Control of Hexamerization, Assembly, and Excluded Strand Specificity for the Sulfolobus solfataricus MCM Helicase

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Results and Discussion

There has been increasing evidence supporting a steric exclusion and wrapping (SEW) model for DNA unwinding in which hexameric helicases interact with the excluded single-strand DNA (ssDNA) in addition to the encircled strand. Interactions with the excluded ssDNA have been shown to be mediated primarily by electrostatic interactions, but base stacking with surface exposed tyrosine residues is an alternative hypothesis. Here, we mutated several external tyrosine and positively charged residues from full-length Sulfolobus solfataricus MCM (SsoMCM) along the proposed path of excluded strand binding and assessed their impact on DNA unwinding. Four of the five tyrosine residues had significant decreases in unwinding, and one, Y519A, located within the α/β-α linker region of the C- terminal domain (CTD), had the most severe perturbation attributed to disruption of hexamerization. The Y519 mutant exhibits an enhanced and stabilized secondary structure that is modulated by temperature, binding DNA with greater apparent affinity and suggesting a pathway for hexameric assembly. HDX-14.5 T FT-ICR MS was used to map deuterium uptake differences between wild-type and Y519A apo structures highlighting global differences in solvent accessible areas consistent with altered quaternary structure (see Figure 1). Two of the five electrostatic mutants had significantly reduced DNA unwinding and combined with previous mutations better define the exterior binding path. The importance of the electrostatic excluded strand interaction was confirmed by use of morpholino DNA substrates that showed analogous reduced unwinding rates. These results better define the hexameric assembly and influence of the excluded strand interactions in controlling DNA unwinding by the archaeal MCM complex.

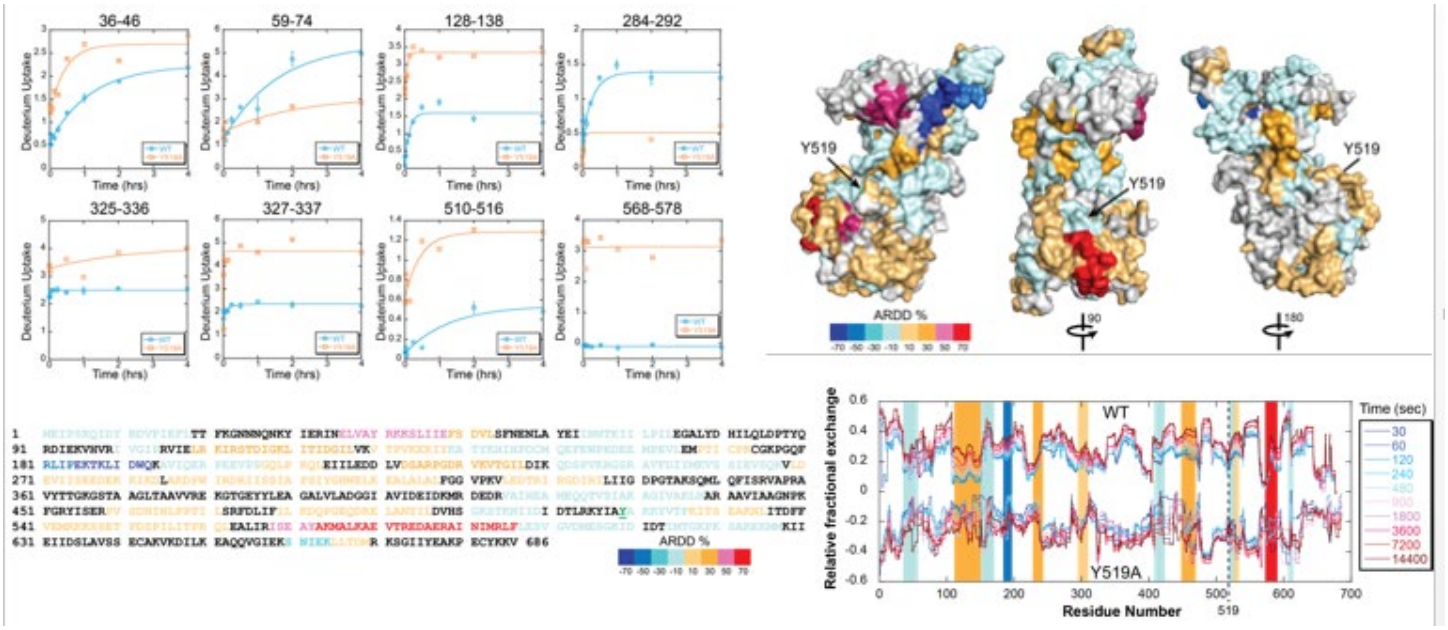

Fig. 1. HDX Deuterium uptake profiles for SsoMCM WT vs. Y519A. Top Left: Deuterium incorporation during the H/D exchange period for representative peptides, showing differences between the two conditions. Average relative deuterium uptake difference (ARDD) % difference color coding for (Bottom Left) primary sequence of SsoMCM (Y519 is underlined green) and (Top Right) peptide regions with significant ARDD % differences (Y519A minus WT) are color-mapped onto the surface of the SsoMCM monomer. (Bottom Right) HDX butterfly plots for SsoMCM WT (positive values) vs. Y519A (negative values) showing relative fractional exchange as a function of amino acid residue number. Lines are color-coded from shades of blue to red from 30 to 14,400 s deuterium exchange period. Dashed line: Y519A. Shaded regions correspond to significant changes in relative fractional change kinetics correlated with ARDD colors.

Acknowledgments

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References

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