ISAS - INTERNATIONAL SCHOOL
FOR ADVANCED STUDIES

Protein Motors

Thesis submitted for the degree of
Magister Philosophiæ

Candidate:
Gianluca Lattanzi

Supervisor:
Prof. Amos Maritan

October 1999

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Contents

Preface

The aim of this thesis is to focus on the problems raised by the study of the phenomenon of energy transduction in living organisms.

Movement is possible because the energy provided by chemical reactions can be transduced into mechanical work. This phenomenon leads to very important questions not only for muscle physiology or molecular biology, but even in non equilibrium thermodynamics and statistical physics.

The structure of this thesis is inspired by the interdisciplinarity of the subject.

In the first chapter we give an introduction to the problem, focussing on the relevant biological issues.

In the second chapter we review a recent theoretical simplified model and we also claim that any coarse–grained model should be used to calculate quantities which can be directly compared with experiments.

The third chapter contains our results both for the simplified model introduced in the second chapter and for a slight modification of it.

Finally, in the conclusion, we point out our findings and outline our future perspectives.

Chapter 1

The biological problem

In this chapter we introduce the biological background for the problem under study. Starting from the definition of the problem we focus on the qualitative cross-bridge model pertaining to the working cycle of molecular motors. We also provide a short description of the experiments performed in this area of research.

1.1 Motility in the cell

Many important biological processes in the cell rely on transport of material, organelles and vesicles; in some cases these transport phenomena are due to physical effects, such as pressure or concentration gradients; in the majority of cases the transport of material goes in directions which are opposite to the gradients themselves.

Human bodies and all living organisms in general need to perform work *against* external generalized forces.This is evident in the contraction of muscles, but also in the processes leading to cell division or *mitosis*, in the passage of neurotransmitters from the terminations of nerve cells (or axons) to the adjacent synaptic channels.

Specific enzymes provide these essential functions: these enzymes work as *motors*, i.e. they transduce the energy excess in the chemical reaction of ATP (adenosinetriphosphate) hydrolysis into mechanical work. They are known as protein motors or molecular motors [1].

1.2 Classification and structure of the enzymes

Many different *families* of protein motors have been detected so far and for each family it is possible to further classify the proteins into *classes*, see Fig. 1.1.

The motion of these enzymes can be linear or rotary. Examples of linear motors can be found in the families of *myosins*, *kinesins* and *dyneins*. An example of rotary motor is the F_0F_1-ATP-ase which is used to synthesize ATP molecules

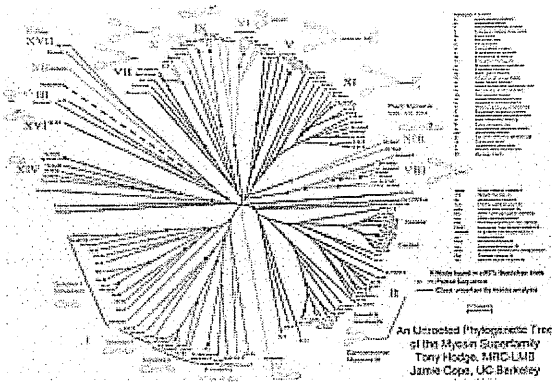

Figure 1.1: An example of one family of motor proteins

starting from the products of the hydrolysis reaction when working in one sense, or to induce rotations of parts of the cells when working in the opposite sense, as for instance is required in the swimming of cilia and flagella.

The structure of molecular motors is different for proteins belonging to different families and also among proteins in the same family, but in different classes. Nevertheless it is usually possible to detect some specific common features.

These proteins are usually made of one *head* or more (myosins from striated muscles are one-headed motors, kinesins are usually two-headed, but it is even possible to find three heads); the head is attached to a very flexible region which can be more or less complex and is globally indicated as the *neck*. Finally a long *tail* is attached to the neck and can be rigid or semi-rigid, made of one, two or more chains, called *essential* or *regulatory* depending on their function, see Fig. 1.2.

The motor protein can work as a single enzyme, for the transport of material or can work in bundles, as is the case for muscle myosins; in the latter case the tails are attached together to form a more or less flexible backbone. The dimensions of the head of a typical motor protein are of about 5 nm, while the neck region is about 2 nm wide, and finally the tail can be several nanometers long (10 or more). Globally the protein size is about some tens of nanometers, while the number of residues belonging to the same protein can vary from approximately one thousand (in the smallest one–headed myosin) to more than four thousand in a complex two-headed myosin or dynein.

1.3 The filaments

Linear protein motors move upon tracks or networks of tracks. The tracks are called filaments, while the whole network of filaments forms the so called cytoskeleton which is the mechanical sustain of the entire cell. The modifications in the cytoskeleton account for the mobility and plasticity of the entire cellular

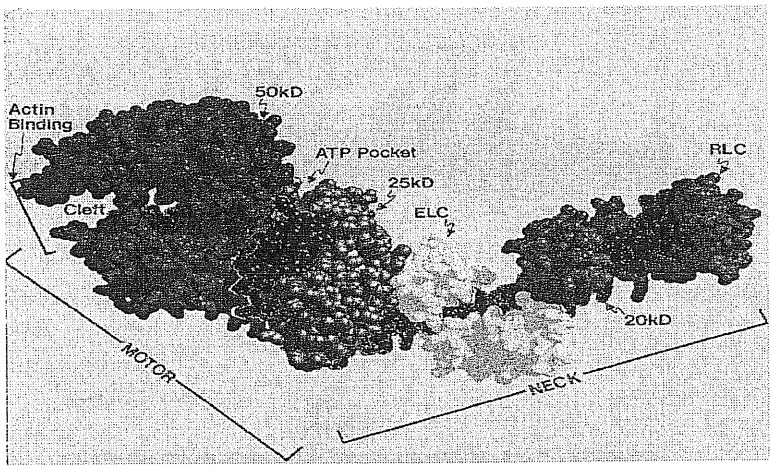

Figure 1.2: The myosin crystal structure and its distinctive regions: the head (red), the neck (green), part of the tail (blue), the essential (yellow) and the regulatory (purple) light chains. The ATP and the actin binding sites are also shown.

body in processes like mitosis or phagocytosis. The filaments are complex structures obtained by polymerization of a single monomer or dimer. They can be essentially divided in two major classes:

- The actin filament is formed by the polymerization of the actin monomer which is about 5 nm in size. The shape of this filament is usually given by a double helix, as shown in Fig. 1.3(a). The two ends of the actin filament differ in the velocity of the polymerization process. The end which polymerizes faster is referred to as the "plus" end, while the slower end is the "minus" end. This property seems to be essential to the movement of motors, since conventional myosins always move in the positive sense[1].

- Microtubules are formed by the polymerization of a dimer (formed by the two proteins $\alpha-$ and $\beta-$ tubuline) which is 8nm long. Their structure is more complicated than in the case of the actin filament, see Fig. 1.3(b).

  They form a hollow tube $\approx$ 30 nm in diameter and several hundreds of nanometers long, depending on the function. Also for these filaments it is possible to distinguish one end from the other according to the different velocities of the polymerization process. Also in this case, the "plus" end is the end which grows faster. Kinesins usually move in the positive sense, while dyneins move in the opposite direction.

