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Temperature-dependent activity of kinesins is regulable

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Abstract

Cytoskeletal transport in cells is driven by enzymes whose activity shows sensitive, typically Arrhenius, dependence on temperature. Often, the duration and outcome of cargo transport is determined by the relative success of kinesin vs. dynein motors, which can simultaneously bind to individual cargos and move in opposite direction on microtubules. The question of how kinesin and dynein activity remain coupled over the large temperature ranges experienced by some cells is one of clear biological relevance. We report a break in the Arrhenius behavior of both kinesin-1 and kinesin-3 enzymatic activity at 4.7°C and 10.5°C, respectively. Further, we report that this transition temperature significantly changes as a function of chemical background: addition of 200 mM TMAO increases transition temperatures by ~6°C in all cases. Our results show that Arrhenius trend breaks are common to all cytoskeletal motors and open a broad question of how such activity transitions are regulated in vivo.

INTRODUCTION

Mechanochemical enzymes of the kinesin and dynein families enable active transport of cargos along microtubules (MTs) which is essential for eukaryotic cell function (1). Cargo motility is often driven by motor ensembles, which may result in saltatory, biased bidirectional or unidirectional motility on a single microtubule, or a variety of cargo navigation phenotypes at MT intersections. The balance of ensemble motor activity, which is critical to the cargo navigation outcome, received significant experimental and theoretical attention but almost always assuming fixed environmental conditions (2). However, temperature changes have clear biological relevance even for mammalian thermoregulated organisms, e.g. extreme variations during hibernation (3). Crucially, for a single cargo, driven by a motor ensemble, a temperature-driven change in an underlying parameter such as motor velocity can result in a qualitative change in the character of motility (4).

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Author Contributions

All authors designed research and co-wrote the manuscript; F.D. performed research; K.M.O.-M. and R.J.M. contributed new reagents; and M.V. supervised research.

Competing Interests

The authors declare no competing interests.

Therefore, quantitatively understanding collective motor activity across temperatures is an issue of rising importance in cytoskeletal biophysics.

The simplest temperature-dependence of enzymatic rate is an Arrhenius trend, corresponding to a chemical reaction proceeding forward by crossing an energy barrier along a one-dimensional reaction pathway. The Arrhenius trend has a characteristic value (activation energy) which corresponds to the height of this energy barrier, and is also a readout of the rate-limiting step of the enzymatic cycle. However, piecewise Arrhenius trend is extremely common (5, 6), and is reflective of a temperature-dependent change in the enzymatic cycle. Arrhenius breaks (transitions between distinct Arrhenius trends) have been previously observed for two of the three major families of molecular motors: in conventional myosin at 5°C, and in various dyneins at 15°C and 17°C (6, 7). Previous studies of kinesin-1 were conducted above 5°C and have not observed a break (4, 8). However, prior report of Arrhenius break for myosin encouraged our search for the same in kinesins due to high homology of motor architecture (9). Here, by extending experiments into a lower temperature range, we show that kinesins do exhibit Arrhenius breaks and we further show that the temperature at which the break occurs is dependent on environmental/chemical conditions.

Results

Prior reports show no Arrhenius break for kinesin-1 above ~5°C (4, 10, 11). Here we extended motility measurements down to 0°C. Motility was steady and distributions of motor velocities were approximately Gaussian in all measured conditions (Fig. 1). The data across all temperatures (Fig. 2A, black) was best fit to a piecewise-Arrhenius trend, with a break at ~4.7°C. The activation energies were significantly different above and below the break.

We next aimed to perturb the enzymatic cycle of kinesin by using saturating amounts of GTP to drive motor activity. This reduced average velocity at room temperature by approximately threefold relative to ATP background, consistent with a previous report (12), but still allowed for accurate velocity measurements at low temperatures. We again observed an Arrhenius break at ~3.7°C (Fig 2A, red) – not significantly different from ATP results. In the GTP assays, the activation energies were significantly different above and below the break, and greater than in the ATP background, consistent with ATP being the preferred nucleotide for driving mechanochemical activity (12).

We then investigated how chemical factors may impact the Arrhenius breaks. We focused on tri-methyl amine oxide (TMAO), which is a crowding mimic at near-molar concentrations (13) and stabilizes kinesin activity at elevated temperatures (14). Under saturating ATP conditions in 200 mM TMAO background the Arrhenius break was found at ~11°C (Fig. 2B). Activation energies were significantly different above and below the break.

