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The effects of osmolytes on motor-driven microtubule mobility

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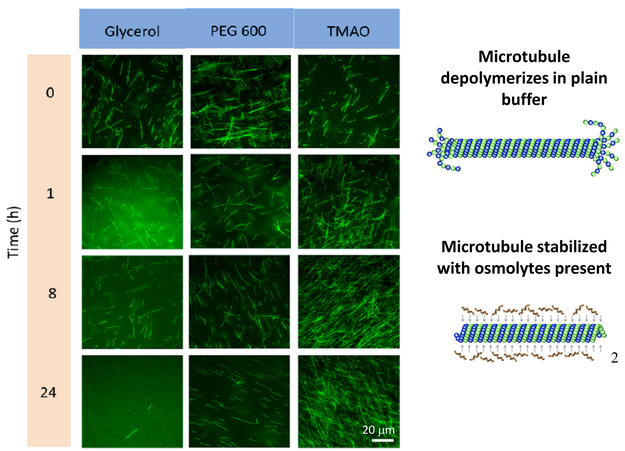
Abstract

Osmolytes, such as TMAO, PEG, and glycerol, have been shown to affect the material properties of microtubules, including their stiffness and resistance from depolymerizing. These osmolytes could potentially be used in place of paclitaxel (Taxol) for conferring microtubule stability in *in vitro* assays, which have been investigated for use in microdevices and as a model system for studying active self-assembly. To this end, we have examined the concentration dependent effects of these osmolytes on the motility of microtubules using motility assays powered by KIF5B. We find that the osmolytes affect the velocity and trajectories of the microtubules in a concentration dependent manner, with higher osmolyte concentrations reducing the microtubule velocity. These data suggest that, while osmolytes can stabilize microtubule against depolymerization, they adversely affect interactions and transport by kinesin motors.

Osmolytes can stabilize microtubules

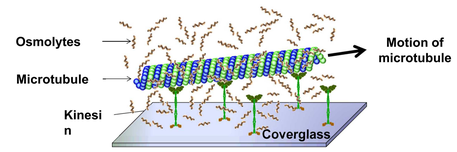
The polymerization/depolymerization of microtubules is regulated by a variety of stabilizing and destabilizing factors, including microtubule-associated proteins and therapeutic agents (e.g., paclitaxel, nocodazole).[1] Certain osmolytes, including polyethylene glycol (PEG 600) and trimethylamine-N-oxide (TMAO), can inhibit the depolymerization of individual microtubule filaments for extended periods of time (up to 30 days).[2] PEG has been shown to stabilize microtubules against both temperature- and calcium-induced depolymerization. The inhibition may be related to combination of the kosmotropic behavior and excluded volume/osmotic pressure effects associated with PEG and TMAO.[2]



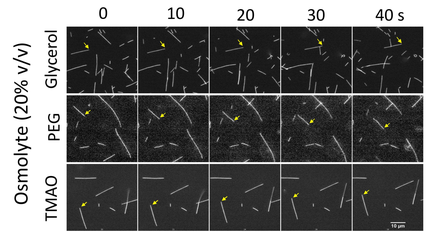
Osmolyte-stabilized microtubules in motor-driven motility assays

The kinesin motor and microtubule system can be reconstituted *in vitro* with the surface-adhered motors transporting the filaments along the surface. In this format, the system has been used to study active self-assembly [3] and to power microdevices or perform analyte detection.[4]

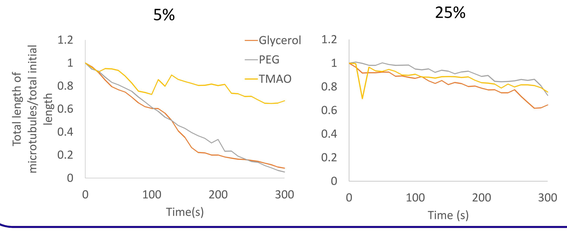
Inverted kinesin motility assay – effect of osmolytes?



Typically, microtubules *in vitro* are stabilized with paclitaxel (Taxol). Now that we know that osmolytes can be used to stabilize microtubules *in vitro*, we want to investigate the effects of these osmolytes on a kinesin powered motility assay without Taxol. We performed motility assays with Glycerol, PEG, and TMAO at 5%, 10%, 15%, 20%, and 25% percent concentration.