Globally the filaments can be described as periodic and fairly rigid structures with a polar symmetry, i.e. it is possible to define a "plus" and "minus" extremity

[1]some myosins can move in the negative direction, indeed. It is true in general that each motor protein moves either in one direction or the other

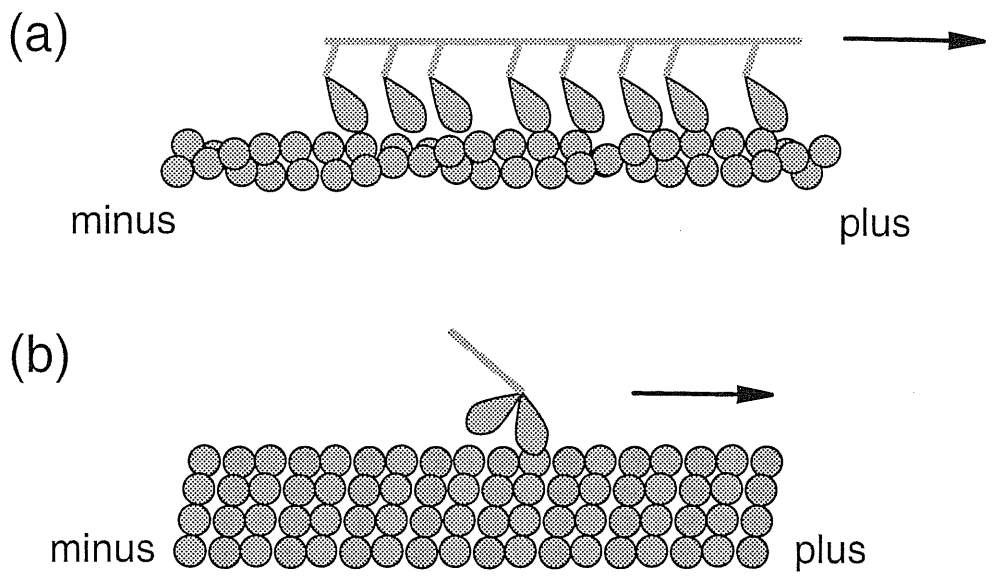

Figure 1.3: Essential structure of the actin filament (a) and the microtubule (b).

(by matter of convention). Polarity is essential to guarantee the unidirectionality of motion.

1.4 The hydrolysis reaction

Adenosine triphosphate or ATP is the only organic molecule with three phosphate atoms. At the end of the hydrolysis reaction, one phosphate group gets lost, and the product is the adenosine diphosphate or ADP for short and one inorganic phosphate group or P_i. The reaction scheme can be represented in the following way:

$$ATP \rightarrow ADP + P_i \tag{1.1}$$

This reaction is catalyzed by the presence of molecular motors. If the catalyst is absent, the reaction can take up to several days to reach full thermodynamic equilibrium. The free energy difference between reacting species and products is approximately $60pN \cdot nm$ which is about $15K_BT$, the thermal energy at ordinary working temperatures (or body temperature). This energy is converted into mechanical work performed against loads attached either to the tail, to the backbone (in myosin bundles) or to the filaments themselves. It is possible to calculate the difference in chemical potential for the reaction:

$$\Delta\mu = \mu_{ATP} - \mu_{ADP} - \mu_P \tag{1.2}$$

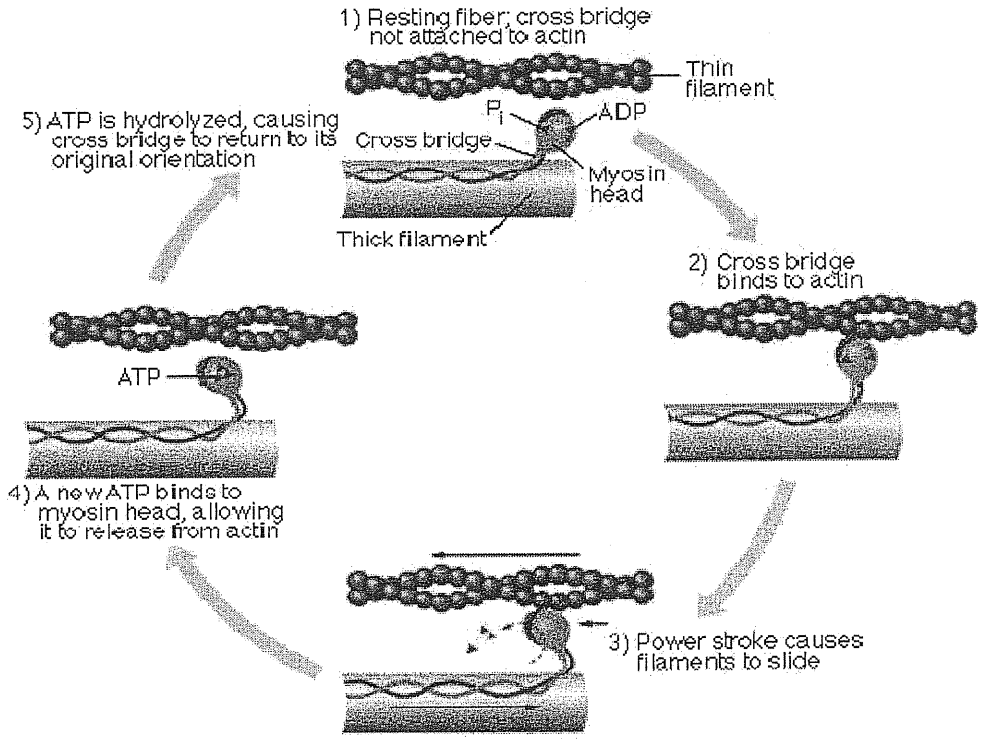

Figure 1.4: The Cross-bridge cycle.

where μ_i is the chemical potential for the i–species. From mass-action law in ordinary chemistry:

$$\frac{[ATP]}{[ADP][P]} = \exp(\Delta\mu) \tag{1.3}$$

where we use the notation $[x]$ to indicate the normalized concentration of the chemical species x.

1.5 The crossbridge model

This model is the qualitative framework for all the proposed mathemical models. Its original formulation was proposed by Huxley in the late '50s, while it is now known in a revised form due to Hill which dates back to 1974. In this model it is assumed that the protein can assume different conformations according to the different steps in the chemical reaction.

In Fig. 1.4 a myosin head is attached to the actin filament while the tail is linked to the backbone (state 3). The binding of an ATP molecule in the active site of the protein (located in the head) induces the detachment of the head from the filament. The protein is now in the "Released" state (state 4) and the hydrolysis reaction can take place. The reaction induces a conformational change of the protein, in particular it strongly changes the conformation of bounds in the neck region which causes the head to move (state 1); the protein is now in a "cocked" state, ready to produce force. In facts the release of phosphate (one of the products) forces the motor protein to bind again to the filament (state 2), in a position which is different from the one from which it detached, and also in a different configuration, which is the "force generating" state. The release of ADP accounts for the "power stroke", the fast movement which brings the protein in the initial "attached" conformation by pulling the filament (state 3). This scheme is of course just a simplified model and many questions are still substantially unanswered until now. For instance the conformational changes are still under investigation, since it is very difficult to obtain crystal sructures corresponding to the different states depicted in Fig. 1.4. It is not clear for instance whether the re–attachment of the myosin head depends on the release of phosphate or not. It is then difficult to obtain information on this length-scale, while this can be done on a larger scale.

1.6 Motility assays

The experiments performed on these proteins are usually referred to as *motility assays*. It is usually possible to measure the velocity developed under an excess of ATP or an external load. They are sketched in Fig.1.5. In experiment (a) a microneedle is used to detect the force exerted on an actin filament by a myosin bundle moving upon it in presence of ATP. The (b) assay makes use of a recently developed experimental device (the *optical trap*) to monitor the displacements of an actin filament. This system is calibrated so as to measure even single displacements. The silica bead below the actin filament is in fact covered with myosins at a low concentration so that at each time only a single myosin interacts with the filament. The optical trap is also used in experiment (c) to monitor the velocity of a kinesin walking on a microtubule. In this case the optical trap is simply attached to the kinesin tail. In experiment (d) an electric field is used to exert a given force on the actin filament which can move under the constraint of a linear groove. It is possible to measure the velocity of the actin filaments under external loads in presence of ATP. This kind of experiment is particularly useful to detect collective effects due to the cooperation of many myosins.

