Revealing the stepping mechanism of kinesin by means of a thermodynamically consistent model

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Introduction

In the case of larger structures (e.g.membrane vesicles or cellular organelles) or bigger distances (e.g.axon, flagellum) diffusion is not sufficient for explaining the observed intracellular transport. In the 80's a new group of proteins was discovered that specialized on solving this problem [1]. The new family of proteins was given the name „kinesin".

The homodimer motor protein kinesin-1 can attach to a microtubule with its two heads and is able to walk on it and pull the cargo attached to its tail. It was shown experimentally that kinesin-1 uses the „hand over hand" mechanism to walk along microtubules in a manner that the microtubule-bound head is overtaken from the left by one head, and from the right by the other. However, the detailed mechanism of the movement is not known. The questions waiting for answer include the precise role of the neck linker, the free energy change of neck linker docking, the number of hydrolysed ATPs during one step or an average trajectory in the state space of kinesin-1.

Goals

The kinetic models of kinesin available in literature use too simple kinetic networks and/or are not consistent thermodynamically in giving the kinetic constants. In the last few years enough data has accumulated to make possible the creation of a model with well established input parameters and that includes all the dimer states constructed from the direct product of monomer states. The goal of our work was to create a thermodynamically consistent model that satisfies these criteria.

To fit the parameters and check the model we used the velocity, dwell time, hydrolysed ATP/step and randomness data from the literature. We reproduced experiments with mutant kinesins and tried to clarify some details of the kinesin-1 stepping mechanism that are experimentally difficult to access. Further goal was to find the answers to the questions in the introduction.

Methods

In our model we distinguished six monomer states. They differed in the state of the neck linker, in the contained nucleotid and in the affinity to microtubules. We assumed a fast equilibrium between the docked and undocked states. The dimer states were constructed from the direct product of the monomer states. Since we included all dimer states we call our model complete.

The kinetic constants between the states originate from experiments, polymer model calculations and thermodynamic considerations.

The force dependence of a kinetic constant is always connected to a molecular structural displacement. If this displacement is small the force dependence cannot be strong. Since great structural changes in kinesin-1 occur only in case of the docking of the neck linker we can assume that the kinetic constants of a given state are force independent. However, this also means that the kinetic constants of the docked and undocked states may differ significantly.

The model was implemented as a kinetic Monte Carlo algorithm written in C. The end-to-end vector density function of the free neck linker was calculated using Mathematica.

Polymer model

To model the neck linker connecting the two heads we used the freely jointed chain polymer model. This model has the feature that the extension of the neck linker is always finite. Using this model we could quantify the rate of a head binding to the microtubule and the probability of the docked and undocked neck linker.

The rate of a head binding to the microtubule was assumed to be proportional to the density of the end-to-end vector of the neck linker at the binding site. To find the force dependence we weighted the density of the neck linker of the head bound to the microtubule with a Boltzmann factor. The external force does not act on the neck linker of the free head. We obtain the density

of the free head on the forward and backward binding site by convoluting the two neck linkers.

The force dependence of the docking probability was calculated from the partition functions of the docked and undocked neck linkers.

Thermodynamic consistency

Thermodynamic consistency means that between any two states on any path the product of the equilibrium constants is equal to the Boltzmann factor constructed from the free energy difference of the two states. Using this equation on one hand makes the model physically valid and on the other hand enables us to quantify the rates of some transitions that are otherwise hard to calculate or access experimentally. Such transitions include the docking of the neck linker in a two head bound state or while binding a nucleotide. We could calculate the rate of ATP synthesis by using the free energy change during an ATP hydrolysis.

Results

1. *Our model lays down principles (completeness, thermodynamic consistency, placing the values of the kinetic parameters on an experimental or theoretical ground) that can be and should be a guide line for future modelling of motor proteins or other similar systems.*

   Models on kinesin-1 were often thermodynamically contradictory and dealt with only a part of the state space.

2. *The results of the polymer model suggest that the values of the free energy differences measured by Rice et al. [2] are too small.*

   Our polymer model calculations [3] indicate that to keep the dwell time low the docked state of the ATP containing head must be more favorable than measured. Kinetic modelling has shown that in the case of an ADP containing head it must be more unfavorable.

3. *Using our simulation we could reproduce the results of Carter and Cross [4] and Block [5] with good fidelity. Furthermore, our results are in good accordance with measurements of mutant kinesins done by Yildiz et al. [6].*

4. *We obtained a somewhat different result for the ratio of the number of forward and backward steps from that of Carter and Cross. However at high force values the force dependence of this observable is well established theoretically and our results can reproduce that.*

5. *With the fitted parameters the kinesin-1 does futile hydrolysis during backward force. Also in case of zero external force slightly more than 1 ATP is used for one step.*

   The efficiency of kinesin has been debated for a long time. The most widely accepted view is 1 ATP/step. However, the ATP dependence and increase in dwell time at high backward loads is readily explained by futile hydrolysis and is also confirmed by our simulations.

6. *Only the part of the futile hydrolysis that appears also in the dwell time appears in randomness.*

   The dwell time and thus randomness is always affected by the hydrolysis in only one of the heads.

7. *The stepping mechanism of kinesin-1 is between „biased diffusion" and „power stroke".*

   According to our simulations the free energy change during neck linker docking is not small but not very big either.

8. *Calculating the rate of ATP synthesis from a thermodynamic box we got a value close to the measured one of Hackney [7].*

Hivatkozások

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