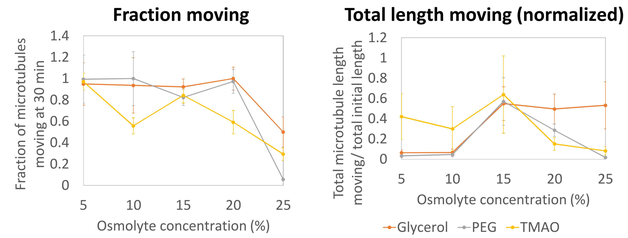


As seen above, motility assays can function with osmolytes used in place of paclitaxel (Taxol) to stabilize microtubules. However, the concentration of the osmolytes is critical to achieving a functioning assay. As seen in the graph left below, low concentrations of the osmolytes do not provide stabilization and most of the microtubules depolymerize by 5 min into the assay for glycerol and PEG. At 25% osmolyte, most of the microtubules that were present at the beginning of the assay remain at 5 min.



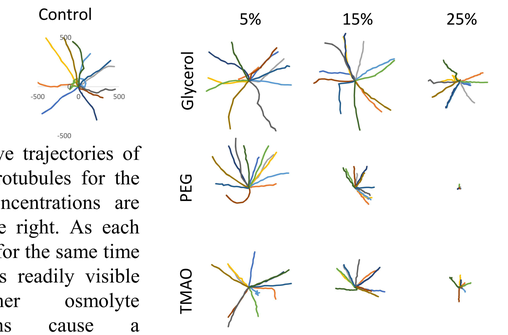
Osmolyte effects

In motility assays, microtubules sometimes become "pinned," stuck to the surface-bound kinesin motors and cease to move. The concentration of osmolyte affected the fraction of microtubules that were stuck, with higher concentrations leading to more pinning. This effect is show in the graph below left, which fraction of microtubules moving at 30 min.



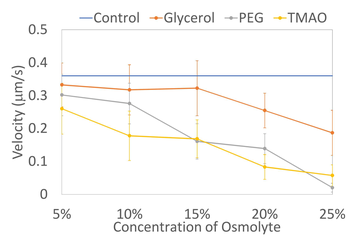
By looking at the fraction of the total length of microtubules present in the frame that is moving at 30 min (above right, normalized to initial total length), one observes a combination of the effect of microtubule depolymerization at low osmolyte concentration, good stabilization and motility at middle osmolyte concentrations, and poor motility at high concentrations. The good motility range is different for the various osmolytes, with TMAO having an optimal range at a lower concentration than PEG and glycerol.

Trajectories



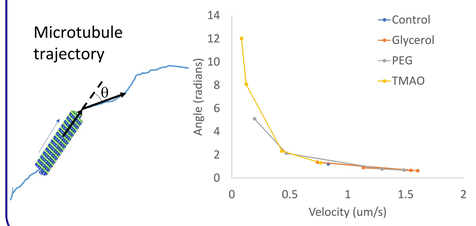
Representative trajectories of moving microtubules for the osmolyte concentrations are shown to the right. As each trajectory is for the same time (150 s), it is readily visible that higher osmolyte concentrations cause a decrease in the velocity of the microtubules that are moving, especially for PEG and TMAO. Thus using PEG or TMAO as a replacement for Taxol requires using the lowest concentration that will stabilize microtubules, or using osmolytes in addition to Taxol to control velocities, as explored by Munmun et al.[5]

Velocities



Trajectory analysis

Finally, we observed the trajectories of the microtubules at high concentrations of PEG and TMAO to be more rough. We attempted to quantify this effect by tracking path of the tip of the microtubule, calculating the scalar product of the vectors made by the microtubule path from one frame to the next, and then averaging the calculated angle over the whole trajectory. This jerky motion could be caused by the osmolytes interfering with the binding of the kinesin to the microtubule, in effect making the microtubule display motion reminiscent of microtubules moving on surfaces with a low kinesin surface density..



References

1) Dumontet and Jordan, Nat. Rev. Drug Discovery, 2010; 2) Bachand et al., Biomacromolecules, 2018; 3) Lam et al., Soft Mat., 2016; 4) Saper and Hess, Chem. Rev, 2020; 5) Munmun et al., Sens. Actuators B Chem., 2020.

Acknowledgements

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