All these experiments can give us useful information on the way chemical energy is transduced into mechanical work. They are not intended to provide information about the specific conformational changes of the protein structure,

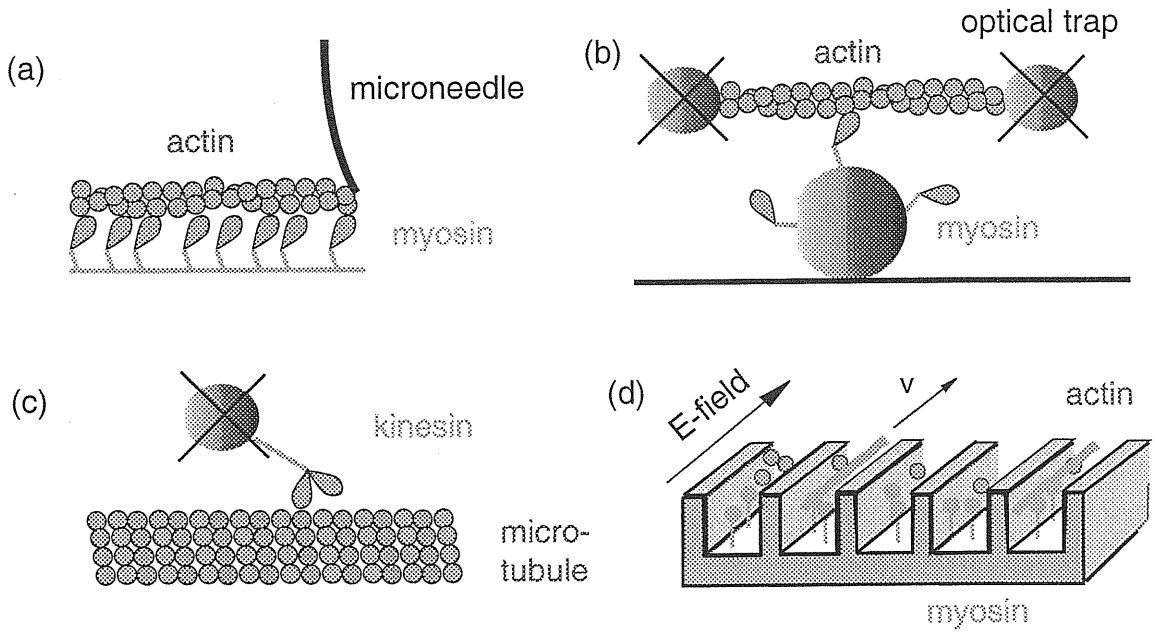

Figure 1.5: Experimental techniques.

nor they account for the coupling of the external force to the chemical reaction. However the extracted information can be used to discriminate between realistic models and mathematical exercises. As long as experimental data get more and more accurate, the theoretical picture of the process is being restricted and clarified.

1.7 The need for models

This is the usual way in which biologists describe the process of chemical energy transduction. The transition rates among different states can be tuned so as to reproduce the experimental results. In the past decades many models have been proposed with increasing number of states (up to 5 or 6). Nevertheless these models are strongly protein–dependent, and not only for the numerical values of transition rates, but also for a tentative explanation to the whole transduction process. A general feeling of the physicist is that for each process occurring in different forms, there is only one explanation and this needs to be as general as possible. In this field the process is the energy transduction and then it should be possible to give a precise physical picture in general terms and under general conditions. This reductionist need has recently found very clear answers in the work by the group led by prof. J. Prost at University of Paris VI [2], but it has also been addressed in many other models and at the moment there is not a reference model for this process. The next chapter will clarify this issue.

Chapter 2

Modelling molecular motors

In this chapter we focus on the continuous two-state ratchet model, first introduced by F. Jülicher, A. Ajdari and J. Prost, which will be our framework for the calculations in the subsequent chapter.

2.1 Common assumptions

Several models have been proposed so far to explain the energy transduction process, dating back to the early reviews by A.F.Huxley (1957) and Hill (1974) [3, 4], usually referred to as cross–bridge models.

In all these models the motors may exist in different states (up to 5 or 6 [5]), within each of which the system reaches *local thermodynamic equilibrium* on time scales small compared to the exchange rates between the states themselves. This hypothesis relies on the fact that characteristic times of the motors (measured through transient response [6]) are of the order of ms, while thermal equilibrium on a lenght scale of about 10 nm occurs in 10-100 ns.

As seen in the previous chapter, the fuel for the motor is provided by the ATP hydrolysis, so that the models should take into account also the chemical potential difference in this reaction ($\Delta\mu$).

Another important issue is that the models should take into account the essential features of the filaments: their periodicity and their asymmetry, at least.

2.2 Discrete and continuous models

The models studied so far can be classified as discrete or continuous [7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. In discrete models, the motor coordinate may assume only discrete values (usually in units of filament periodicity), while in continuous ones it may assume any value.

These two approaches are substantially equivalent, one can switch from a discrete description to a continuous one as long as it is possible to associate a Fokker-Planck equation to a process with a Langevin dynamics. Both can be suitable for the problem under study, and in many cases it is useful to compare results obtained using the two different approaches, mostly to gain confidence on the numerical algorithms used.

These models have been studied to reproduce some experimental data, obtained in 'in vitro' motility assays. The experiments contributed to clarify the picture of the cross-bridge model, pointing out the processivity of kinesins and dyneins and the essential characteristics of the interaction between the myosin head and the actin filaments. Still some open questions remain regarding the conformational changes of protein structure due to ATP-binding and product release, or eventually the attachment-detachment of myosin to the actin filament. Yet a clear physical explanation for the force generation mechanism in muscle fibers is still missing, as recently Fisher pointed out [17].

2.3 The two–state model

We chose, as a working framework, the model proposed by Jülicher, Ajdari and Prost. This is a simple continuous two-state model. The model can be easily extended to include more than two states, at the expense of a higher complexity.

The state of the motor will be indicated by the index i, whereas x is the position of the protein center of mass along the track. The chemical reactions force the motor protein to switch from one state i at position x to another state j in the same position x, with a rate given by $\omega_i(x)$. The motor moves under the influence of an effective potential $W_i(x)$ chosen so as to reproduce the interaction between the filament and the motor head and with the same characteristics of the filament: *polarity* and *periodicity*. For this reason the potential is chosen to be asymmetric and periodic with period p for each state i ("ratchet" potential, see Fig. 2.1).

We start from a simple Langevin stochastic overdamped dynamics:

$$\eta_i \frac{dx}{dt} = -\partial_x W_i(x) + \xi_i(t) \tag{2.1}$$

where η_i is the friction coefficient, $\xi_i(t)$ is a random number, usually a Gaussian white noise.

Turning to a Fokker–Planck (FP) description, $P_i(x,t)$ is the probability density for the particle to be in state i at position x at time t, while the probability current $J_i(x,t)$ is defined as:

$$J_i(x,t) = -\frac{D_i}{kT}\left[P_i(x)\partial_x W_i - P_i(x)F_{ext} + kT\partial_x P_i(x)\right] \tag{2.2}$$

