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Full Length Article

Toxic effects of zinc ions on kinesin – Potential molecular cause of impaired intracellular transport

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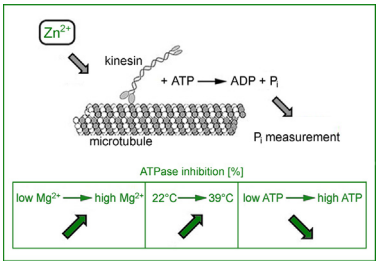
HIGHLIGHTS

- Zinc ions are able to inhibit kinesin ATPase and kinesin-based motility generation.
- Zinc-induced inhibition depends on the zinc/magnesium ion ratio.
- Zinc-induced inhibition depends on temperature.
- Zinc-induced inhibition might be one molecular cause of impaired intracellular transport.

GRAPHICAL ABSTRACT



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ABSTRACT

In healthy organisms the metabolism of the trace element zinc is well balanced. If this balance becomes destroyed the free zinc level might increase and cause toxic effects. The present study demonstrates that under definite conditions zinc ions are able to inhibit the ATPase activity of neuron-specific KIF5A (kinesin-1). Correspondingly, the motility activity of KIF5A also decreased. The inhibition rates have been found to depend on the magnesium ion concentration. Lowering the magnesium concentration weakens the inhibition. In addition, also decreases of temperature or increasing the ATP concentration result in reduced inhibition. Zinc ion-mediated inhibition of KIF5A activity might be one molecular cause contributing to impaired transport processes within brain and other organs in cases of zinc dyshomeostasis.

1. Introduction

Zinc is the second most abundant transition metal in the body behind iron (Canzoniero et al., 1999). Being an essential trace element for most living organisms, it is crucially involved in various physiological events such as mitotic cell division or protein and DNA synthesis (Kawahara et al., 2014). Zinc ions also play an important role in the transmission of nerve impulses, in the regulation of enzymatic activity, and in protein stabilization (McCord and Aizenman, 2014). In healthy organisms the concentration of zinc ions is well balanced. It is known that stressful conditions like oxidative stress, heavy metal load or thiol-modifying substances are able to disturb the intracellular zinc homeostasis leading to increased concentrations of free zinc within the cytoplasm (Kroncke, 2007; McCord and Aizenman, 2014). Increased cellular zinc levels have been found to be toxic (Cuajungco and Lees, 1996; Frederickson et al., 2005; Sensi et al., 2009) and can cause several diseases, including neuronal injury in cases of cerebral ischemia, epilepsy, or brain trauma (Capasso et al., 2005). Recently, the pathogenesis of some neuronal disorders like Alzheimer's disease has been discussed in relation with zinc dyshomeostasis (Kawahara et al., 2014; Li and Wang, 2016; McCord and Aizenman, 2014; Mizuno and Kawahara, 2013).

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So far, the molecular mechanisms underlying the zinc-induced development of diseases and the impairment of cellular processes have not been elucidated sufficiently.

Numerous intracellular transport events are based on the kinesin-based motility-generating system. So, it seems to be well established that KIF5 motors are causally involved in the transport of lysosomes, synaptic vesicles precursors, synaptic membrane precursors, diverse other vesicles and of mitochondria (Hirokawa et al., 2009). Interestingly, zinc ions have been reported to inhibit the mitochondrial transport within neurons, whereby this effect was related to phosphatidylinositol 3-kinase activation (Malaiyandi et al., 2005). It has been well established that mitochondria in neurons are driven along microtubule rails by KIF5. Within the past decade, we reported that aluminum (Bohm et al., 2015), lead (Bonacker et al., 2005) and some transition metal ions like, mercury (Bonacker et al., 2004), cadmium (Böhm, 2014), and copper (Böhm, 2015) are able to inhibit the KIF5A ATPase and motility activity, suggesting that these cations are powerful to impair kinesin-dependent cellular transport events. The question arises whether zinc ions are also potent to affect KIF5A and whether direct attacks of zinc ions on the kinesin might be a further molecular cause of disturbances of the transport of mitochondria and/or other organelles. To answer this question the ATPase activity of human neuron-specific KIF5A, which belongs to the kinesin-1 group, was measured. The results, which were complemented by motility data, might indicate that the direct interaction of zinc ions with kinesin could be another potential molecular cause of failed intracellular transport processes, which might underlie neuronal diseases.

2. Material and methods

The KIF5A (Niclas et al., 1994) was expressed in *E. coli* as truncated tag-free construct including the *N*-terminal amino acids 1–560 and purified as described formerly (Kalchishkova and Böhm, 2008). Like the complete KIF5A (consisting of 1032 amino acids), this construct forms dimers and has been proved to be functionally intact concerning its ATPase activity and its ability to bind to and to transport cargoes in cell-free environment (Dreblow et al., 2010; Kalchishkova and Böhm, 2008).

