysis or for structural studies. It may be possible to use **1** in cellular studies with delivery by microinjection, permeabilizing reagents, or by linkage to cell penetrating peptide sequences.[18] Furthermore, in the future identification and characterization of novel demethylases, compounds related to **1** may play a useful role in proteomics analysis.[19] In this regard, the recent discovery of a non-heme iron-dependent histone demethylase[20] suggests that the known enzymatic strategies for methyl−lysine cleavage may be incomplete.

Acknowledgment. We would like to thank Ramin Shiekhattar and Min G. Lee for helpful discussions. We thank the NIH (U54RR020839) for financial support as well as the JHMI mass spec core facility. This paper is dedicated to Prof. C. H. Robinson on the occasion of his 77th birthday.

Supporting Information Available: Experimental details are available for the solid phase assembly of **1**−**4**, expression and purification of LSD1, measurement of LSD1 activity, and MALDI-TOF analysis of the FAD conjugate. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Schreiber, S. L.; Bernstein, B. E. *Cell* **2002**, *111*, 771.
(2) Santos-Rosa, H.; Caldas, C. *Eur. J. Cancer* **2005**, *41*, 2381.
(3) Martin, C.; Zhang, Y. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 838.
(4) Shi, Y.; Lan, F.; Matson, C.; Mulligan, P.; Whetstine, J. R.; Cole, P. A.; Casero, R. A.; Shi, Y. *Cell* **2004**, *119*, 941.
(5) Binda, C.; Mattevi, A.; Edmonson, D. E. *J. Biol. Chem.* **2002**, *277*, 23973.
(6) Metzger, E.; Wissman, M.; Yin, N.; Müller, J. M.; Schneider, R.; Peters, A. H. F. M.; Günther, T.; Buettner, R.; Schüle, R. *Nature* **2005**, *437*, 436.
(7) Forneris, F.; Binda, C.; Vanoni, M. A.; Battaglioli, E.; Mattevi, A. *J. Biol. Chem.* **2005**, *280*, 41360.
(8) Edmondson, D. E.; Mattevi, A.; Binda, C.; Li, M.; Hubálek, F. *Curr. Med. Chem.* **2004**, *11*, 1983.
(9) Weiss, G. A.; Valentekovich, R. J.; Collins, E. J.; Garboczi, D. N.; Lane, W. S.; Schreiber, S. L.; Wiley, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10945.
(10) While **2** could in principle be an alternate substrate, MALDI shows no evidence of processing by LSD1 (see Figure S1).
(11) Walsh, C. T. *Annu. Rev. Biochem.* **1984**, *53*, 493.
(12) Silverman, R. B. *Methods Enzymol.* **1995**, *249*, 240.
(13) Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245.
(14) Maycock, A. L.; Abeles, R. H.; Salach, J. I.; Singer, T. P. *Biochemistry* **1976**, *15*, 114.
(15) Binda, C.; Hubalek, F.; Li, M.; Herzig, Y.; Sterling, J.; Edmondson, D. E.; Mattevi, A. *J. Med. Chem.* **2005**, *48*, 8148.
(16) Hellerman, L.; Erwin, V. G. *J. Biol. Chem.* **1968**, *243*, 5234.
(17) Hubálek, F.; Binda, C.; Li, M.; Herzig, Y.; Sterling, J.; Youdim, M. B. H.; Mattevi, A.; Edmondson, D. E. *J. Med. Chem.* **2004**, *47*, 1760.
(18) Zheng, Y.; Thompson, P. R.; Cebrat, M.; Wang, L.; Devlin, M. K.; Alani, R. M.; Cole, P. A. *Methods Enzymol.* **2004**, *376*, 188.
(19) Saghatelian, A.; Cravatt, B. F. *Nat. Chem. Biol.* **2005**, *1*, 130.
(20) Tsukada, Y.; Fang, J.; Erdjument-Boramge, H.; Warren, M. E.; Borchers, C. H.; Tempst, P.; Zhang, Y. *Nature* **2006**, *439*, 811.

JA0602748