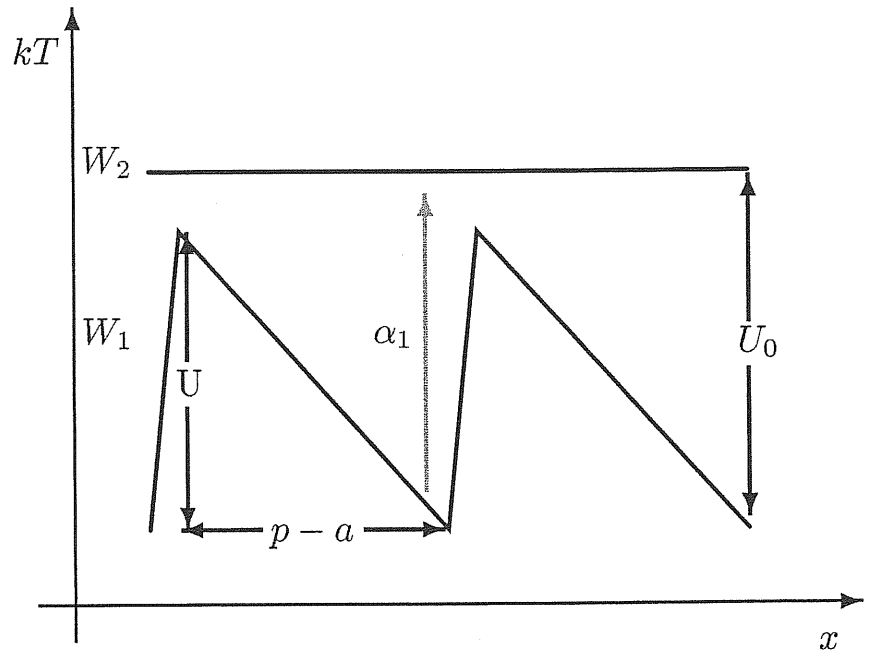

Figure 2.1: Model (a): ratchet potential (W_1) and diffusive state 2. The parameters are chosen so that $a/p = 0.1$, $U = 10kT, U_0 = 12kT, \omega/D = 50$.

where D_i is a diffusion coefficient which is taken to be equal in both states, T is the temperature, k is the Boltzmann constant and F_{ext} is the external force.

2.3.1 The chemical reaction

Since we are dealing with a simple two-state model, the chemical reaction cycle is compressed in a two step process:

$$M + ATP \underset{\alpha_2(x)}{\overset{\alpha_1(x)}{\rightleftharpoons}} M \cdot ADP \cdot P$$

$$M + ADP + P \underset{\beta_2(x)}{\overset{\beta_1(x)}{\rightleftharpoons}} M \cdot ADP \cdot P$$

where M means motor while α_1, α_2 (β_1, β_2) are the transition rates for the first (second) chemical reaction and they may depend on the coordinate x. The state $M \cdot ADP \cdot P$ corresponds to a state in which the motor head is detached from the fiber by binding of ATP. In this state the motor may move more or less freely upon the filament. This state will be called "free" state, or state 2. All the other terms in the equations refer to a state in which the motor is attached to the filament and therefore its motion is strongly dependent on the motor–filament interaction. This is the so called bound state or state 1. In this case we are

dealing essentially with a single headed motor, although the mechanism for a two head motor protein is similar.

2.3.2 The choice of the potential

We chose a very simplified shape of the potential. Since we are assuming that in state 1 the motor is attached to the filament, we choose a ratchet potential to represent this interaction, while for state 2, corresponding to a simple diffusion, we choose a flat potential, see Fig. 2.1.

2.3.3 Detailed balance

We assume detailed balance to hold for each chemical reaction:

$$\frac{\alpha_1(x)}{\alpha_2(x)} = \exp\left(\frac{W_1(x) - W_2(x) + \Delta\mu}{kT}\right) \tag{2.3}$$

$$\frac{\beta_1(x)}{\beta_2(x)} = \exp\left(\frac{W_1(x) - W_2(x)}{kT}\right) \tag{2.4}$$

where $\Delta\mu = \mu_{ATP} - \mu_{ADP} - \mu_P$ is the difference in chemical potential or the chemical driving force.

The total transition probabilites ω_i are simply given by:

$$\omega_i(x) = \alpha_i(x) + \beta_i(x) \tag{2.5}$$

It is important to observe that we are not assuming detailed balance to hold also for the total transition probabilites; indeed only when $\Delta\mu = 0$, i.e. the chemical reaction is at equilibrium, the detailed balance condition will be satisfied by the total transition rates.

2.3.4 The system of equations

From the assumptions above we are left with a system of two coupled FP equations:

$$\begin{aligned} \partial_t P_1 + \partial_x J_1 &= -\omega_1 P_1 + \omega_2 P_2 \\ \partial_t P_2 + \partial_x J_2 &= \omega_1 P_1 - \omega_2 P_2 \end{aligned}$$

The model can be easily extended to include more than two states, at the expense of an higher complexity. The main advantage of this approach is in the clear role played by eventual external forces, directly inserted in eq.(2.2) without any need for further assumptions (for a discussion of this point see [17]).

It is also important to observe that the parameters are strictly related to some measurable quantities; one is able to model the potential and the transition rates, under certain conditions, but this is the only freedom, while most of the parameters are settled out by the experimental conditions one wishes to reproduce.

2.3.5 Transition probabilities

Using eq. (2.3) and (2.4) only two of the four functions $\alpha_1(x), \alpha_2(x), \beta_1(x)$, and $\beta_2(x)$, can be chosen arbitrarily once W_1 and W_2 are fixed. Since the release of products is just a thermal process and does not involve chemical reactions (see eq. (2.3.1)), we assume it to be position independent as in [18], so that $\beta_2(x) = \omega = const.$ Various choices of W_i and α_2 can be done [2, 18].

Transitions due to chemical reactions are usually chosen to be localized, i.e. they may take place only in a certain position of the motor along the filament period and therefore they are not distributed over the whole period. This in some way corresponds to the "active site" concept in biology and in [2] the former hypothesis is shown to agree with experimental data. To take this effect into account we also define:

$$\alpha_2(x) = \begin{cases} \omega & \text{for } p-\delta < x < p, \\ 0 & \text{otherwise.} \end{cases} \tag{2.6}$$

with $\delta/p = 0.05$.

If the external force is independent of time, it is possible to look for a stationary solution.

2.4 Conditions for motion

For each pair $(\Delta\mu, F_{ext})$, there is a uniquely defined average velocity

$$v = \frac{D}{kT}\int_0^p dx[P_1(F_{ext} - \partial_x W_1) + P_2(F_{ext} - \partial_x W_2)] \quad , \tag{2.7}$$

where the P_is satisfy the normalization condition

$$\int_0^p dx[P_1 + P_2] = 1 \quad . \tag{2.8}$$

Similarly, we can introduce the ATP hydrolysis rate r which denotes the number of chemical cycles performed per unit time:

$$r = \int_0^p dx[\alpha_1 P_1 - \alpha_2 P_2] = \int_0^p dx[\beta_2 P_2 - \beta_1 P_1] \quad . \tag{2.9}$$

If $\Delta\mu = 0$, the total transition rates satisfy

$$\omega_1/\omega_2 = e^{-\Delta W/kT} \quad , \tag{2.10}$$

where

$$\Delta W(x) = W_2(x) - W_1(x) \quad . \tag{2.11}$$

This condition of detailed balance for the total transition rates indicates that transitions are just thermal fluctuations and that the system is not driven chemically. If the external force also vanishes, the steady state is a thermal equilibrium with $P_i = Ne^{-W_i(x)/kT}$ for which $v = 0$ and $r = 0$. For $\Delta\mu > 0$, the system is chemically driven. If no external force is applied spontaneous motion with $v \neq 0$ can occur, however only if the system is polar. For a symmetric system with $W_i(x) = W_i(-x)$ and $\omega_i(x) = \omega_i(-x)$ the steady state distributions are also symmetric $P_i(x) = P_i(-x)$. Since $\partial_x W_i$ is antisymmetric in this case, $v = 0$ by symmetry according to Eq. (2.7). On the other hand, r is in general nonzero in this case (the functions α_i are symmetric). For spontaneous motion to occur two requirements have to be fulfilled: detailed balance of the transition has to be broken, which corresponds to $\Delta\mu \neq 0$ and the system must have polar symmetry. In the case of motor proteins the polar filaments play this role.

