BINDING OF DYNEIN LIGHT CHAINS LC8 AND TCTEX-1 TO INTRINSICALLY DISORDERED INTERMEDIATE CHAIN

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Introduction

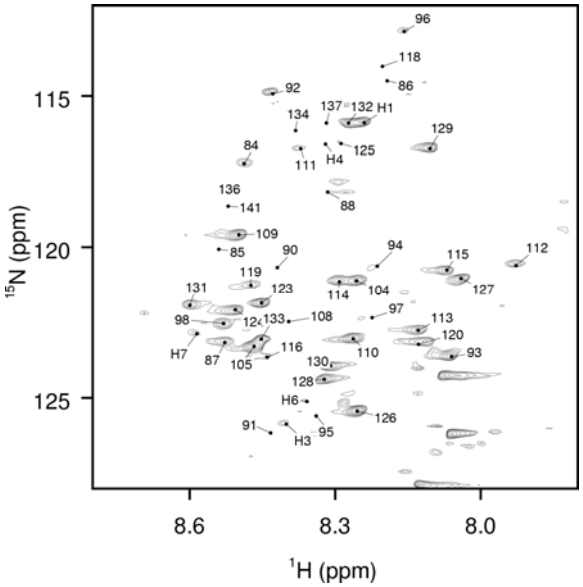
Cytoplasmic dynein is a microtubule-based retrograde motor responsible for intracellular transport of membranous vesicles and multiple cargo proteins. It is a large multisubunit complex composed of dimeric heavy chains which contain the microtubule binding domain and the ATPase activity, and five smaller subunits which comprise the cargo attachment site and include two copies each of light intermediate and intermediate chains and three dimeric light chains. Our approach to address the question of dynein assembly and its attachment to cargo is to characterize the interactions of the intermediate chain of dynein, IC74, with dimeric light chains LC8 and Tctex-1. To provide insights into the structural and dynamic changes that occur in the intermediate chain upon light chains binding, we have used NMR spectroscopy to compare the properties of two distinct sub-domains of the intrinsically disordered N-terminal domain of IC74: IC84-143 which is the light chains binding domain, and IC198-237 which contains a predicted coiled coil necessary for the increase in ordered structure upon light chain binding. Neither construct has stable secondary structure when probed by circular dichroism, amide chemical shift dispersion, and steady state heteronuclear NOEs. IC84-143 remains primarily unfolded even after binding to LC8 and Tctex-1 [1].

Experimental

NMR spectra were collected on the 900 MHz Bruker spectrometer at the NHMFL facility, on a ^{15}N labeled IC84-143 at a protein concentration of 0.5 mM in complex with 1 mM unlabeled LC8 at pH 5.5, in 50 mM citrate phosphate, 50 mM NaCl, 10 % D_2O, 1 mM sodium azide and a protease inhibitor cocktail (Roche).

Results and Discussion

^{1}H -^{15}N Steady state heteronuclear NOEs were collected for the IC/LC8 complex. Similar experiments were previously collected at 600 MHz and showed that the IC84-143 peaks involved in binding completely disappear with addition of LC8 and the heteronuclear NOE experiments showed that all peaks were negative. In contrast, data collected at 900 MHz show that there are several residues with positive NOEs. The spectrum on the right collected at 900 MHz shows only the positive peaks in the proton saturation experiment. Positive peaks are the most intense for those assigned to the residues involved in binding but at chemical shifts corresponding to the free. For example, 126, 128, 130, 133 are the most intense, while 118, 125, 116, 95, are negative (not shown in this spectrum). It is possible that the peaks that disappear at 600 but are still present at 900 show positive NOEs reporting on a more ordered conformation of the bound form in exchange with the free. Another reason for the positive NOEs is the dependence of NOE on $\omega_{\tau c}$, which increases at higher field, causing borderline dynamics to switch from fast to slow tumbling. To test this, we need to record similar spectra for free IC84-143 at 900 MHz, and the answer is more likely a combination of both possibilities.



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References
[1] Benison, G., *et al.*, (2006). J. Mol. Biol. **362**, 1082-1093.