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Rapid Report

The Glutamate Switch Is Present in All Seven Clades of AAA+ Protein†

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ABSTRACT: Recent work has identified a "glutamate switch" in six of the seven clades of AAA+ ATPases. The glutamate switch acts to transduce information regarding substrate binding to the ATPase active site. We provide biochemical evidence that a highly conserved threonine residue acts as a glutamate switch in the replicative helicase, MCM, and, thus, reveal that the glutamate switch is a feature common to all seven AAA+ clades.

The hyperthermophilic archaeon *Sulfolobus solfataricus* encodes a single homologue of the eukaryotic replicative helicase, MCM. All functional archaeal MCMs studied thus far form homomeric assemblies with a homohexamer believed to be the active form of the protein (*1*, *2*). The recent determination of the X-ray crystal structure of the *Sulfolobus* MCM (SsoMCM) has been an important step in unveiling the structural basis of the helicase activity of the enzyme (*3*). A second crystal structure of an archaeal MCM has also been determined, that of *Methanopyrus kandleri* MCM2 (*4*). However, that protein is inactive and appears to have accumulated a number of mutations in residues important for ATP binding and hydrolysis or for productive interactions with DNA (*5*).

MCMs are members of clade 7 of the AAA+ superfamily of ATPases. AAA+ proteins can be classified into one of seven distinct clades and generally function to harness the energy supplied during the ATPase cycle to effect conformation alterations upon a substrate macromolecule (*6*, *7*). In the case of MCM, this effects unwinding of duplex DNA to generate the single-stranded templates for DNA replication. In many cases, the ATPase activity of the AAA+ protein is modulated by binding to the substrate macromolecule. A recent study has proposed a mechanism for this phenomenon (*8*). During the catalysis of hydrolysis of the $\beta-\gamma$ phosphoanhydride bond in ATP, the glutamate in the conserved DExx motif of AAA+ proteins activates a water molecule to attack the γ-phosphate. Analysis of the crystal structures of a range of AAA+ proteins that were caught at different stages in the ATP cycle allowed comparison of the position of the glutamate in these different states. This revealed that this residue can adopt a variety of orientations. Furthermore, the glutamate was juxtaposed to a conserved polar or basic residue. Significantly, this second residue lay within a secondary structure element implicated in binding of the AAA+ proteins' target ligands. Thus, binding of the ligand has the potential to influence the ATPase activity of the AAA+ protein's active site via the polar residue. Accordingly, this polar or basic residue has been termed the glutamate switch (*8*). Structural and biochemical evidence of the presence of the glutamate switch in six of the seven clades of AAA+ proteins exists. However, to date there has been no report of the characterization of this feature in clade 7, a family of AAA+ proteins that includes MCM.

With the elucidation of a 4.35 Å resolution structure of residues 7−601 of SsoMCM, it became possible to superimpose the structure of SsoMCM with that of the clade 6 AAA+ family protein, PspF (Figure 1a−c). Analysis of the PspF has revealed that an asparagine residue (N64) acts as the glutamate switch, modulating the conformation of the crucial glutamate (E108) (*8*).

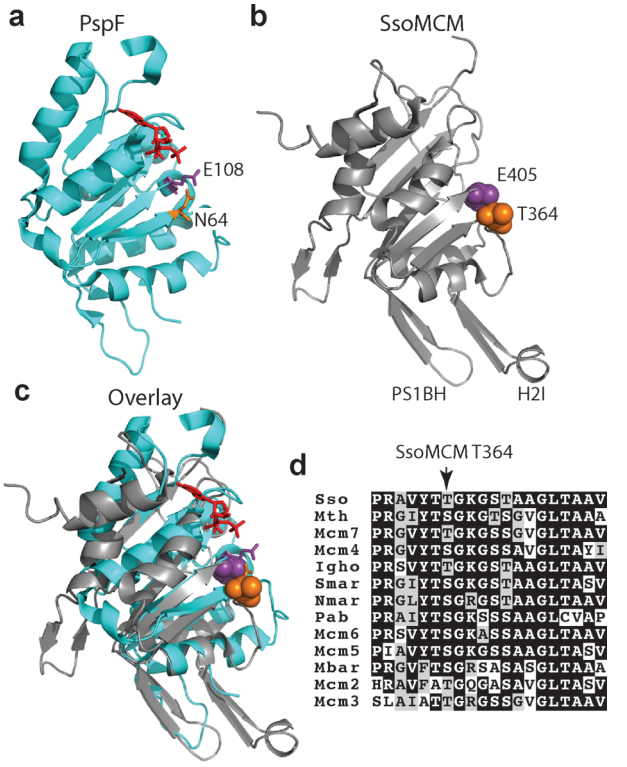

FIGURE 1: (a) Structure of the clade 6 AAA+ ATPase, PspF (residues 8−182). Asparagine 64 (N64) and glutamate 108 (E108) are colored orange and purple, respectively. This panel was generated from Protein Data Bank (PDB) entry 2C96. (b) Structure of SsoMCM residues 285−485 with the positions of threonine 364 (T364) and glutamate 405 (E405) colored orange and purple, respectively. The positions of the H2I element (*9*) and presensor 1 β-hairpin (PS1BH) (*10*) are indicated. This panel was generated from PDB entry 3F9V. (c) Overlay of panels a and b. Panels a−c were generated with Pymol (www.pymol.org). (d) Conservation of the candidate glutamate switch residue in a range of archaeal MCMs and budding yeast Mcm2-7: Sso, *S. solfataricus*; Mth, *M. thermautotrophicus*; Igho, *Ignicoccus hospitalis*; Smar, *Staphylothermus marinus*; Nmar, *Nitrosopumilus maritimus*; Pab, *Pyrococcus abyssi*; Mbar, *Methanosarcina barkeri*].