In the presence of an external force F_{ext}, the system can perform mechanical work, i.e. it operates as a motor. The work performed per unit time against the external force is

$$\mathcal{W} = -F_{ext}v \tag{2.12}$$

while the chemical energy consumed per unit time is given by

$$\mathcal{Q} = r\Delta\mu \quad . \tag{2.13}$$

We can therefore define the efficiency of energy transduction

$$\eta = -\frac{F_{ext}v}{r\Delta\mu} \tag{2.14}$$

This quantity is useful for forces applied opposite to the direction of motion where $0 \leq \eta \leq 1$. Note, that this definition relies on the fact that a bulk solution exists which plays the role of a thermodynamic reservoir and allows to define the chemical potential difference of fuel and products. In situations where reservoirs are small the efficiency would be more difficult to define.

2.5 Measurable quantities: Leibler's point of view

It is possible to model the transition rates so as to keep into account the fact that the transition due to chemical reaction takes place in a certain position of the motor along the filament period and is therefore not distributed over the whole period. In [2] it is shown that this former hypothesis agrees with experimental results. It is then desirable to proceed further in the search for quantities which can be directly compared with experiments. According to Leibler [19] it is possible to identify some directly or indirectly measurable quantities. Any

model constructed to describe the action of motor proteins and the phenomenon of chemical transduction should provide accurate predictions for these quantities. The accordance between predictions and values from experiments will be the indicator of a realistic modellization of the process under study. These quantities are described below.

2.5.1 Probabilities of different states

The probabilities:

$$P_i = \int_0^p P_i(x)dx \tag{2.15}$$

According to Leibler and his model, they should depend on the ATP concentration ($[ATP]$) through a simple law, which is known for enzymatic catalysis as the Michaelis law and will be described later. For large $[ATP]$, the probability of the attached state becomes arbitrarily small, while the others saturate at extremum values. Particularly interesting are the two limiting quantities for large $[ATP]$: P_2^{max} which measures the fraction of time the motors spend detached from the fiber, and P_1^{max}, often called the *duty ratio*, which measures the fraction of time spent by the motors in the working state.

2.5.2 Average ATPase activity, r

It is possible to calculate the numbers of ATP molecules consumed per second per motor, r. In Leibler's model this quantity obeys a simple Michaelis law. One can define a Michalis-like constant, K_m, as the ATP concentration for which the rate is half maximal.

2.5.3 Average velocity of the fiber, v

In the absence of external load, the dependence of the fiber velocity, v, on $[ATP]$ should be similar to Michaelis law, with the velocity increasing linearly for small $[ATP]$ and saturating at a maximal value for large $[ATP]$.

Also in this case it is possible to define a Michaelis-like constant, L_m. This constant is analogous to K_m for the ATP hydrolysis rate r.

2.5.4 The ratio of Michaelis-like constants, ψ

It is important to notice that the ratio of the Michaelis-like constants for the velocity and ATPase activity,

$$\psi \equiv \frac{L_m}{K_m} \tag{2.16}$$

does not need to be equal to 1 even if one supposes the tight–coupling mechanism for motor proteins.

If only one motor interacts with the fiber, the velocity curve will generally follow the hydrolysis curve, i.e., $\psi = 1$, however if many motors are present, the velocity v can saturate at much larger ATP concentrations than does the hydrolysis rate, r. Indeed, for large $[ATP]$, the motors can spend a large part of their cycle time, P_2^{max}, detached; during that time the fiber can be moved by other motors which are in the working state.

2.5.5 Effective step size, δ

One can define the average distance by which the fiber is moved between two hydrolysis events taking place on one motor. In Leibler's model this effective step size,

$$\delta \equiv \frac{v}{r} \tag{2.17}$$

is always equal to the molecular step size, δ, for small ATP concentrations, but at large $[ATP]$, he obtains:

$$\delta \propto \psi \tag{2.18}$$

and thus the effective step size can be bigger than the molecular one.

In the next chapter, we will calculate these quantities for the continuous two–state ratchet model.

Chapter 3

Results

In this chapter we outline the procedure used to obtain some results for measurable quantities. The Michaelis–Menten mechanism is also described. Finally we show our results for the continuous two–state ratchet model and for a slight modification of it.

3.1 The stationary solution: numerical method

Starting from the two coupled dimensionless equations for the continuous two–state ratchet model:

$$\partial_t P_1 + \partial_x J_1 = -\omega_1 P_1 + \omega_2 P_2 \tag{3.1}$$
$$\partial_t P_2 + \partial_x J_2 = \omega_1 P_1 - \omega_2 P_2 \tag{3.2}$$

we seek the stationary solution ($\partial_t P_i = 0$) using a ratchet potential:

$$\frac{W_i}{K_B T} = \overline{W_i}\left[\frac{x}{a}\theta(a-x) + \frac{p-x}{p-a}\theta(x-a)\right] + \Delta_i. \tag{3.3}$$

where $\overline{W_i}$ is the maximum height of the potential, Δ_i is an offset potential both in dimensionless units.

We write:

$$\omega_2(x) = \alpha(x) + \omega \tag{3.4}$$

$$\omega_1(x) = \omega_2(x)\exp\left[W_1(x) - W_2(x)\right] + \Omega(x) \tag{3.5}$$

with:

$$\Omega(x) = \alpha(x)\left(e^{\Delta\mu} - 1\right)\exp[W_1(x) - W_2(x)] \tag{3.6}$$

so that in chemical equilibrium ($\Delta\mu = 0$), $\Omega(x) = 0$. We try to integrate the system of FP equations, defining:

$$\begin{cases} y_1 = P_1 \\ y_2 = P_2 \\ y_3 = -P_1\partial_x W_1 - \partial_x P_1 \\ y_4 = -P_2\partial_x W_2 - \partial_x P_2 \end{cases}$$

We use the method of shooting to a fitting point: we start with four values at one boundary (0 or p); these values are chosen so as to represent a good initial guess to the solution we are looking for. We use the same values at the other boundary in order to guarantee periodicities of both currents and probabilities.

We then integrate using a Runge-Kutta fifth order method (Cash and Carp) with adaptive step-size [20] starting from boundaries up to the discontinuity in $x = a$ and we define:

$$f_i = y_i(a^+) - y_i(a^-) \tag{3.7}$$

$$\overline{y_i} = \frac{y_i(a^+) + y_i(a^-)}{2} \tag{3.8}$$

Since we want the solution to be continuous in $x = a$, we change the initial conditions by means of the Newton-Raphson method with line searches and backtracking [20] until all the f_i's are sufficiently close to zero.

Some problems in the numerical integration of the equations may lead to situations where the f_i's still remain quite far from zero. We chose to disregard these points and kept only the points for which:

$$F = \frac{\sum_i f_i^2}{\sum_i \overline{y_i}^2} < 10^{-3}$$

We solved the FP equations for different values of $\Delta\mu$, we also changed some parameters and verified that our results are consistent with those obtained by Parmeggiani et al. [18]

3.2 $\Delta\mu$ or ATP concentration?

From mass-action law we know that at equilibrium:

$$\frac{[ATP]_{eq}}{[ADP]_{eq}[P]_{eq}} = e^{\beta\Delta\mu_0} \tag{3.9}$$

where $\Delta\mu_0$ is the free energy difference for standard concentrations. In a real motility assay, the experimenter provides the system with excess ATP so as to raise the difference in chemical potential to the value $\Delta\mu$ (under physiological

conditions $\Delta\mu \approx 10kT$). The ATP concentration can be written in terms of the difference in chemical potential:

$$[ATP] = [ATP]_{eq} e^{\beta\Delta\mu} \tag{3.10}$$

so that our results can be plotted in terms of ATP concentration rather than chemical potential difference. We will use equivalently the parameter:

$$q = e^{\beta\Delta\mu} \tag{3.11}$$

which is directly proportional to $[ATP]$, at least at high ATP concentrations, where depletion problems are not implied.

