Temperature Compensation in Biomolecular Motor-Powered Nanomaterials and Devices

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Proposal Title:
Temperature compensation of kinesin-powered molecular shuttle velocity (Henry Hess)

Research Achievement:

The use of kinesin motor proteins to transport synthetic nanomaterials[1-3] and assemble hybrid nanocomposites has been widely explored.[4-6] While these biomolecular machines offer unique functions, a number of fundamental challenges arise from this approach, particularly the stabilization of motor proteins against significant temperature changes. Two useful strategies for temperature compensation are common in biological systems: (1) feedback loops used in metabolic pathways, and (2) the ability to maintain constant enzymatic activity at subsaturating substrate concentrations. As an example of the latter, the Michaelis constant (K_m) of many enzymes has been shown to increase with temperature and compensate for the increased maximum turnover rate (v_{max}).[7,8] The goal of this User Project was to discern whether kinesin motor proteins use this temperature compensation strategy, and if so, to understand how this strategy applies to the integration of kinesin motors in hybrid nanomaterials and devices.

Methods

The temperature dependent, kinetic properties of two different kinesin motors (mesostable Drosophila kinesin-1 and thermostable Thermomcyes kinesin-3) were evaluated across a wide range of substrate concentrations using the gliding motility assay. These assays consisted of the adsorption of kinesin motors to the surface of microfluidic flow cells, followed by the introduction of fluorescently-labeled microtubule filaments and varying levels of ATP (i.e., kinesin motor substrate). Temperature of the flow cell was tightly controlled using a heated stage on an inverted microscope. Time-lapse images were captured and used to estimate microtubule transport velocities as a function of substrate concentration and temperature.

Results & Discussion

The ATP-dependent transport velocities for both the Drosophila and Thermomyces kinesins followed typical Michaelis-Menten kinetics (*not shown*), as expected. Curves fit to these data demonstrated a limited temperature dependence on the K_m across the temperature range tested (19-34ºC) for both kinesins (Figure 1A). The lack of temperature dependence was somewhat surprising as previous work with the Thermomcyes kinesin demonstrated a significant increase in the K_m at 50ºC (the thermal optimum for this kinesin).[9] The v_{max} for both kinesin displayed a relatively linear increase as a function of increasing temperature (Figure 1B), which is consistent with prior reports for kinesin and other enzymes.[7-10] Based on this relationship, the Q_{10} (temperature coefficient = increase in velocity per 10ºC change in temperature) for Drosophila and Thermomyces kinesin were estimated at 2.04 and 1.67, respectively. Overall, the incoherence between the temperature responses in the K_m and v_{max} parameters indicate that

temperature compensation in kinesin motor proteins is achieved by a different mechanism than has been observed in thermo- and psychro-stable enzymes.[7,8]

Future Work

The results of these experiments suggest that temperature compensation in kinesin motors does not occur through changes in the intrinsic kinetic properties of the enzyme. Thus, future work is focused on developing alternate strategies (e.g., feedback networks) to compensate for temperature-dependent effects on kinesin transport in hybrid materials and devices with applications in motor protein-powered biosensors.

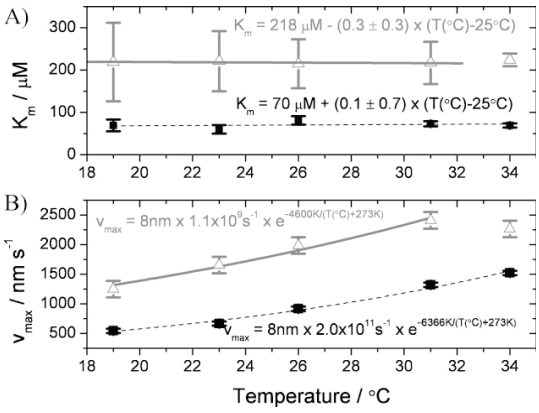

Figure 1. (**A**) K_m and (**B**) V_{max} of Drosophila kinesin-1 (black squares) and Thermomyces kinesin-3 (open grey triangles) as function of temperature. Figure taken from Tucker et al.[11]

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Publications

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