Effect of Osmotic Stress and D_2O on Kinesin Activity


"Kiney"

Open Notebook Science

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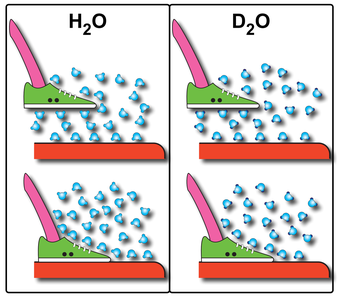
Motivation

Kinesin and microtubules have been proposed as components for chemical and biological sensors. In order to fully understand the dynamics of kinesin and microtubules, fundamental questions must be answered related to how we observe those interactions. One way to observe their interactions is by using a ***gliding motility assay*** which can easily be visualized as a molecular "crowd surfing" for microtubules. Visualizing the steps kinesin take along microtubules via fluorescence microscopy show that kinesin's motility (the ability of kinesin to walk along microtubules) is strongly affected by water's chemical potential. Changing the osmotic pressure of the aqueous environment the kinesin and microtubules are in, affects the speed at which kinesin walks along the microtubules.



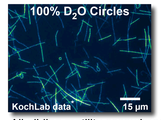
Using kinesin and microtubules as chemical and biological sensors necessitates investigating how kinesin's motility is affected by osmotic stressors. Initial studies with deuterium oxide (D_2O or heavy water) have shown that kinesin's motility is affected by solvent properties. We have shown that changing the fraction of D_2O to H_2O does affect kinesin's velocity. This is a first step into understanding how we can use kinesin as a molecular reporter for chemical and biological sensors. It also introduces a new experimental "knob" if you will that will allow us to investigate the role of water on kinesin's stepping abilities.

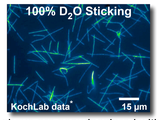
D_2O facts

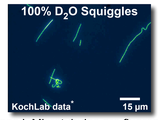
- Hygroscopic.
- 11% denser than H_2O.
- Toxic to eukaryotes. The toxic effects are similar to chemotherapeutic drugs[1].
- A mixture of 50% D_2O and 50% H_2O will dissociate to 50% HDO and 25% H_2O and 25% D_2O[9].
- D_2O is used to stabilize viral vaccines[10].
- D_2O stabilizes tubulin and microtubules[2].
- D_2O stimulates tubulin assembly formation[2].
- No previous work with kinesin has been found.

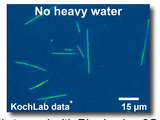
Microtubule shape differences

The differences between heavy water and light water on microtubules are stark. Some interesting shape differences include: circles, sticking, and squiggles.

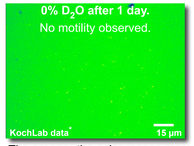


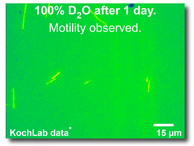


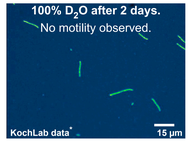




All gliding motility assay images above are pseudocolored with ImageJ. Microtubules are fluorescently tagged with Rhodamine 6G.







The greener the color means more fluorescence.

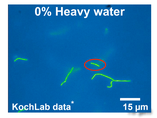
Microtubule & kinesin stability

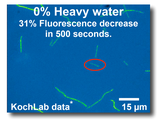
Combined with the new and interesting features microtubules have in heavy water, they are also more stable[1]. Slides last for 2 days with motility ending somewhere between day 1 and day 2. As a reference point, motility only lasts for a few hours in light water.

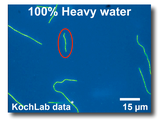
It has been shown that tubulin is also more stable if packed in heavy water[2, 3].

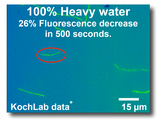
Fluorescence stability

Another amazing fact about heavy water and fluorophores is that heavy water will prolong fluorescence[4]. Prolonging fluorescence actually helps the lifetime of microtubules. Photodegradation of attached fluorophores on tubulin, aid in microtubule depolymerization.



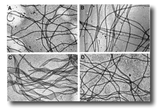




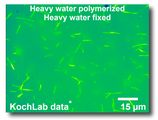


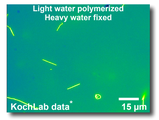
A. Polymerization in light water with 8% DMSO.
B. Polymerization in heavy water without DMSO.
C. Polymerization in heavy water with DMSO.
D. Polymerization in both heavy water and light water with 8% DMSO[2].

