The dominant effect of mutant spastin subunits in wildtype background

Spastin is a hexameric ring AAA ATPase that severs microtubules. To see whether the ring complex funnels the energy of multiple ATP hydrolysis events to the site of mechanical action, we investigated the cooperativity of spastin. Several lines of evidence indicate that interactions among two neighbor subunits dominate the cooperative behavior: (i) the ATPase activity shows a sigmoidal dependence on the ATP concentration; (ii) ATP-γS displays a mixed-inhibition behavior for normal ATP turnover; and (iii) inactive mutant subunits inhibit the activity of spastin in a hyperbolic dependence, characteristic for two interacting species. A quantitative model based on neighbor interactions fits mutant titration experiments well, suggesting that each subunit is mainly influenced by one of its neighbors. These observations are relevant for patients suffering from SPG4-type hereditary spastic paraplegia, and explain why single amino acid exchanges lead to a dominant-negative phenotype. In severing assays, wildtype spastin is even more sensitive towards the presence of inactive mutants than in enzymatic assays, suggesting a weak coupling of ATPase and severing activity. To understand the biophysical mechanism of severing, the interaction between spastin and microtubules was studied. A stretch of three K-residues was found to be responsible for microtubule binding and diffusive motility along the microtubule. Together, our observations suggest that as little as 5% of inactive spastin in a mixture with wildtype is able to reduce the severing activity by 50%, implying that therapeutic strategies have to aim at removing defective subunits as efficiently as possible.

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