# MEP50 Simulation of PRMT5 Methyltransferase Activity is Substrate-Dependent

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## Abstract

PRMT5 (Protein Arginine Methyltransferase 5) modifies a variety of protein substrates and plays roles in chromatin remodeling, RNA processing, regulation of gene expression, cell growth and differentiation. Its pro-proliferative effects and association with multiple cancers (lung, breast, ovarian, lymphoid) has led to increased interest in its possible targeting for anti-cancer therapy. PRMT5 can form a hetero-oligomeric complex, (PRMT5/MEP50), with MEP50 (Methylisoyaspartic Protein 50), which is itself a component of multiple macromolecular complexes (e.g. 20S Methylisoyaspartic, Swi5/Snf) located in both the nucleus and cytoplasm. It is unclear to what degree, if any, the activity of PRMT5 in the absence of MEP50 plays a role in vivo. The in vitro reconstituted activity of recombinant PRMT5 can be weak relative to that of (PRMT5/MEP50), but this has been assessed primarily with histone peptide or histone protein substrates (e.g. H2A, H4). Working in insect cell-derived recombinant PRMT5 and MEP50, we have surveyed the activity of PRMT5 and (PRMT5/MEP50) against a panel of substrates (Hela clipo- and moni-nucleosomes, chicken core histones, recombinant human histones H2A, H2B, H3.3 and H4 and GST (GST fused to glycinine arginine rich domain of fibrillarin, residues 139-331), in a protein substrate assay (GST, ubiquitin and tubulin b1), which was not reported to include MEP50. [M. Yanagida et al. J. Biol. Chem. 2014 279 1607]. The GAR domain of fibrillarin is methylated, although the methyltransferase responsible for that in vivo has not been identified. The activity of PRMT5 with GST in our in vitro assays would suggest that such a role could be played by PRMT5, even in the absence of complex formation with MEP50.

## Methods

Enzyme activity assays were carried out by determining methyltransferase activity of PRMT5 and PRMT5/MEP50 for a fixed substrate with 1 µM or 50 µM [3H]-SAM with the following reaction conditions: 50 mM Tris-HCl, pH 8.5, 50mM NaCl, 1mM DTT, 1mM PMSF, 30°C. All substrates assayed were E. coli expressed and purified recombinant proteins except for Hela nucleosome, core histones (chicken). Activity was measured as [3H]-precipitated counts of [3H]-SAM in a scintillation/wet filter plate assay (Multiscan FB, TopCount). Initial velocities were determined from the slope of the linear portions of time courses comprising multiple points between 0 and 90 minutes. Assay calibration with [3H]-BSA determined a conversion of 19.710 CPM/mol of [3H]-methyl. Data was fitted to three separate kinetic equations (Michaelis-Menten, General Velocity, and Bisubstrate Rapid Equilibrium) using Kaleidagraph. Kinetic parameters and curves are shown for fits with lowest sum of squares. Differential. The C elegans PRMT5 has been reported to act by a rapid equilibrium, random substrate binding mechanism.4

1. Michaelis-Menten Equation

   \[
   \frac{V_{\text{max}}}{K_m + S} = \frac{V_{\text{max}}}{K_m} + \frac{S}{K_m}
   \]

2. General Velocity Equation

   \[
   \frac{V_{\text{max}}}{K_m} \left( \frac{S}{K_m} + 1 \right) = \frac{V_{\text{max}}}{K_m} + \frac{S}{K_m}
   \]

3. Bisubstrate Rapid Equilibrium Equation

   \[
   \frac{V_{\text{max}}}{(K_m + K_a + K_b + 1)} \left( \frac{K_m + K_a + 1}{(K_m + K_a + B + 1)} \right) = \frac{V_{\text{max}}}{(K_m + K_a + K_b + 1)} + \frac{S}{K_m + K_a + K_b + 1}
   \]

## Results and Discussion

The initial rate kinetic results indicate that H2A/B methylation by PRMT5 is MEP50-dependent, H4 core histone methylations are MEP50-independent and GST methylation is MEP50-dependent. PRMT5/MEP50 has higher activity than PRMT5 on the protein substrates in the panel except for GST-GAR. Neither form of PRMT5 has methyltransferase activity on monoi- or oligo-nucleosomes. (PRMT5/MEP50), has methyltransferase activity on all histones, but displays substrate specificity particularly toward histone H2A and histone H4. In contrast, PRMT5 alone shows significantly less activity on individual histones; while displaying substantially similar kinetics to the complex on GST-GAR (somewhat higher K_v and V_max). The high activity of (PRMT5/MEP50) compared to PRMT5 on histone H2A and histone H4 is likely due to the significantly greater V_max of (PRMT5/MEP50) on these substrates as the roughly equivalent K_v values do not indicate significantly different binding affinities of (PRMT5/MEP50) and PRMT5 for histone H2A and histone H4. (PRMT5/MEP50) has a lower K_v than PRMT5 on GST-GAR with both saturating and non-saturating [3H]-SAM, indicating a possible tighter binding affinity to GST-GAR. Due to this K_v difference, and despite somewhat higher V_max's, the catalytic efficiency (K_v/V_max) of the complex is ~2x higher (1.7 vs. 0.92 saturating [3H]-SAM ). Since a region of fibbrilin outside the GAR domain is reported to interact with PRMT5, it will be of interest to reassess the kinetics of PRMT5 alone and the (PRMT5/MEP50) complex with the full-length protein, as opposed to the GAR domain. We are currently preparing protein from GST-tagged and untagged full-length fibbrilin constructs for this purpose.

## References

2. Ho et al. (2013) PLOS One 8 e57008
4. Wang et al. (2013) Biochemistry 52 9430