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The DEAD Box Helicase YxiN Maintains a Closed Conformation during ATP Hydrolysis

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ABSTRACT: DEAD box helicases unwind RNA duplexes at the expense of ATP hydrolysis. Recently, unwinding has been demonstrated in the absence of ATP hydrolysis. Herein, we show that ADP·BeF_x supports RNA unwinding by YxiN, a DEAD box helicase that specifically recognizes a hairpin in 23S rRNA. ADP·AlF_x and ADP·MgF_x do not promote RNA unwinding, but all ATP analogues induce a closed conformation of the helicase core as required for RNA unwinding. Our results show that the interdomain cleft in the helicase core closes upon ATP binding at the beginning of the cycle. Reopening occurs after ATP hydrolysis, most likely coupled to phosphate release.

DEAD box helicases use the energy of ATP hydrolysis for unwinding and remodeling of RNA (reviewed in ref *1*). The molecular mechanism of how ATP hydrolysis is coupled to structural changes in the RNA substrates is not fully understood. Nonhydrolyzable ATP analogues have been employed to dissect the effects of nucleotide binding and hydrolysis. ATP analogues are useful tools to investigate the mechanism of ATPases and to understand the role of ATP hydrolysis for ATP-driven processes. The nonhydrolyzable ATP analogue ADPNP does not support RNA unwinding by DEAD box helicases (*2*, *3*), and it has been assumed for quite some time that ATP hydrolysis is required to provide the energy for the endergonic process of duplex separations. However, recent studies have demonstrated that the ATP analogue ADP·BeF_x allows for RNA unwinding by the DEAD box helicases Ded1p, Mss116p, and eIF4A under single-turnover conditions (*3*). ADP·BeF_x has been used as an ATP analogue in studies with different classes of ATPases and is commonly believed to mimic ATP in a prehydrolysis state (*4*−*6*). Interestingly, Ded1p-mediated RNA unwinding under multiple turnover conditions strictly requires ATP (*3*). Hence, ATP hydrolysis appears not to be required for unwinding itself, but for resetting the enzyme for a new round of catalysis (*3*). ADP·AlF_x, an ATP analogue that can act as a transition state or a product analogue for ATPases (*4*−*6*), does not support RNA unwinding by Ded1p (*3*).

To define the conformations in the catalytic cycles of DEAD box helicases during ATP hydrolysis, we investigated the effect of the ATP analogues ADP·BeF_x, ADP·AlF_x and ADP·MgF_x on the catalytic cycle of the DEAD box helicase YxiN. ADP·MgF_x exhibits similar properties as ADP·AlF_x and mimics a state after hydrolysis, but presumably closer to hydrolysis than ADP·AlF_x (*8*−*11*).

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The *Bacillus subtilis* helicase YxiN consists of a helicase core and a C-terminal RNA binding domain (RBD) that contains an RNA recognition motif and mediates specific and high affinity binding to the hairpin 92 in 23S rRNA (*12*−*14*). The YxiN helicase core adopts an open conformation in the absence of ligands, but undergoes a conformational change toward a closed, compact conformation in response to RNA and ATP binding (*2*, *15*). Mutant studies have shown that the closure of the interdomain cleft is necessary, but not sufficient for RNA unwinding by YxiN (*15*).

Dissociation constants of the YxiN/nucleotide analogue complexes were determined in fluorescence equilibrium titrations (Figure 1). All nucleotide analogues displaced the fluorescent nucleotide mant-ADP from a preformed mant-ADP/YxiN complex, resulting in a fluorescence decrease. The K_d values of the complexes are 20 ± 2 μM (ADP), 38 ± 4 μM (ADP·BeF_x), 47 ± 16 μM (ADP·AlF_x), and 44 ± 4 μM (ADP·MgF_x), and 308 ± 16 μM (ADPNP). For comparison, the corresponding value for the ATP complex is 346 ± 29 μM (*16*). ADP·BeF_x, ADP·AlF_x, and ADP·MgF_x thus bind more tightly to YxiN than ATP, but comparable to ADP.

