Measuring the Activity of Histone Lysine Methyltransferases in Enzymatic Assays Using LC-MS/MS

Olufola O. Ige[1], Wenxia Luo[1], Thordur Hendrickson-Rebizant[1] and Ted M. Lakowski[1]

[1]Pharmaceutical Analysis Laboratory, College of Pharmacy, University of Manitoba, Winnipeg, Manitoba, Canada

INTRODUCTION

Post translational modifications to histones are catalyzed by histone lysine methyltransferases (HMTs) and this epigenetic modification can alter gene expression. Epigenetic events are vital for normal cell functions and development. However, any aberrations in such events like HMT can yield phenotypic manifestations like cancer.

OBJECTIVE

- Develop a LC-MS/MS method to quantify HMT activity
- Determine the kinetic parameters of recombinant SET domain-containing lysine methyltransferase- 7/9 (SET 7/9)

METHOD

- SET7/9 and histone H3 were expressed in E coli cells.
- Immobilized metal affinity chromatography for SET7/9 purification
- HPLC-based Histone H3 purification
- LC-MS/MS method to measure mono- di- and tri- methyllysine from hydrolyzed methylated histones
- Determination of linearity of the reaction in terms of time and enzyme concentration, the pH optimum and apparent Km.

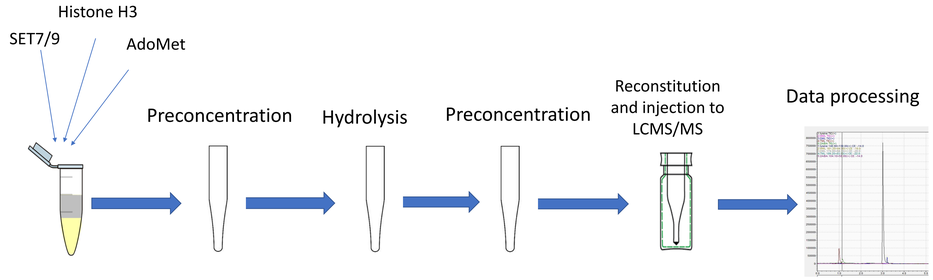

Fig 1. Sample processing scheme for the LC-MS/MS analysis

RESULTS

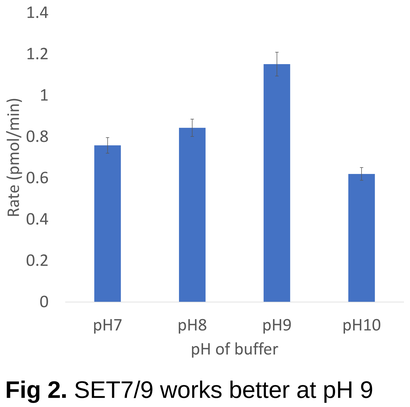

Fig 2. SET7/9 works better at pH 9

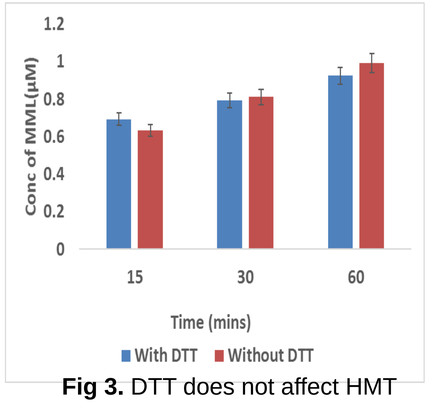

Fig 3. DTT does not affect HMT activity

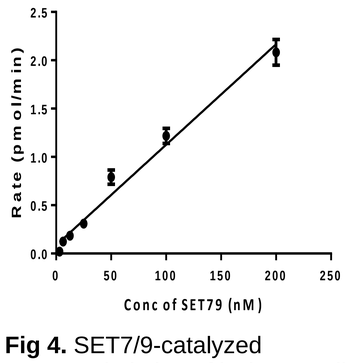

Fig 4. SET7/9-catalyzed monomethylation of lysine was linear up to 200 nM

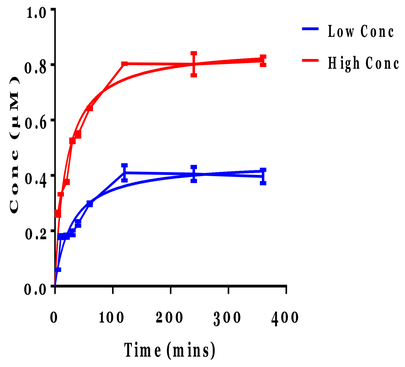

Fig 5. 60 min linear kinetic profile regardless of substrate levels

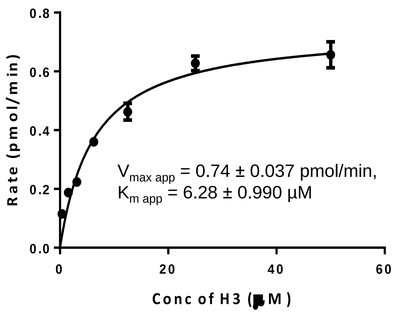

Fig 6. SET 7/9 kinetics as f([Histone H3])

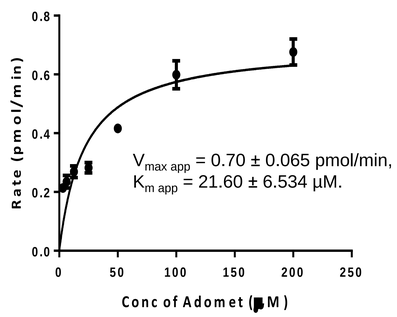

Fig 7. SET 7/9 kinetics as f([AdoMet])

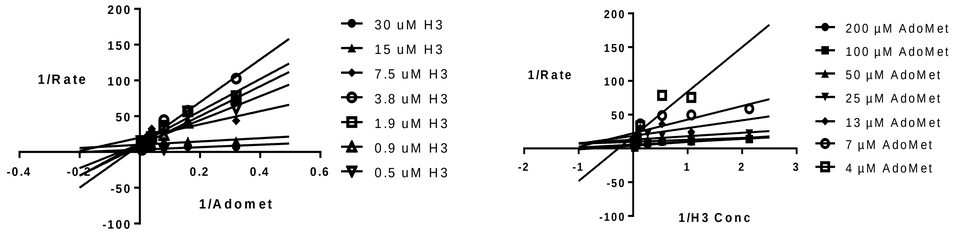

Fig 8. Sequential Bi Bi mechanism of SET7/9 was observed, as presented by the two inverse plots above

CONCLUSION

- We developed an LC-MS/MS method with three order of dynamic range
- The method is suitable for enzyme kinetics and HMT inhibitor assays

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