Finally, we sought to determine whether this break was specific to kinesin-1 or a more general feature of kinesin motors. We repeated our experiments with dimeric hKIF1A, a kinesin-3 family motor known to be involved in fast axonal transport (15). Previous studies

of fungal kinesin-3 did not observe an Arrhenius break down to ~5°C (10), but the temperature dependence of human KIF1A has not been previously studied. We did identify an Arrhenius break at ~10.5°C for hKIF1A with significantly different activation energies above and below the break (Fig. 3, red). In 200 mM TMAO background the Arrhenius break temperature was found at ~16°C, with significantly different activation energies above and below the break (Fig 3, black).

Discussion

Here, we report an Arrhenius break in both the kinesin-1 and kinesin-3 families. It is tempting to speculate that this feature is a general property of kinesins, and likely of all cytoskeletal motors (4, 6). Prior work on kinesin's randomness parameter has established that there are at least two steps in the enzymatic cycle of kinesin-1 which are either rate-limiting or close (16). If the durations of these steps have differing temperature dependence, then the nature of the rate-limiting step could change abruptly as a function of temperature as is the case for e.g. myosin (6). Further work is necessary to provide definitive insight into the atomic-scale mechanism of the observed behavior and likely shed further light on the nature of rate-limiting steps in kinesin's enzymatic cycle.

The observed significant shift in the transition temperature in the presence of 200 mM TMAO shows that chemical/environmental regulation of Arrhenius breaks is possible for cytoskeletal motors. Notably, shifting the Arrhenius break in Kinesin-1 from 4.7°C to 11°C brings its temperature-dependent trend much closer to that of mammalian cytoplasmic dynein (7) hinting that kinesin and dynein motors in cells may be better matched across a wider temperature range than previously appreciated. Our TMAO work also raises the question of how other families of motors (particularly cytoplasmic dyneins) would perform under the same environmental conditions. Overall, our findings open a new set of hypotheses bearing on how transport regulation depends on temperature.

Materials And Methods

Motility Assays:

Assays were performed similar to (11). Upon MT attachment, surfaces were passivated with 1% Ficoll solution. Motors were adsorbed to ∅1 μm beads via streptactin conjugation (30 min streptactin then 15 min. kinesin incubations).

Motor purification:

KIF1A motors were purified as previously described (17). Amino acid residues 1-556 of human KIF5A (top) were cloned into the pET28a vector followed by an mScarlet-strepII tag using isothermal assembly. The construct was verified by sequencing. To express the protein, BL21 (DE3) RIPL cells were transformed and grown in LB medium to an OD of ~0.4. Cells were induced with 0.1mM IPTG overnight at 18°C. Cells were harvested and resuspended in lysis buffer: 50mM Tris pH 8.0, 150mM KAce, 2mM MgSO4, 1mM EGTA, 10% glycerol along with 1mM PMSF and 1mM DTT. Cells were disrupted via high-pressure homogenization using an Emulsiflex C3 (Avestin). The lysate was centrifuged at 28,000 x g for 20 min and the supernatant was pumped over a column of Streptactin XT

resin (IBA) for ~1 hour at 4°C. The column was then washed with excess lysis buffer to remove unbound material and the motors were eluted in lysis buffer containing 50 mM biotin. Eluted protein was further purified via anion exchange chromatography using a TSKgel SuperQ-5PW (Tosoh bioscience) 7.5 mm ID × 7.5 cm. column equilibrated in HB buffer (35 mM PIPES-KOH pH 7.2, 1 mM MgSO4, 0.2 mM EGTA, 0.1 mM EDTA). Bound proteins were eluted with a 45mL linear gradient of X mLs from 0-1M KCL in HB buffer. Fractions containing the motor were combined and concentrated on amicon spin filters with a 50 KDa cutoff after addition of 0.1 mM ATP and 10% glycerol. Concentrated motors were frozen in LiN2 and stored at −80°C.

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Statement of Significance

Many cytoskeletal motors studied to date follow Arrhenius kinetics, at least from room temperature up to mammalian body temperature. However the thermal dynamic range is typically finite, and breaks in Arrhenius trends are commonly observed at biologically relevant temperatures. Here we report that the thermal dynamic range of kinesins is also limited and moreover that the location of the Arrhenius break for kinesins can shift significantly based on chemical backgrounds. This implies that the balance of multiple motor cargo transport along the cytoskeleton is far more tunable as a function of temperature than previously appreciated.