We have previously shown that the interdomain cleft in the YxiN helicase core closes in response to ATP and RNA binding (*2*, *15*). The closed conformation of the helicase core has been observed in structures of the DEAD box proteins Vasa, eIF4A-III, and Dbp5/Ddx19 in complex with short single-stranded RNA oligonucleotides and ADPNP (*17*−*21*), and in the structure of eIF4A-III in complex with ADP·AlF_3 and a short single-stranded RNA (*7*). In all structures, including the ADP·AlF_3 complex, a kink in the backbone of the RNA bound to the helicase core is observed, and as this backbone conformation is

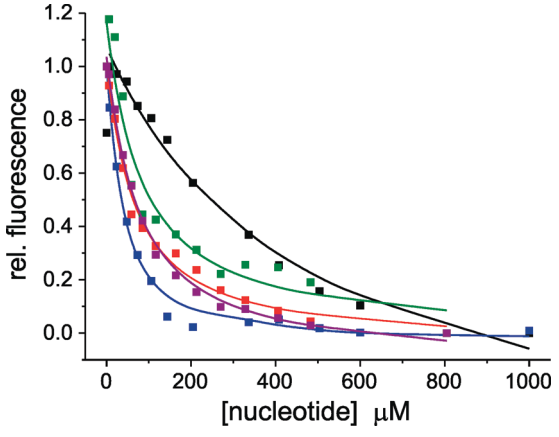

FIGURE 1: Binding of ATP analogues to YxiN. A complex of mant-ADP/YxiN was titrated with ADPNP (black), ADP·BeF_x (red), ADP·AlF_x (green), ADP·MgF_x (purple), and ADP (blue). Mant-ADP displacement was monitored via the decrease in mant-fluorescence.

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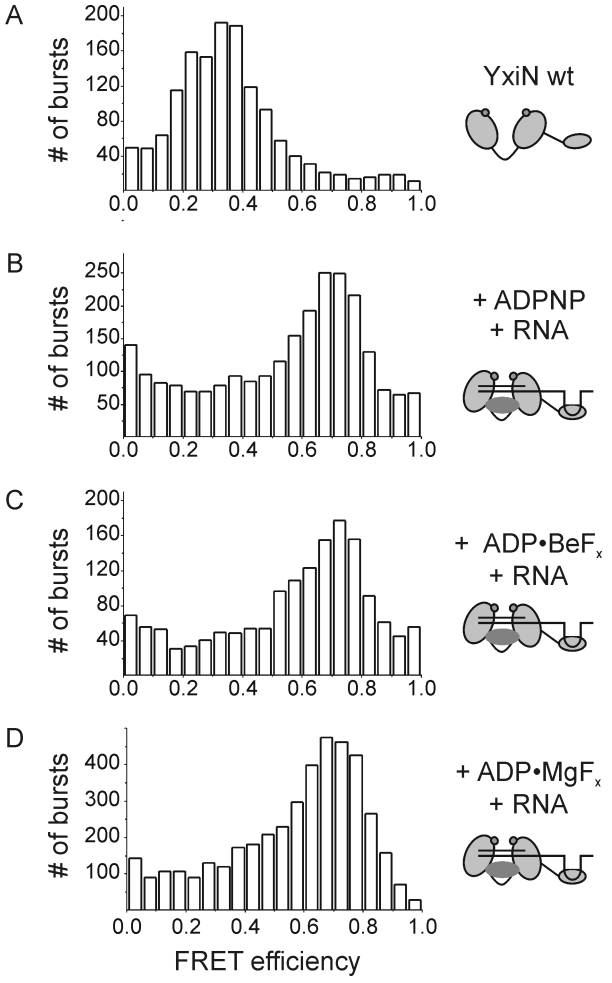

FIGURE 2: Conformation of YxiN in the presence of RNA and different nucleotides. Single molecule FRET experiments were performed with donor/acceptor-labeled YxiN using a confocal microscope. FRET histograms are shown for YxiN only (A), YxiN in the presence of RNA and ADPNP (B), ADP·BeF_x (C), and ADP·MgF_x (D). YxiN adopts the closed conformation with ADPNP, ADP·BeF_x, ADP·MgF_x. The cartoon depicts the conformation of the helicase core, and indicates the interaction of the hairpin with the YxiN RNA binding domain, and of the adjacent ds (or ss) region with the core.