3.3 The Michaelis–Menten mechanism

In 1913 Michaelis and Menten developed the theories of earlier workers and proposed the following scheme:

$$E + S \overset{K_S}{\rightleftharpoons} ES \rightarrow EP \tag{3.12}$$

The catalytic reaction is divided into two processes. The enzymes and substrate first combine to give an enzyme-substrate complex, ES. This step is assumed to be rapid and reversible with no chemical changes taking place; the enzyme and substrate are held together by physical forces. The chemical processes then take place in a second step with a first-order rate constant k_{cat} (the turnover number). The rate equations are solved in the following manner:

$$K_S = \frac{[E][S]}{[ES]} \tag{3.13}$$

and

$$r = k_{cat}[ES]. \tag{3.14}$$

Also the total enzyme concentration $[E_0]$ and the free enzyme $[E]$ are related by:

$$[E] = [E_0] - [ES] \tag{3.15}$$

Thus:

$$[ES] = \frac{[E_0][S]}{K_S + [S]} \tag{3.16}$$

and finally:

$$r = \frac{[E_0][S]k_{cat}}{K_S + [S]} \tag{3.17}$$

It is indeed found experimentally in most cases that the initial rate r of formation of products or destruction of substrate by an enzyme is directly proportional to the concentration of enzyme $[E_0]$. However, r generally follows saturation kinetics with respect to the concentration of substrate, $[S]$, in the following way: at sufficiently low $[S]$, r increases linarly with $[S]$. But as $[S]$ is increased this relationship begins to break down and r increases less rapidly than $[S]$ until at sufficiently high or saturating $[S]$, r reaches a limiting value termed r_{max}. This is expressed in the Michaelis–Menten equation.

3.4 Our results

The Michaelis–Menten mechanism, as shown before, is typical of enzymatic catalysis, so it is not surprising that experimentally this law is verified also for molecular motors. In fact the spontaneous hydrolysis of ATP occurs on time scales which are many orders of magnitude longer than the ones observed in presence of myosin or protein motors in general, so that protein motors can be considered catalyst for the ATP hydrolysis reaction.

3.4.1 Rate of ATP consumption

The rate of ATP consumption follows the Michaelis-Menten behaviour for $\Delta\mu$ up to approximately $15K_BT$ where our method fails to converge, see Fig.3.1.

It is indeed striking that the model proposed by Jülicher, Ajdari and Prost is also in agreement with this prediction and the experimental observations. The constants involved are difficult to express in terms of the parameters of the model, neverthelesss it is possible to calculate them when solving the equations.

The equation for the rate of ATP consumption is simply given by:

$$r = \frac{q \cdot r_{MAX}}{K_M + q}. \tag{3.18}$$

where K_M represents the Michaelis constant for the rate of ATP consumption.

3.4.2 Velocity

In Fig. 3.2 we show our results for velocity.

Also in this case we obtain substantially a Michaelis law, given by:

$$v = \frac{q \cdot v_{MAX}}{L_M + q} \tag{3.19}$$

where L_M is the Michaelis constant for velocity.

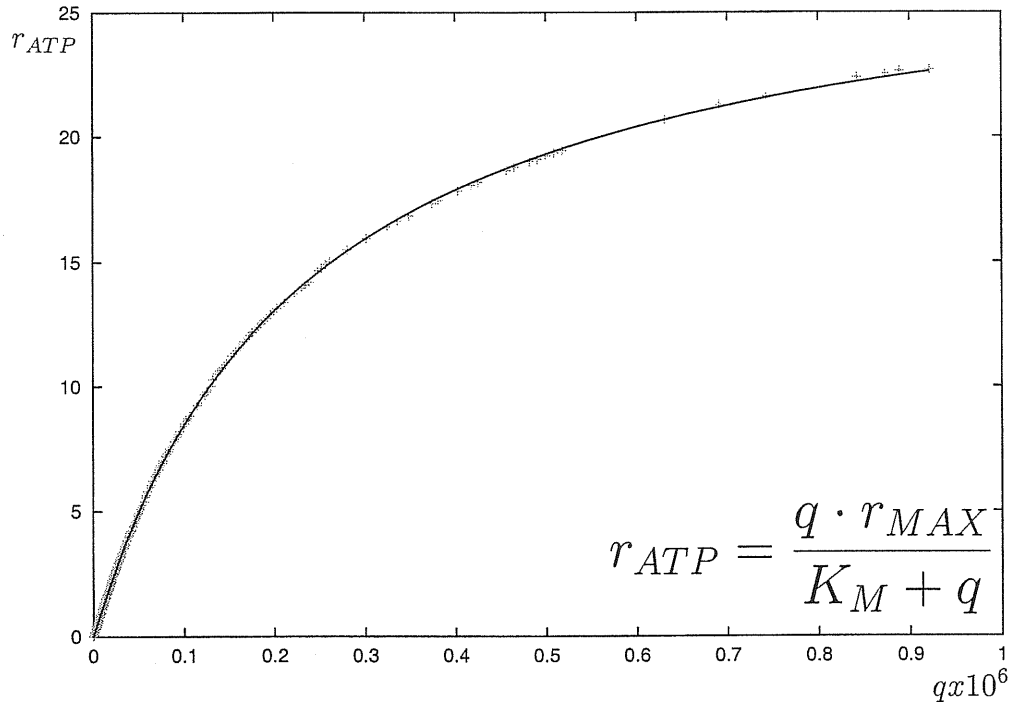

Figure 3.1: The rate of ATP consumption plotted versus the parameter q. Crosses stand for the points calculated by the numerical solution, the straight line is our best fit.

3.4.3 Duty ratio

In Fig. 3.3 we show our results for the duty ratio. We obtain again a Michaelis behavior in the form:

$$P_1 = 1 - \frac{q \cdot P_0}{M_M + q} \tag{3.20}$$

where M_M is the Michaelis constant. It is interesting to observe that the duty ratio even at high ATP concentration is not very low: the protein motor is in the attached state for a substantial time, showing a behavior which is typical for "porters", i.e. individual motors. This is confirmed by the ratio of the Michaelis constants:

$$\frac{K_M}{L_M} = 1. \tag{3.21}$$

3.4.4 Effective step-size

As shown in Fig. 3.4 the step-size saturates for $\Delta\mu \approx 8K_BT$ to the value $0.13p$ for the chosen set of parameters. This implies that many hydrolysis events are

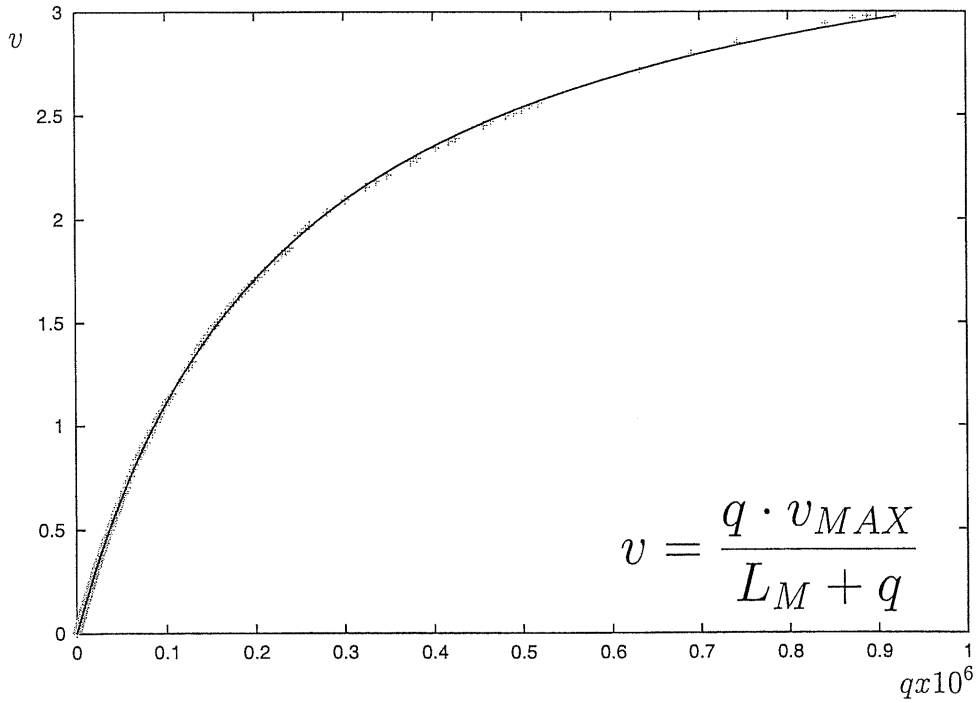

Figure 3.2: . The same plot as in Fig. 3.1 for velocity.