- Kinesin-1 has an Arrhenius break at low temperatures
- Kinesin-3 has an Arrhenius break at low temperatures
- The Arrhenius break temperature can be regulated

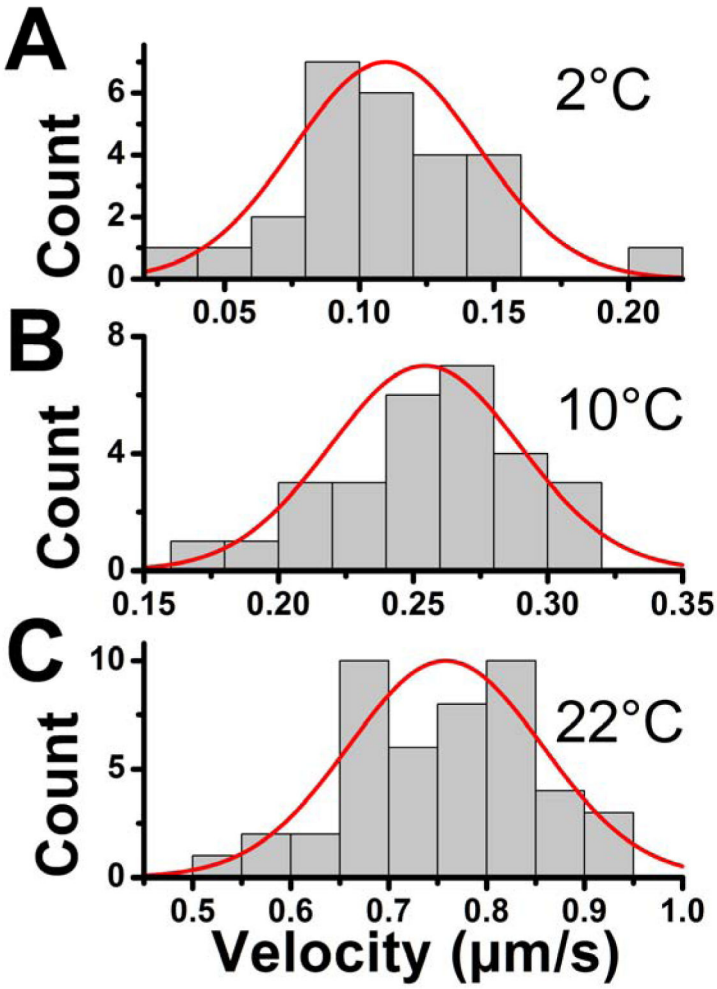

Fig. 1.

KIF5a-driven cargo motility at varying temperatures. Velocity distributions at (A) 2°C, (B) 10°C, and (C) 22°C have a well-defined peak which is approximately Gaussian (red). Velocity distributions appear similar for assays with and without TMAO, and for KIF1a-driven motility both with and without TMAO (data not shown). Microtubules exhibited no excessive bending or kinking and were able to support robust motility over many microns, consistent with largely intact polymeric filament.

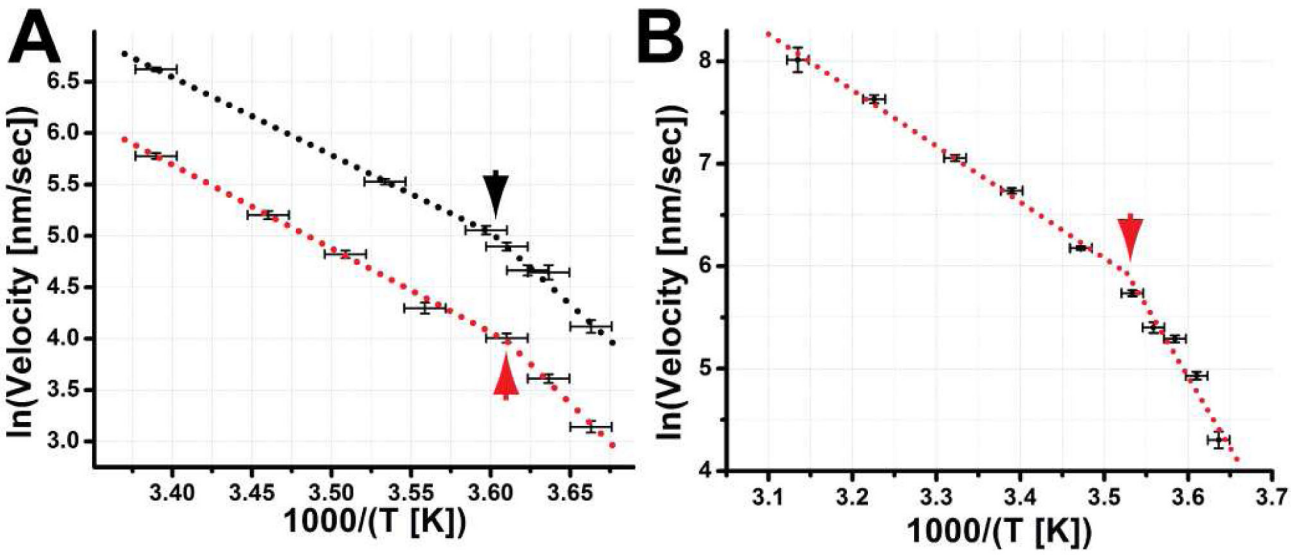

Fig. 2.

