Comparative Analysis of Kar3Vik1 and Kar3Cik1 ATPase Mechanism

by

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ABSTRACT

Kar3, a kinesin-14 microtubule (MT) minus end-directed and non-processive motor, was one of the six kinesin-related proteins identified in the budding yeast, *S. cerevisiae*. It functions in nuclear fusion during yeast mating (karyogamy) and meiosis l, as well as in vegetative growth during mitosis. Recent work on the homodimeric kinesin-14 Ncd using structural, cryo-electron microscopy (cryo-EM), and mechanistic methodologies reveals that this class of motors displays an asymmetry between the two heads, even though identical structurally, for nucleotide binding and MT interaction and performs a powerstroke mechanism for its MT minus end-directed force generation. The powerstroke mechanism is similar to the lever arm rotation described for myosin and is hypothesized to generate the MT minus end-directed force for stabilizing, crosslinking, and sliding MTs during mitosis. On the other hand, Kar3, *in vivo*, functions as heterodimers by associating with either Cik1 or Vik1 through a central α-helical coiled-coil domain. Vik1 and Cik1 by analogy through protein sequence homology, show no evidence of a nucleotide binding site at the head domain. However, the protein structure of the C-terminal globular domain of Vik1 (Vik1MHD) folds similarly to that of the Kar3 motor domain (Kar3MD), forming an unusual heterodimeric kinesin with one functional motor head and one "dead" head incapable of ATP turnover. Therefore, Kar3Vik1 and Kar3Cik1 represent extremely rare and valuable entities for further investigating the asymmetrical behaviors of the kinesin-14 heads in terms of the ATPase cycle and MT binding pattern. My dissertation work, therefore, focuses on the mechanochemical characterization of Kar3Vik1 and Kar3Cik1 using transient state kinetics, thermodynamic equilibrium binding, and TIRF fluorescence microscopic methodologies. My pre-steady state kinetics experiments reveal that Kar3Cik1's association with the MT occurs at 4.9 $\mu M^{-1}s^{-1}$, followed by a 5-6 s^{-1} structural transition that limits ADP release from the Kar3 head. MantATP binding occurs at 2.5 $\mu M^{-1}s^{-1}$, and the pulse-chase experiments indicate an ATP-promoted isomerization at 69 s^{-1}. ATP hydrolysis is observed as a rapid step at 26 s^{-1} and is required for the Kar3Cik1 motor to detach from the MT. The conformational change at 5-6 s^{-1} that follows Kar3Cik1 MT association and precedes ADP release is hypothesized to be the rate-limiting step for the

steady-state ATP turnover. We propose a model in which Kar3Cik1 interacts with the MT lattice through an alternating cycle of Cik1 MT collision followed by Kar3 MT binding. On the other hand, Kar3Vik1 binds to MTs at 2.4 $\mu M^{-1}s^{-1}$ possibly through the Vik1 head. ADP release after Kar3-MT interaction occurs at 14 s^{-1}, leading to a two heads bound state with Vik1 weakly associated or destabilized from the MT. ATP binding to Kar3 is measured at 0.8 $\mu M^{-1}s^{-1}$, and the pulse-chase experiments reveal an ATP-promoted isomerization at 55 s^{-1} associated with the powerstroke of the coiled-coil stalk rotation, visualized through a cryo-EM approach. ATP hydrolysis at 26 s^{-1} occurs prior to the MT•Kar3Vik1 dissociation at 6 s^{-1}, which is the rate-liming step for the Kar3Vik1 ATPase cycle. Overall, for both Kar3Vik1 and Kar3Cik1, multiple lines of evidence indicate that head-head communication is mediated through the adjoining coiled-coil and modulated by an intramolecular strain resulting from different nucleotide states. In addition, our collaboration with structural groups uncovers a novel MT binding pattern, in which Kar3 and Vik1 are bound across adjacent protofilaments rather than along a single protofilament, as observed for other dimeric kinesin motors studied to date.