Cryo-EM Structures Of The DEAH-Box Helicase DHX36 Reveals The Initiation Of Unwinding DNA And RNA G-Quadruplexes

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G-quadruplexes (G4s) are four-stranded nucleic acid structures formed by the self-assembly of guanines adopting a helical arrangement of stacked tetrads. These structures function as regulatory elements for numerous fundamental biological processes and aberrant formation of G4s are associated with multiple human diseases. Various helicases have been identified with G4-resolving activity, but the mechanistic details of unwinding G4s by these molecular motors still remain largely unknown. The DEAH-box helicase 36 (DHX36) is a ~110 kDa G4- specific helicase that preferentially associates with guanine-rich sequences in vivo and exhibits high affinity for DNA and RNA G4 structures (Kd ~1 pM). Our lab has solved multiple X-ray crystal structures of the Bos taurus DHX36, including a structure bound to a DNA G4 found in the promoter region of the cMyc oncogene. In the absence of ATP, the crystal structure illustrated the DNA G4 was pulled by a single nucleotide, suggesting that ATP-independent conformational changes induced destabilization of the G4 structure (Chen et. al., Nature, 2018). Previous single-molecule FRET (smFRET) studies of DHX36 supported this proposed initiation mechanism by demonstrating ATP-independent conformational fluctuations of DHX36 bound to a DNA G4, which corresponds to the distance of a single nucleotide (Tippana et. al., PNAS, 2016). Contrary to the ATP-independent behavior of DNA G4s, similar measurements of DHX36 with a RNA G4 described a single conformational state (Tippana et. al., Nat. Comm., 2020). These results indicated that initiation of unwinding by DHX36 progresses differently for DNA and RNA G4s. Here, we determined multiple single particle cryo-EM reconstructions of DHX36 bound to a DNA (DHX36/DNA) and RNA (DHX36/RNA) version of the cMyc G4 that represents the initiation of unwinding. These reconstructions contain an average resolution range (FSC-curve = 0.143) between 3.8 – 2.6 Å. In all cryo-EM reconstructions, the core of the helicase adopts a similar conformational state that was described in the X-ray crystal structure. Unexpectedly, the RNA G4 was not observed being pulled by a single nucleotide, which implies that DHX36 bound to RNA G4s predominantly exists in a non-pulled state. Furthermore, our cryo-EM reconstructions, along with unwinding kinetics and smFRET studies, reveal the interplay between helicase domains in the N-terminus and protein core that contribute to ATP-independent unwinding of G4 structures. Altogether, our results provide additional mechanistic insights into the differences of ATP-independent remodeling of DNA and RNA G4s by DHX36, which can be also extended to other DEAH-box helicases that possess G4-resolving activity.