Arrhenius plots for KIF5A velocity data as a function of temperature. (A) In the ATP background (A, black), activation energy is 62.9 kJ/mol (1σ CI: [51.7 kJ/mol, 63.2 kJ/mol]) above ~4.7°C, and 117.9 kJ/mol (1σ CI: [93.0 kJ/mol, 122.5 kJ/mol]) below. In the GTP background (A, red), activation energy is 68.4 kJ/mol (1σ CI: [66.8 kJ/mol, 72.0 kJ/mol]) above ~3.7°C and 135.6 kJ/mol (1σ CI: [111.6 kJ/mol, 146.2 kJ/mol]) below. (B) In the ATP and 200mM TMAO background activation energy is 46.5 kJ/mol (1σ CI: [41.8 kJ/mol, 51.9 kJ/mol]) above ~11°C and 105.4 kJ/mol (1σ CI: [73.0 kJ/mol, 122.3 kJ/mol]) below. Data for 28°C, 37°C, and 46°C is reproduced from previous work (17). CI estimates were obtained via bootstrap analysis. Arrows highlight Arrhenius breaks.

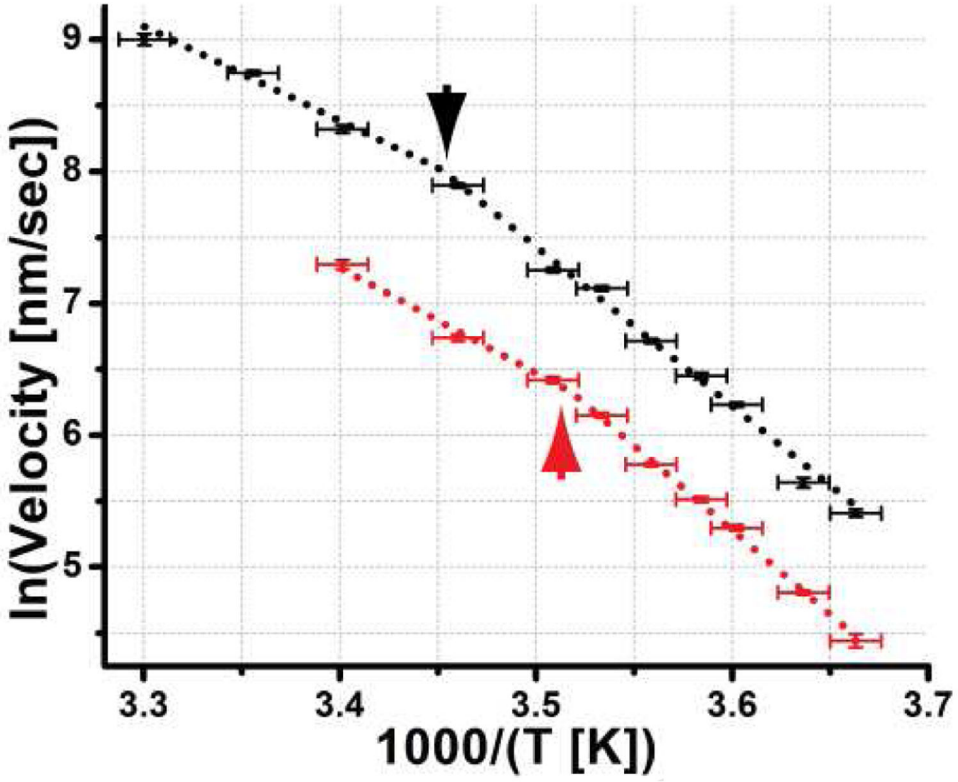

Fig. 3.

Arrhenius plots for KIF1A velocity data as a function of temperature, with 200mM TMAO (black) and ΔTMAO (red, offset down for clarity). In ΔTMAO buffer, activation energy is 68.1 kJ/mol (1σ CI: [57.4 kJ/mol, 75.3 kJ/mol]) above 10.5°C and 107.5 kJ/mol (1σ CI: [101.1 kJ/mol, 113.9 kJ/mol]) below. With TMAO, activation energy is 60.4 kJ/mol (1σ CI: [55.2 kJ/mol, 77.5 kJ/mol]) above 16°C and 107.5 kJ/mol (1σ CI: [98.7 kJ/mol, 114.8 kJ/mol]) below. CI calculated via bootstrap analysis.