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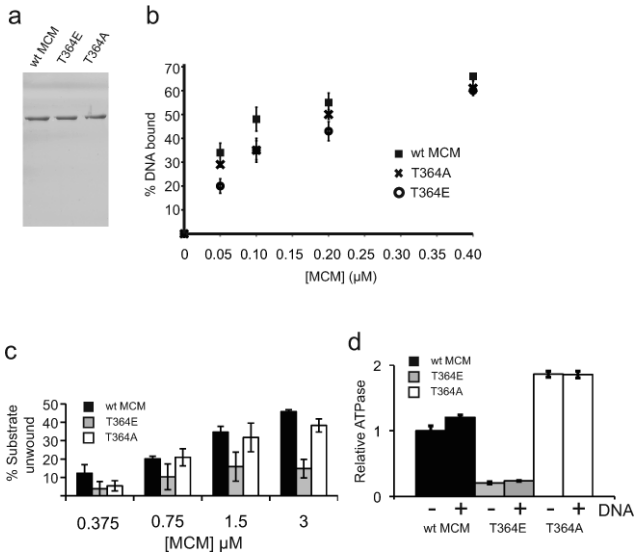

FIGURE 2: (a) Wild-type (wt), T364E, or T364A SsoMCM (2 μg) was electrophoresed on 10% SDS−PAGE gels and stained with Coomassie. (b) DNA binding by wt and mutant SsoMCM proteins. EMSAs were performed as detailed in the Supporting Information, and the fraction of the Y-shaped DNA substrate bound was quantified with a phosphorimager. (c) DNA helicase activity of wt and mutant proteins. Helicase assays were performed as detailed in the Supporting Information, and the fraction of the Y-shaped DNA substrate melted was quantified with a phosphorimager. (d) Relative ATPase activities of wild-type and mutant proteins in the presence and absence of DNA. The assays were performed with 2 μM SsoMCM (as a monomer). Values are expressed relative to the ATP hydrolysis rate of wt MCM in the absence of DNA [1.5 pmol of ATP hydrolyzed (pmol of MCM monomer)$^{-1}$ min^{-1}]. The assays presented in panels b−d were performed at least three times, and the standard error of the mean is indicated by the error bars.

Superposition of the SsoMCM and PspF structures reveals that the analogous positions in SsoMCM are occupied by T364 and E405. Comparison of the sequence of a range of archaeal MCMs, together with budding yeast MCM2-7, reveals absolute conservation of serine or threonine at the position corresponding to SsoMCM T364. Significantly, the glutamate switches in the clade 5 AAA+ proteins Hs1U and RuvBL1 are threonine and serine residues, respectively. Threonine and serine are polar residues and thus can interact with the catalytic glutamate. In MCM, the T364 residue is located in the proximity of the H2I element. The H2I element is a $\beta-\alpha-\beta$ insert within the AAA+ fold. Interestingly, studies of *Methanothermobacter thermautotrophicus* MCM revealed that deletion of the H2I element leads to an MCM with highly elevated double-stranded DNA-stimulated ATPase activity and increased affinity for DNA but which has no detectable helicase activity (*9*).

To test the contribution of T364 to the activities of SsoMCM, we purified two mutant proteins, containing T364A and T364E substitutions (Figure 2a). The proteins were heat-stable and hexameric (data not shown). Next, we assayed the ability of the wild-type (wt), T364A, and T364E proteins to bind to a Y-shaped DNA substrate. As one can see in Figure 2b, the mutant proteins retained DNA binding activity, although in both cases this was very slightly less than that displayed by the wt protein. We then assessed the ability of the proteins to perform helicase activity on a model Y-shaped DNA substrate (Figure 2c). The T346E mutant exhibited significantly reduced helicase activity, at least 2-fold, at all concentrations tested. In contrast, the T364A mutant revealed an only modest reduction, barely detectable beyond the error in these measurements.

Finally, we tested the ability of the proteins to hydrolyze ATP in the presence and absence of DNA (Figure 2d). As we have described previously, the ATPase activity of SsoMCM is modestly stimulated in the presence of DNA, rising to 1.2 times the basal level observed without DNA (*10*). Analysis of the T364E protein revealed a 5-fold reduction in ATPase activity compared to that of the wild-type protein, but importantly, no significant stimulation by DNA could be detected. In contrast, the T364A protein exhibited elevated ATPase activity, at 1.8-fold the level of the wild-type protein. However, as with the T364E protein, DNA did not stimulate the ATPase activity of the T364A protein. From these data, we infer that the T364A mutant phenocopies an active glutamate switch conformation, while the T364E mutation positions the catalytic glutamate suboptimally. We note that the reduction in the helicase activity of T364E is 2-fold, a less severe effect than the 5-fold reduction in ATPase activity. This is in agreement with our previous observations of probabilistic versus cooperative modes of ATP usage for ATPase and helicase activities, respectively, and indicates that a significant proportion of the ATP hydrolyzed by MCM is not harnessed for productive helicase action (*10*).

Thus, mutation of T364 abolishes the DNA-dependent stimulation of MCM ATPase activity. These data, in combination with the structural analyses shown in Figure 1, support the identity of threonine 364 as a glutamate switch residue in MCM and, thus, reveal that the glutamate switch is a feature present in all seven clades of the AAA+ protein.

SUPPORTING INFORMATION AVAILABLE

Detailed experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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