The ATPase activity has been determined by measuring the amount of inorganic phosphate released from ATP by the kinesin (Bohm et al., 2015) at the following standard conditions: 55 nM $KIF5A_{560}$, 1.5 μM tubulin (added as fragmented paclitaxel-stabilised microtubules), 1.25 mM ATP, 2.5 mM $MgCl_2$, 50 mM Pipes, pH 6.8, 37 °C. Corresponding experimental variations from these conditions are indicated in the legends to the figures. Motility activity has been determined using the conventional so-called gliding assay, in which the velocity of microtubules moving across a kinesin-coated glass surface is measured under microscopic control (Bohm et al., 2015).

The zinc chloride (Sigma-Aldrich (99.999% trace metals basis) used in this study was added from a 10 mM stock solution in 50 mM Pipes buffer (pH 6.8).

3. Results and discussion

Zinc ions were found to inhibit the ATPase activity in concentration-dependent manner with an IC_{50} of 42.7 μM ± 3.1 μM at standard condition (Fig. 1a). Remarkably, the inhibition strength was observed to depend strongly on the magnesium ion

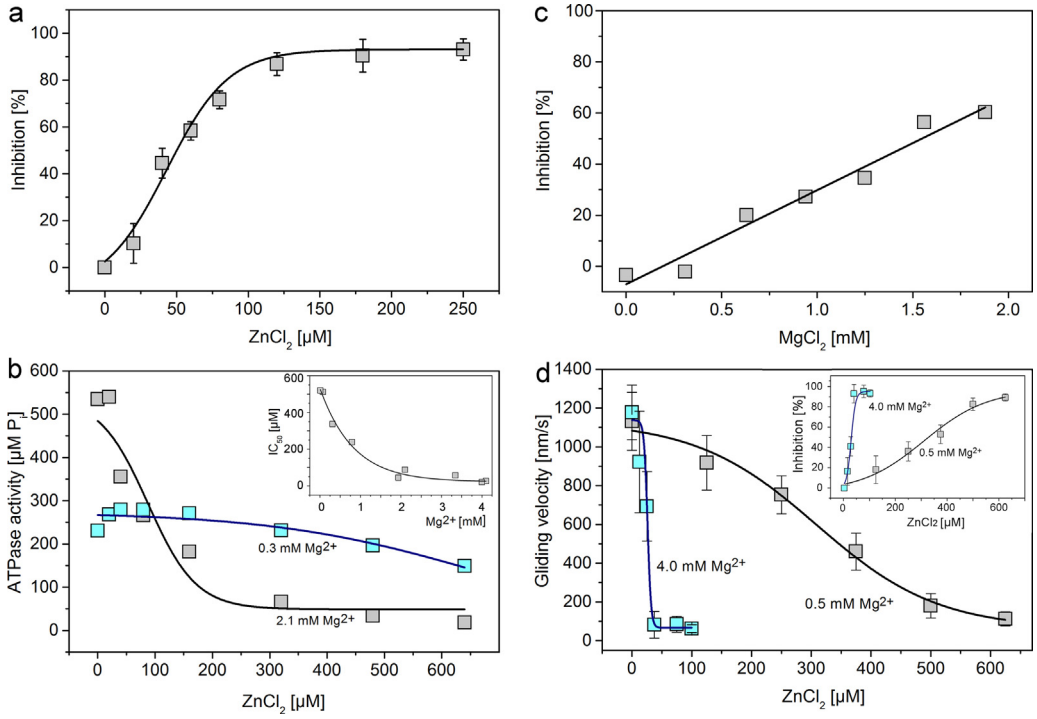

Fig. 1. Zinc ion-caused inhibition of the kinesin activity.
(a) Dependence of ATPase activity on zinc chloride concentration at standard conditions. Mean values with SD, calculated from six independent measurements. (b) ATPase activities (given as concentration of inorganic phosphate released within 30 min incubation in a constant volume) at 0.3 and 2.1 mM $MgCl_2$. *Insert*: Dependence of the IC_{50} values on magnesium ions. (c) Rate of zinc ion-induced inhibition dependent on magnesium ion concentration. (d) Kinesin-mediated motility in the presence of zinc ions. The data are based on the measurement of the velocity of microtubules gliding across a glass surface covered by $KIF5A_{560}$. 1.14 μM $KIF5A_{560}$, 0.4 μM tubulin. Further experimental details are described formerly (Bohm et al., 2015). *Insert*: Percent inhibition. The velocities determined in the absence of zinc ions (1132 nm/s ± 149 nm/s at 4.0 mM $MgCl_2$ and 1178 nm/s ± 141 nm/s at 0.5 mM $MgCl_2$, respectively) were set as 0% inhibition. Mean values with SD, calculated from two independent measurements.