Mechanical modelling of motor protein myosin II

Summary and Theses of PhD dissertation

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Budapest, 2012

1 The objectives of the dissertation

Ezymes play crucial role in living organisms. These macromolecules participate in certain reactions, but unlike reagents, they go through a cycle and get back to their initial state at the end of the reaction. In terms of chemistry, they can be considered as catalysts, but their role is usually much more important, than that of 'classiclal' catalysts: in many biological processes, the acceleration of the reaction is only a secondary objective, or even not an objective at all. Their main role is very often the utilization of the energy liberated during the reaction for an important aim of the organism.

A special group of enzymes is the group of motor proteins, capable of transforming the chemical energy liberated from the reagent (called *substrate* in enzimology) to mechanical energy at a high rate of efficiency.

The objective of this dissertation is the mechanical modelling of skeletal muscle myosin II. This enzyme is responsible for the contraction of skeletal muscle. (There are other types of myosin II, however: heart muscle myosin, smooth muscle myosin, etc. In this dissertation, we just dealt with skeletal muscle myosin II, a single exception is Chapter 2, where the results can be applied for any motor protein that have two functional sites.) In the basic functional unit of muscle, called *sarcomere*, there are about 300 myosin molecules acting together in an arrangement reminescent to a team of tug-of-war. The myosin molecules – in a bundle-like structure – walk along a filamentous protein polimer called *actin* in a cyclic way, while in each cycle, they transform an adenosine-triphosphate (ATP) molecule into adenosine-diphosphate (ADP) and an inorganic phosphate ion (Pi). In terms of chemistry, ATP is a substrate, but from the point of view of mechanics, it can be considered as the fuel for contraction. Myosin works in a four-stroke like manner: one of its movements is the *powerstroke* – this is when the current head actively contributes to force generation –, while the remaining serie of movements is reminscent to the process, when a member catches a new hold of the rope in a game of tug-of-war. Meanwhile, the current molecule passively accompanies the 'team' with the help of heads just performing their powerstrokes.

The examination of myosin II and its cycle is hindered not just by its little size, but also by the complexity of the interactions during the cooperation in the sarcomere which are not easy to be measured or not measurable at all.

Many models have been developed for the description of the behaviour of myosin II, from macroscopic mechanical models through enzyme kinetic models, to molecular dynamic simulations. The mechanical models prevailing in the XIXth century with such 'classical' elements as linear springs dashpots, etc. are ignored nowadays, as they are no more consistent with our present knowledge about muscle tissue.

The permanently progressing enzyme kinetic models have given answers to many questions, but they have the disadvantage of requiring a more or less arbitrary selection between the states of the enzymatic cycle. In these models the continuous motion of the molecule is described by a discrete set of states, where the values of the forward and backward rates draw out the most probable pathway. In case of motor proteins, the pathway strongly depends on the loads applied on the molecules, which are considered by load-dependent rates in enzyme kinetic descriptions, but these models are nearly or totally unable to consider such crucial mechanical aspects as the *angle* of load.

The mechanical aspects of the comlicated behaviour of myosin II are much more precisely revealed by molecular dynamic simulations, which try to describe the motion of the atoms

based on dynamic equations. Even though these models follow more precisely the motion of the molecule, there are many unresolved problems and questions inhibiting the application of these methods. On one hand, the number of atoms in myosin is about 10 thousand (this implies a system with 30 thousand degrees of freedom), and due to the strong non-linearities caused by large displacements and collisions, these calculations are time-consuming. On the other hand, the thermal fluctuations prescribe very little timesteps, and this leads to a further increase in computation time. Another difficulty with these simulations is the existence of several local minima on the potential energy function, which triggers that the simulations can easily get lost on the energy landscape and stop at a local minimum, while in reality myosin can easily overcome these local minima with the help of thermal collisions.

In view of these facts, it became clear for us that there are many opportunities in a model that tries to describe the motion of myosin with minimal degrees-of-freedom. Based on the results of researches and the open questions, we summarized the objectives of our reseach in the following:

1. We desired to build up a system capable of modelling the motion of a motor protein with two mechanical functional sites. (In case of myosin these two functions are the attachment to actin and the rotation of the lever arm.)

2. We desired to minimize the number of arbitrary influences into our system, and we aimed to build up a model that follows the four states of myosin II, with continuous transitions from one state to another.

3. We required the capabilty of advancing to be an internal property of our model, as the previous models used a non-symmetrical geomertical constraint that could not be justified by any experiment up to now.

4. We required from our model a mechanical behaviour consistent with the experimental results of myosin II.

5. By the consideration of thermal fluctuations, we desired to answer the question, wether the powerstroke is a thermal ratchet mechanism or a global relaxation mechanism.

6. By answering the questions concerning the powerstroke, we desired to give a clarifying explanation for the contradiction that the efficiency results (55-60 percent) from macroscopic measurements on muscle tissue are much higher, than the values obtained from single-molecule experiments, which reported values below 15 percent.