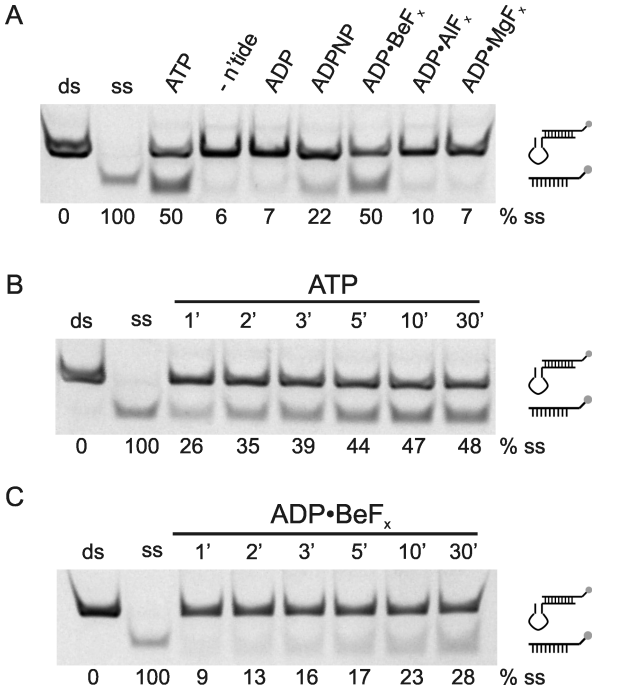

FIGURE 3: (A) RNA unwinding in the presence of different nucleotides and nucleotide analogues. ADP·BeF_x supports unwinding of a 32/9mer model substrate by YxiN, whereas no or little unwinding is detected in the presence of ADP·MgF_x, ADP·AlF_x, ADPNP, or ADP. ds and ss denote the controls for double-stranded 32/9mer and single stranded 9mer. (B) RNA unwinding by the motif III mutant (SAT to AAA) in the presence of ATP. The observed rate constant k_{obs} is 0.012 s^{-1}. (C) RNA unwinding by the motif III mutant in the presence of ADP·BeF_x. The observed rate constant k_{obs} is 0.004 s^{-1}. Compared to the ATP-dependent reaction, unwinding occurs 3-fold more slowly in the presence of ADP·BeF_x.

not compatible with double-stranded RNA, introduction of the kink has been suggested as a first step of RNA unwinding (*17*). However, the closure of the interdomain cleft, and thus introduction of the kink, is necessary for RNA unwinding, but not sufficient (*15*). To delineate the conformations of DEAD box proteins at different states in the nucleotide cycle, we performed single molecule fluorescence energy transfer (FRET) experiments in a confocal microscope on freely diffusing donor/acceptor-labeled YxiN carrying AlexaFluor488 (donor) and AlexaFluor546 (acceptor) on opposite sides of the interdomain cleft.