We do not polymerize our microtubules with DMSO instead, we use glycerol.



Chakrabarti et al[2].



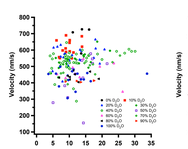


Microtubule polymerization

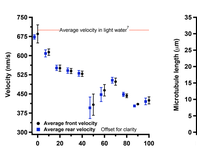
Microtubules can be polymerized in heavy water. The background fluorescence is larger in the heavy water polymerized microtubules than the light water polymerized ones. This is thought to come from ribbon structures of tubulin forming[2].

Kinesin velocity

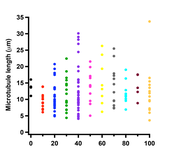
We have a joint DTRA project with a theoretical physics group lead by the PI, Susan Atlas from UNM. We are developing a new charge-transfer force field for atomistic modeling of biological systems. As a first step in our experimental tests of their new force field, we have set out to gain fundamental understanding of the effects of water activity on the enzymatic activity of kinesin. We plan to do this using osmotic stress and solvent isotope effects. Here we report our first results for the change in gliding motility assays as a function of substitution of D_2O for H_2O.



Velocity differences are not due to microtubule length. Microtubules were polymerized in light water.



Velocity dependence on D_2O concentration. Microtubules were polymerized in light water.

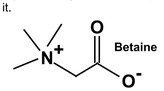


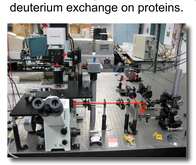
Microtubule length does not depend on D_2O concentration. Microtubules were polymerized in light water.

Future work

Continued investigation of how kinesin is affected by solvents and osmolytes will include studies that incorporate:

- **Betaines.** Betaines are zwitterionic osmolytes. They will change the osmotic pressure of the solution kinesin is in without interacting with it.
- **$H_2{}^{18}O$.** This is a form of "heavy water" except that there is an isotope of oxygen instead of hydrogen. This may lead to interesting physics since we no longer deal with hydrogen deuterium exchange on proteins.

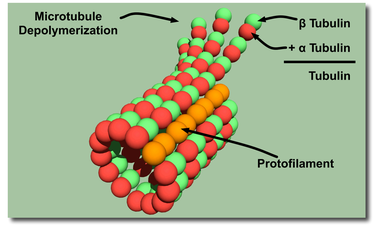




- **Optical tweezers.** All experiments thus far have been executed in a light microscope using gliding motility assays. The next step is to use an optical trap (designed and built by Maloney and Salvagno) to investigate kinesin attached to a dielectric bead. The optical trap will track the kinesin's motion along a microtubule thus giving us single molecule resolution of kinesin's processivity.

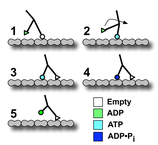
Microtubules

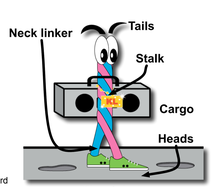
- Heterodimer of tubulin subunits α and β. One α and one β subunit together is called tubulin
- Tubulin forms polymers called protofilaments.
- Microtubules are made from 13 - 17 protofilaments.
- They are hollow and are an average of 25 nm in width.
- Calcium causes depolymerization.



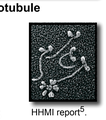
Kinesin

- Dimer that consists of two heavy chains and two light chains.
- The heavy chains form the "head" group or the motor domains.
- The light chains form the "tail" group where cargo binds. The kinesin supplied to us from Dr. Liu does not have light chains. It is a truncated heavy chain Drosophila kinesin-1.
- The chains are connected by a "neck linker" and an intertwined stalk region.
- Uses ATP to generate motion.





Kinetic cycle of kinesin
Kinesin uses ATP to propel itself forward along a microtubule. The exact steps kinesin takes to complete this cycle is still debated, however, we do know that the rear motor will not swing forward until an ATP binds to the front motor. Binding and unbinding of kinesin motor domains to microtubules will be affected by osmotic stress and isotope water substitutions. These are experimental knobs we wish to turn in order to understand more fundamentally the kinesin catalytic cycle.



HHMI report[5].

Acknowledgments

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- Haiqing Liu (CINT)
- Evan Evans
- CINT[6] is a user facility partnered with Los Alamos and Sandia National Laboratories.
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* Images false colored with ImageJ.