2 The structure of the dissertation, methods

The first chapter of the dissertation summarizes the researches about myosin II, from the first experiments and models to the results achieved up to now. In the same chapter, we present the different experimental and theoretical methods and approaches of the different disciplines. At the end of the chapter, we summarize the questions for which we got an answer by the research of this dissertation.

In the second chapter, by taking into account the fact that motor proteins are overdamped, we present a simple mechanical model, which is capable of modelling all motor proteins that

have two mechanical functional sites. We show that by the consideration of the damping center, a spatial and temporal separation can be achieved, by which different displacement functions with different decays can be produced at the two sites of the protein.

In the third chapter, we apply the results of the second chapter for myosin II: by incorporating a separating device into the head of myosin, we model its motion on a symmetrical geometric constraint. The number of the degrees of freedom varies between 2 and 4 according to the state of the head depending on the contact with the geometric constraint.

During the enzymatic cycle of myosin II – and also in our model – large displacements occur, thus the motion is described by a first order, non-linear, ordinary differential equation system. In terms of matrix-calculation, the non-linearity appears in the form of displacement dependent damping and stiffness matrices, so in our system, we have tagent matrices, instead of constant ones. Even though the numerical method – due to the low degrees of freedom of our system – does not use any matrix-calculation, but solves the equation system directly, in fact, it determines the tangent damping and stiffness matrices in one timestep for calculating the displacement vector of the next timestep. I wrote in C language the simulation of the system, which approximates the solution of the equation system with fourth-order Runge-Kutta method.

After finishing the simulation code, I began the investigation of parameters, by which we obtained a system consistent with the observed behaviour of myosin II, both in aspects of stiffnesses and of energetics. At the end of the third chapter, we show that this model with low degrees of freedom mimics well the dynamics of myosin, it follows the cyclic motion of myosin II observed in experiments listed in the second thesis.

In the last, fourth chapter I built up a model that takes into account the real shape of the discovered subdomains of myosin II with seven rigid elements. A remarkable progress compared to the preceding chapter is that we consider the effect of thermal collisions, which are always present at molecular level. Even though the degrees of freedom did not increase with the help of a very simple assumption concerning the internal geometric constraints, the system has become more complex due to the previously ignored non-linearities arising from the real shape of the newly introduced elements. For the solution of the system, two different alternatives have emerged. The first way could have been that of the previous chapter, i.e. the derivation of the reduced equation system, but this turned out to be so complicated, that we decided to solve the system in another way: the thermal fluctuations prescribed a very little timestep which permitted the application of the simplest Euler-method. This way, we could substitute the previously totally rigid geometric constraint with a set of stiff springs which led to a solution of sufficient accuracy from the point of view of our investigations.

After having written the simulation software, I began the investigations of parameters. Most of the damping coefficients were determined by the real shape of the elements and the viscosity of the surrounding solvent, while the choice of the stiffnesses of the springs was determined by the energy levels of the known states of the enzymatic cycle. We obtained a system reproducing the experimental observations listed in the third thesis.

Also with the help of the extended model of 7 elements, we got an answer for the nature of the powerstroke, i.e. wether it is a ratchet-like or a relaxation mechanism. As revealed by the simulations, the two mechanisms coexist: from a geometric point of view, the relaxation mechanism can be considered as dominant, as about 85 percent of the rotation of the lever arm occurs during the period of relaxation, but the relaxation is preceded by a ratchet mechanism when high forces act accompanied by little displacements. So, from the the point of view of forces, the ratchet mechanism turned out to be dominant. Besides these results, the simulations

released the apparent contradiction between macroscopic efficiency measurements and the values obtained from experiments performed on individual molecules. Based on the simulations that gave a deeper insight into the process of the powerstroke, we announced the fourth thesis of the dissertation.

3 Results, theses

As a result of the research, we can announce the following theses:

Thesis 1 *I built up an overdamped mechanical system with negligible inertial forces capable of splitting the spatial and temporal scales of a motor protein with two mechanical functional sites. I introduced the concept of the* damping center*, which can be considered as an analogy with the concept of the center of mass in systems of non-negligible mass.*

Based on [1].

Thesis 2 *I created an internal lever arm model for a single myosin head composed of three rigid elements with a geometrical constraint that is capable of modelling the four-stroke enzymatic cycle of myosin II. It provides piecewise exponential time-displacement curves consistent with the following experimental results:*

- *Energy is supplied only once in each cycle when ATP is hydrolyzed.*
- *The enzymatic cycle cannot be completed away from a binding site, inhibiting the molecule to make futile cycles.*
- *The frequency of cycles decreases as the load increases due to the slow-down of the enzymatic cycle.*
- *The ratio of the bound state per the overall periodicity (i.e. the duty ratio) increases as the load increases.*
- *There exists a stall force, a value of the load when the cyclic behaviour stops.*
- *In lack of ATP, myosin II stays strongly bound to actin, and only a huge force can pull the molecule away from a binding site.*
- *During powerstroke, the contact force between actin and the motor domain increases.*
- *The energy profile of the system is consistent with the values obtained from experiments.*

Based on [3].

