Structural Dynamics of the NSD Family of Histone Methyltransferases

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Nuclear SET domain containing protein 2 (NSD2) is a member of the NSD family of oncogenic histone methyltransferases (HMts) known for the methylation of histone 3 lysine 36 (H3K36) in vivo. NSD2 is involved in 20% of multiple myeloma cases and is associated with poor prognosis. Therefore, NSD2 is a high-profile target for inhibition. In previous studies, different constructs of NSD2 have been shown to have different activities on varying substrates (peptide, nucleosome, etc.). This variation in activity has great impact on the high-throughput screening aspect of drug discovery. In the absence of a crystal structure, we employed hydrogen-deuterium exchange mass spectrometry (HDX MS) to probe the conformational dynamics of the peptide backbone of two NSD2 constructs in the presence and absence of different substrates and cofactors.

METHODS

For the baseline dynamics measurement, NSD2 was placed in a deuterated 50 mM Tris buffer with 5 mM MgCl₂ at pH 8.5. The reaction exchange was quenched using 3 M GdmHCl and 0.8 formic acid at pH 2.5. The sample was then introduced to a Waters nanoACQUITY with HDX technology for online digestion and HPLC separation. A Waters Xevo QTof was used to measure the incorporation of deuterium into each peptide and data were analyzed using DynaX software. This process was repeated for the analysis of the effect of peptide binding and SAM binding on the dynamics of NSD2. For each experiment, a Kₐ of 20 μM was assumed. For the peptide binding experiment, 400 pmol peptide was incubated with 84 pmol SUMO-NSD2. For the SAM binding experiment, 420 pmol SAM was incubated with 98 pmol GST-NSD2 for 20 min.

The baseline deuterium incorporation of the larger construct was subtracted from the incorporation of the NSD2-SAM complex. Upon SAM binding, the larger construct experiences a small decrease in deuterium uptake, indicating a small degree of stabilization occurs.

The baseline deuterium incorporation of each protein was measured. The difference in incorporation was obtained by subtracting the uptake of the smaller construct from the uptake of the larger construct. The data is plotted to the left.

A small region of the larger construct (941-1240) experiences a decrease in deuterium uptake, indicating protection in this region (blue on structure).

Other regions of the larger construct experience an increase in uptake, indicating an increase in NSD2 dynamics in these regions (red on structure).

This increase in uptake, and thus dynamics could be indicative of a structural rearrangement required for engaging with the peptide substrate.

There were no significant differences in uptake between the bound and unbound states of the large construct.

The deuterium uptake of the NSD2:peptide complex was subtracted from the uptake of NSD2 alone.

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The baseline deuterium incorporation of the larger construct was subtracted from the incorporation of the NSD2-SAM complex. Upon SAM binding, the larger construct experiences a small decrease in deuterium uptake, indicating a small degree of stabilization occurs.

CONCLUSIONS

• N- and C-terminal extensions induce structural changes in NSD2
• Peptide binding induces structural rearrangement of NSD2
• "Gatekeeper" extensions prevent engagement with peptide
• SAM binding does not influence NSD2 dynamics

REFERENCES


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