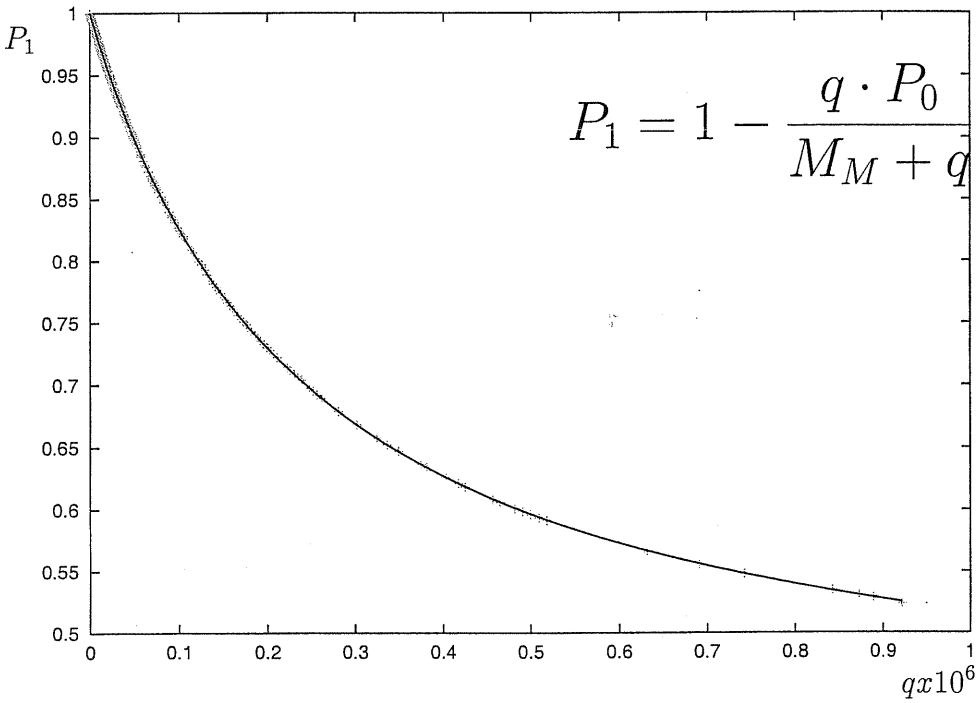

Figure 3.3: . The same plot as in Fig. 3.1 for the duty ratio.

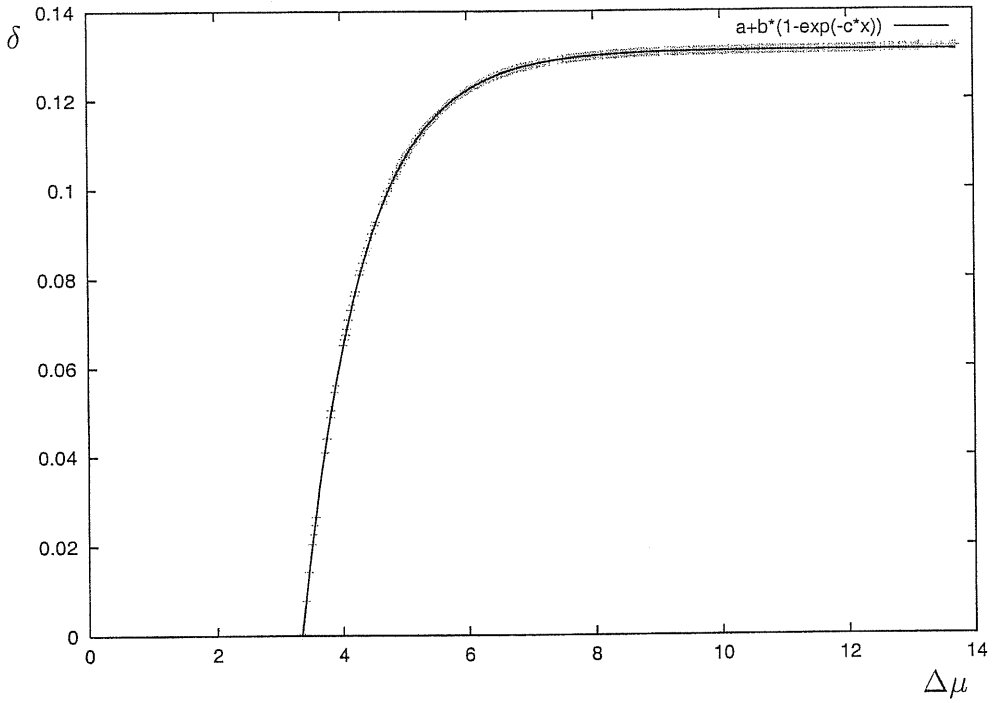

Figure 3.4: . The effective step size is plotted versus the chemical potential.

taking place without corresponding to a net motion of the protein motor. If for each hydrolisis event we expect the protein to proceed for one filament period, the effective step-size should be close to 1. The fact that this is not true for the model proposed by Jülicher indicates that it consumes more ATP than expected. This is not actually a failure of the model, since the weak points are indeed the potential shapes and the transition rates. It is just an indication that although this model reproduces some experimental observations, it is not the end of the story.

3.5 Effect of a trap in the potential

We tried to go a step further in this direction applying some ad hoc changes to the continuous two–state ratchet model.

In the downhill motion of the protein in the potential W_1, the motor moves backward when its position lies in the interval $[0, a)$. We tried to reduce the effect of this backward motion introducing a sine wave in the potential in this interval. The sine wave creates a trap when its amplitude overcomes the limit:

$$A_{min} = \frac{W_1}{2\pi} \tag{3.22}$$

When this happens we call:

$$x_{min} = \frac{a}{2\pi} \arccos\left(-\frac{W_1}{2\pi A}\right) \tag{3.23}$$

the centre of the trap. While in the previous model transitions from state 1 to state 2 were mostly probable in a left neighborhood of the potential minimum, in our modification we use two intervals, one centered in the potential minimum, and the other in x_{min}. These two intervals of equal length are chosen so that:

$$\int_0^p \omega_2(x)dx = \text{constant} \tag{3.24}$$

i.e. the number of transitions to state 2 per unit time is kept constant.

Our choice of a flat potential for state 2 corresponds to a diffusion process for the motor in the detached state. A necessary condition for a successful hydrolysis event is that after the transition to state 2 the motor diffuses so as to overcome the maximum of potential $W_1(x)$. In this way when the motor attaches to the filament again, it can go downhill the potential in the positive direction of motion.

In the new model some transitions occurr in a position which is very close to $x = a$, so that the diffusive step can be shorter than that in the previous model. Also, when the diffusive step fails to overcome the potential barrier, the protein can be trapped in x_{min} rather than falling to $x = 0$, so that again the probability of a successful hydrolysis event is supposed to increase.

In this perspective, we should obtain an enhancement of successful hydrolysis events leading to an increased effective step–size, when compared to the case without traps.

Indeed we obtained an increase in step-size to about $0.19p$ in the new model even in the absence of traps (when $A = 0$). This is due to the fact that transitions are more probable in a region centered in $x = 0$, so that the diffusive step–size is smaller than before. It is also possible to show that this situation corresponds both to an increase of velocity and a decrease of rate of ATP consumption.

In Fig. 3.5 it is possible to observe the effect of the trap. The step size is plotted versus different amplitudes of the sine wave for a certain value of $\Delta\mu$. It is possible to see a maximum in the step size when the amplitude is about 30–40% of W_1. This corresponds to a trap in the potential which has the effect of increasing the step–size of about 2%.