Thesis 3 *I built up an extended model of seven rigid elements that takes into account the experimentally observed shape of the subdomains of myosin II. Into this extended model, I have incorporated the effect of random forces arising from thermal collisions. It is consistent with the following experimental results:*

- *There is 11° closure of the cleft during the enzymatic cycle. Meanwhile U50 rotates 31° and L50 rotates 20°, both of them counter-clockwise with respect to the N-terminal subdomain.*

- *Energy is supplied only once in each cycle when ATP is hydrolyzed.*
- *The enzymatic cycle cannot be completed (i.e. much less probable) away from a binding site, inhibiting the molecule to make futile cycles.*
- *There exists a stall force, a value of the load when the cyclic behaviour stops.*
- *During powerstroke, the contact force between actin and the motor domain increases.*
- *The energy profile of the system is consistent with the values of experiments.*

Based on [2].

Thesis 4 *I discovered a previously unrevealed hidden part of the powerstroke which released the apparent contradiction since long time between macroscopic measurements on muscle tissue and experimental results performed on individual molecules. I separated two parts of the powerstroke and I showed the existence of a Kramers mechanism at the beginning of the powerstroke, followed by an Eyring relaxation mechanism.*

Based on [2].

4 Aplication of the results, proposal for furthe research

In the dissertation, we modelled the mechanical behaviour of myosin II. A plausible direction of further research is the application of our model for other members of the myosin superfamily, as all myosins meet the requirements described in the first chapter and in the first thesis. Another possible direction of further investigations is the application of our model for *kinesin*, a motor protein that also has two mechanical functional sites.

The model presented in the third chapter revealed that even a very simple mechanical system can reproduce the complicated behavior of myosin II, which traced out a very interesting direction of further reseach: the simplicity of the system emerges the possiblity of the creation of artificial muscle, by which a system with properties very similar to those of real muscle tissue could be obtained. As the system of the second chapter behaves like myosin without thermal effects, we can imagine the design of the artificial muscle on a scale much larger than that of myosin, where the effect of thermal fluctuations is already negligible.

From the results of the fourth chapter, we can see, how myosin II can unify the requirement of the capability of high mechanical work, with the capabilty of acting as a very precise sensor cancelling out the huge noise caused by thermal fluctuations. During the enzymatic cycle the proportion between tensile and fexural bearing of loads strongly changes, and myosin uses this phenomenon for fullfilling the two requirement mentioned above: with a suitable proportion of the axial and tangential stiffness, the molecule is enabled to perform high mechanical work and to be a very precise sensor at the same time.

Thus the structure of myosin II with its lever arm gives a very convincing example how to work in a very efficient way on the scale of nanometers. Even though the author is not well educated in the dynamically progressing field of nano-machines, he has the impression that the way how myosin works gives useful advice to researchers who plan to build up a simple system with high capability of work performance based on a very precise internal sensory mechanism.

Publications on the subject of the dissertation

Articles in foreign language published in Hungary

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[2] Bibó, A., Károlyi Gy., Kovács M. (2012): Fluctuation and dissipation in a mechanical model of myosin II. *Periodica Polytechnica Ser. Civ. Eng.*, accepted for publication.

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[3] Bibó, A., Gy. Károlyi, M. Kovács (2010): Internal Lever Arm Model for Myosin II. *IUTAM Symposium on Dynamics Modeling and Interaction Control in Virtual and Real Environments* Budapest, 2010. június 7-11., pp. 155–163.

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[4] Bibó, A. (2007): Motorfehérjék mechanikai modellezése. *Doktori kutatások a BME Építőmérnöki Karán, 2007. november 14.*, Budapest, pp.139–146.

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[5] Bibó, A., Gy. Károlyi, M. Kovács (2009): A visco-elastic myosin model. *17^{th} Inter-Institut Seminar for Young Researchers*, Krakkó.

[6] Bibó, A., Gy. Károlyi, M. Kovács (2010): Internal Lever Arm Model for Myosin II. *81^{st} Annual Meeting of the International Association of Applied Mathematics and Mechanics*, Karlsruhe, Germany.

[7] Bibó, A., Gy. Károlyi, M. Kovács (2011): Internal Lever Arm Model for Myosin II. *SIAM Conference on Applications of Dynamical Systems*, Snowbird, UTAH.

[8] Bibó, A., Gy. Károlyi, M. Kovács (2011): Internal Lever Arm Model for Myosin II. *Wellcome Trust Focused Meeting – Cellular Cytoskeletal Motor Proteins*, Hinxton, Cambridge, UK.

[9] Bibó, A., Gy. Károlyi, M. Kovács (2011): Internal Lever Arm Model for Myosin II. *Gordon Conference on Muscle & Molecular Motors*, New London, NH, USA.

Other publications

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