In the open conformation in the absence of nucleotide and RNA substrate, the efficiency of FRET between these dyes is 0.3 (refs *2*, *15*, Figure 2A). In the presence of RNA and ADPNP, the FRET efficiency increases to 0.7 (Figure 2B), indicative of the closure of the interdomain cleft (*2*, *15*). A similar FRET histogram is obtained for the ADP·BeF_x complex (Figure 2C), demonstrating that the closed conformer of YxiN is formed. Single molecule events were very scarce after adding ADP·AlF_x, possibly due to quenching effects by Al^{3+}, and no unambiguously interpretable FRET histograms could be obtained. The structure of eIF4A-III/ADP·AlF_3 with RNA shows the closed conformation (*7*), and thus a FRET efficiency of 0.7 would be expected. ADP·MgF_x has been described as a transition state or product analogue similar to ADP·AlF_x (*8*, *9*). Because of its proper charge and geometry, it presumably mimics a state closer to hydrolysis than ADP·AlF_x (*10*). ADP·MgF_x did not have any negative effects on FRET histograms. The FRET efficiency in the presence of ADP·MgF_x was centered at 0.7, again consistent with the closed conformation (Figure 2D). In contrast, the open conformation with a FRET efficiency of 0.3 is predominant in the presence of ADP (*2*).

Altogether, the FRET experiments demonstrate that the helicase core of YxiN is in the open conformation at the beginning of the catalytic cycle, and closes upon ATP binding. During ATP hydrolysis, and in the product complex, it remains in the closed conformation, as exemplified by the high FRET efficiencies of the YxiN complexes with ATP analogues. The ADP state of YxiN, however, is mainly in the open conformation (*2*). It can thus be inferred that reopening of the cleft in the helicase core occurs upon release of phosphate. Phosphate release, together with ATP hydrolysis, is the rate-limiting step in the catalytic cycle of the homologous *Escherichia coli* DEAD box helicase DbpA, and has been suggested as a trigger for RNA release (*22*). In the closed conformation, the RNA is embedded in an extensive network of interactions between residues from both domains (*17*−*21*). A reopening of the helicase core concomitant with phosphate release would disassemble the bipartite RNA

binding site and rationalize the reduced affinity for RNA in the ADP complex.

We next tested the capacities of the ATP analogues to support RNA unwinding by YxiN under single turnover conditions. Interestingly, ADP·BeF_x promotes unwinding of a 32/9mer RNA substrate by YxiN to the same extent as ATP (Figure 3A).

Unwinding by YxiN is rapid, and after 3 min the unwinding reaction is already complete. However, a mutation of the conserved motif III, from SAT to AAA, reduces the RNA unwinding rate, and unwinding can now be resolved in hand-mixing experiments (*15*) (Figure 3B,C). The observed rate constants are 0.012 ± 0.001 s^{-1} for ATP-driven and 0.004 ± 0.001 s^{-1} for ADP·BeF_x-driven RNA unwinding. YxiN-mediated RNA unwinding in the presence of ADP·BeF_x is thus 3-fold slower. In contrast to ADP·BeF_x, no RNA unwinding is observed in the presence of ADP·AlF_x or ADP·MgF_x (Figure 3A). Altogether, these results are in agreement with the observations for Ded1p (*3*).

All ATP analogues tested here induce the closure of the cleft in the YxiN helicase core, but only ATP and ADP·BeF_x support RNA unwinding. In the presence of ADP·BeF_x, only single turnovers are possible, whereas multiple turnovers require ATP (*3*). Our data, in conjunction with previous studies, support the notion that DEAD box proteins alternate between "on" and "off" states in ATP-driven catalytic cycles, allowing for multiple turnovers of RNA unwinding. ADP·BeF_x populates the "on" state, but switching off is not possible. Consequently, only one cycle can occur, and the reaction is restricted to single turnover conditions. In contrast, ADPNP, ADP·AlF_x, and ADP·MgF_x appear to populate a "pseudo-on" state that is structurally very similar to the productive on-state but does not lead to RNA unwinding. Indeed, the structures of Mss116p in complex with RNA and different ATP analogues are virtually identical (*23*), confirming that the structural differences between the productive and nonproductive complexes are small. It is possible that the different capacities of the nucleotide analogues to support RNA unwinding may be linked to kinetic differences of individual steps in the catalytic cycle. Future experiments will therefore address the kinetics of opening and closing.

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SUPPORTING INFORMATION AVAILABLE

Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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