When $\Delta\mu$ is increased, the effect of the trap is still weaker. In facts in Fig. 3.6 it is possible to see that the maximum step–size is obtained when $A = 0$ for sufficiently high $\Delta\mu$. The effect of the trap is so weak that it disappears under normal working conditions for myosin ($\Delta\mu \approx 10kT$).

The trap may also be seen as a metastable state, in which the motor protein is forced after an unsuccessful hydrolysis event. The fact that its effect is so weak could be an indication that the essential mechanism for the energy transduction process is caught by a two step model without any need for further complications as metastable states or multiple states models. This indication is quite strong, we

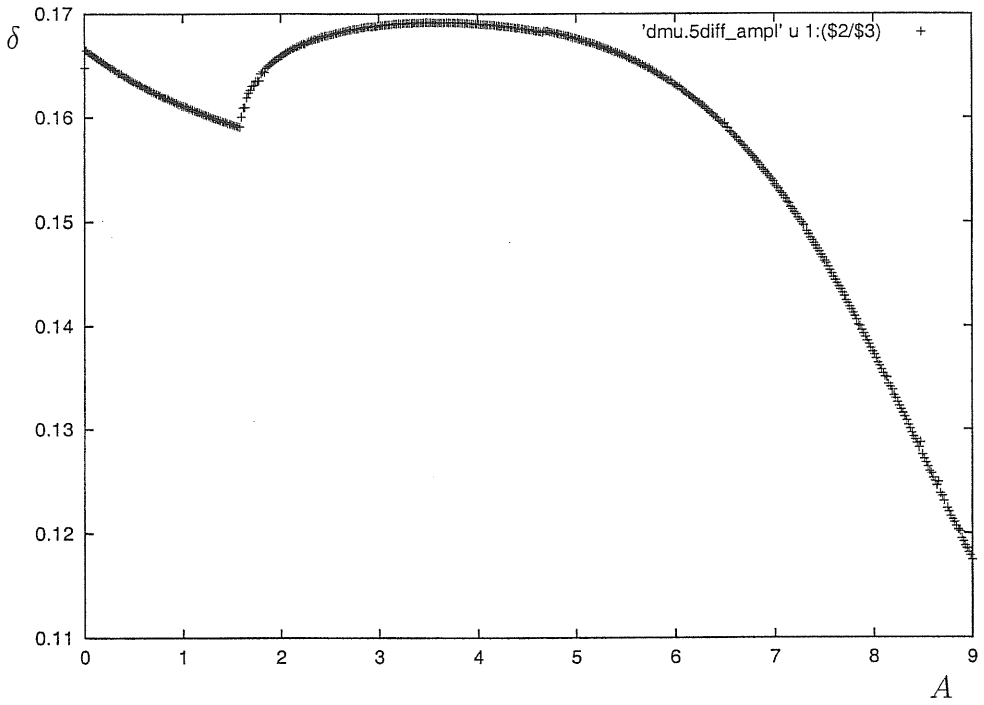

Figure 3.5: The effective step size as a function of different amplitudes of the sine wave. In this case: $W_1 = 10$, $\Delta\mu = 5kT$.

observe that results obtained by Jülicher et al. depend mostly on the transition rates, rather than the shapes of the potentials.

Our conclusion is that the robustness of the model with respect to the change in potential shapes is a clear indication of the validity of the essential description of a two–state model, as long as it is able to reproduce, at least qualitatively, the experimental results.

A coarse–grained description of the energy transduction process has been accomplished by the continuous two–state ratchet model; our results seem to suggest that any variation on this theme may lead only to some minor readjustement of the physical quantities involved, but new original results can be obtained only in a more detailed description, taking into account the conformational free energies of the various states of myosin and the effect of ATP binding and product release.

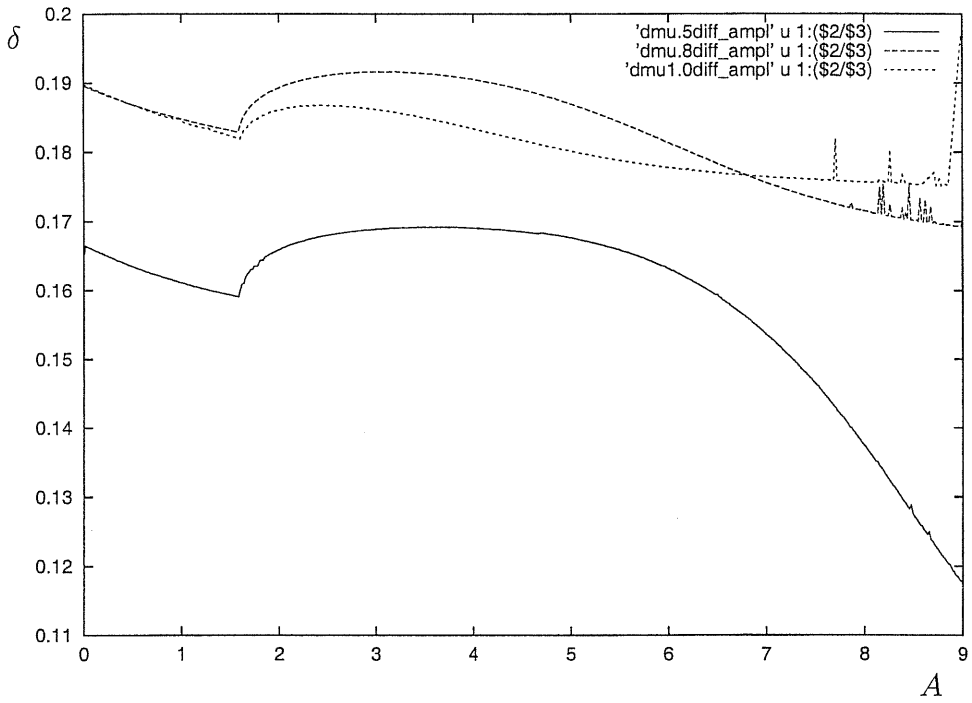

Figure 3.6: The effective step size as a function of different amplitudes of the sine wave for different values of $\Delta\mu$; dmu.5 stands for $\Delta\mu = 5kT$, dmu.8 for $\Delta\mu = 8kT$ and finally dmu1.0 for $\Delta\mu = 10kT$.

Conclusion

The problem of chemical energy transduction in motor proteins was described from a biological point of view and addressed by means of physical simplified models. Our calculations confirm the results obtained so far for continuous two–state ratchet models and extend them to other directly measurable physical quantities.

Our main results are:

- the rate of ATP consumption follows the Michaelis law.
- the velocity of the fiber follows the Michaelis law.
- the duty ratio shows a Michaelis–like behavior and is still appreciable for high ATP concentrations.
- the effective step size saturates to a value which is far below the expected 1, indicating that this model wastes more ATP than expected.
- slight modifications of this model lead to negligible differences in the whole picture.

Of course many points need further clarification and in the next future we hope to address at least some of the issues below.

- Some recent experimental results [21] suggest that the Michaelis constant would increase with applied load and this effect cannot be accounted for by simple discrete models. Therefore we would like to investigate the variations of the Michaelis constant with applied load in continuous two–state ratchet models.
- Discrete models are much simpler to handle mathematically, but of course they cannot reach the completeness and accuracy of continuous ones. More importantly the eventual external force is simply inserted in the FP equations, while in discrete models one has to resort to more complicated force dependent transition rates [17]. For these reasons it would be interesting to introduce a new discrete model, built so as to obtain the continuous one as a limit.

- The conformational changes in the protein structure are still an open question both for structural biology and statistical mechanics. We hope to address also this issue by means of less coarse–grained descriptions involving molecular dynamics or Monte Carlo simulations.

For these reasons we are sure this study deserves further investigation.

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