Vibrations in Microtubules

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Abstract. Vibrations in microtubules and actin filaments are analysed using a method similar to that employed for description of lattice vibrations in solid state physics. The derived dispersion relations show that vibrations in microtubules can have optical and acoustical branches. The highest frequency of vibrations in microtubules and in actin filaments is of the order of 10^8 Hz. Vibrations are polar and interaction with surroundings is mediated by the generated electromagnetic field. Supply of energy from hydrolysis of guanosine triphosphate (GTP) in microtubules and of adenosine triphosphate (ATP) in actin filaments may excite the vibrations.

Key words: Vibrations in microtubules, Vibrations in actin filaments, Microtubule translation symmetry, Dispersion relation, Energy supply to cyto-skeleton, Hydrolysis of GTP, Hydrolysis of ATP, Fröhlich's condensation in cytoskeleton, Nonlinearity in cytoskeleton

1. Introduction

The cytoskeleton is a highly dynamic structure which reorganizes continuously as the cell changes its shape, divides, and responds to the environment [1, 2]. The cytoskeleton is composed of intermediate filaments, actin filaments (which are also called microfilaments), and of microtubules. The filaments and the microtubules are mutually connected and form a three dimensional network in the cell. The center of the cytoskeleton is the centrosome. The cytoskeleton is connected to the transmembrane proteins. The cytoskeleton organizes the cell, mediates transport of molecules, structures, and organelles (by means of motor proteins), receives signals from cellular environment mediated by the membrane proteins, and has a fundamental role in the mitotic spindle.

Intermediate filaments are ropelike fibres with a diameter of about 10 nm. They are composed of fibrous molecules (vimentins, keratins, nuclear lamins, neurofilament proteins, etc.). Their main role is connected with resistance to mechanical stresses.

Actin filaments are two stranded helical polymers with a diameter of about 8 nm. They are composed of globular protein actin. The actin filaments are organized in linear bundles, two dimensional networks, and in three dimensional gels. They are dispersed throughout the cell but mainly concentrated in the cell cortex which

Figure 1. A schematic picture of a microtubule (a), and of conformation change of a tubulin heterodimer from α to β state (b). After [4].

controls the shape and surface movements of most animal cells. They are polarized and their function depends on polarity.

Microtubules are the main organizers of the cytoskeleton. They are formed by cylindrical structures (with a diameter of about 25 nm) composed of 13 (or 14) protofilaments which are one dimensional chains of heterodimer tubulin molecules (Figure 1a). A tubulin molecule is composed of two globular proteins – α - and β -tubulins; each of them has the relative molecular mass of about 55 000 ('molecular weight' in daltons) but the masses are not equal. The heterodimer molecule can change conformation from the α state (non tilted) to the β state (tilted by 29 degrees from the microtubule axis) (Figure 1b). The heterodimer molecule is an electric dipole [3–5]. As a result the microtubules are polar structures with the plus end (fast growing) and the minus end (slow growing) embedded in the centrosome. The half-lifetime of a microtubule is of about 10 min.

Guanosine triphosphate molecules (GTP) are bound to both tubulins in a heterodimer. After polymerization when the heterodimer is attached to the microtubule GTP bound to the β -tubulin is hydrolyzed to guanosine diphosphate (GDP). The majority of the hydrolysis energy is not released but stored in the microtubule [2, 6]. The hydrolysis energy is not used for polymerization. A similar mechanism of hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) is functioning in the actin molecules added to the filament.

The cytoskeleton is an organizing system of the cell and exerts forces and generates movements without any major chemical changes [2]. The mechanisms of the cytoskeleton activity and the sources of energy for them are still not well under-

Figure 2. Translation symmetry in a protofilament.

stood. Physical mechanisms are probably fundamental to cytoskeleton function. One may also assume that the cytoskeleton can use the energy from hydrolysis of ATP and GTP stored in actin filaments and microtubules, respectively. We will analyse vibrations in microtubules as they may be one of possible mechanisms underlying the cytoskeleton function.

2. Dispersion Relation

The heterodimers in a protofilament form a one dimensional chain. The globular tubulins in the chain may be considered as mass units with translation symmetry. These mass units have internal vibrations (exerted by atoms and by large parts of tubulins) as well as external vibrations in the chain (i.e., vibrations exerted by each tubulin as a whole). A similar rationale may be applied to the actin filaments. We will analyse the external vibrations in the protofilaments and in the actin filaments using a method similar to that employed in the solid state physics. Nevertheless, we have to point out certain differences between crystalline solids with translation symmetry and microtubules (or actin filaments). The 'diameters' of atoms in solids are of about 0.1 nm and the distances between their centers are about twice or three times greater. The 'diameters' of tubulins and of actins are of about 4 nm; they are only slightly smaller than the distances between their centers of mass. In contrast with atoms tubulin and actin globules are not rigid but rather elastic particles. First of all we will analyse external vibrations without including dynamic deformations of tubulin and actin globules.

A scheme of the one dimensional chain is shown in Figure 2. The circles denote the centers of mass of tubulins in equilibrium positions, the black spots show the theoretical equidistant control points (the distance between them is a), Δ_1 , Δ_2 are distances of tubulins from the corresponding control points, m_1 , m_2 are masses of tubulins, and f_1 , f_2 are the elastic force constants which need not be equal. The

interactions between the closest neighbours will be assumed. We will derive the dispersion relation [7]. The one-dimensional equations of motion can be written in the form

$$\begin{aligned} m_1 \ddot{q}_j &= f_2(q_{j-1} - q_j) + f_1(q_{j+1} - q_j), \\ m_2 \ddot{q}_{j+1} &= f_1(q_j - q_{j+1}) + f_2(q_{j+2} - q_{j+1}) \end{aligned} \quad (1)$$

where q_j , q_{j+1} and $\ddot{q}_j$, $\ddot{q}_{j+1}$ are the displacements from equilibrium positions and their second time derivatives, respectively. (The equations given by (1) have linear form but for $f_1 \neq f_2$ the vibrations are anharmonic as will be discussed further.) The solution of Equation (1) is assumed in the form of propagating waves

$$\begin{aligned} q_{j-1} &= A_2 \exp\{-i[\omega t - k(n-1)a - k\Delta_2]\}, \\ q_j &= A_1 \exp\{-i[\omega t - kna - k\Delta_1]\}, \\ q_{j+1} &= A_2 \exp\{-i[\omega t - k(n+1)a - k\Delta_2]\} \end{aligned} \quad (2)$$

where k is the absolute value of the wave vector.

From Equations (1) and (2) one gets the dispersion relation

$$\omega_{\pm}^2 = \frac{(f_1 + f_2)(m_1 + m_2)}{2m_1m_2} \pm \sqrt{\left[\frac{(f_1 + f_2)(m_1 + m_2)}{2m_1m_2}\right]^2 - 4\frac{f_1f_2}{m_1m_2} \sin^2 ka} \quad (3)$$

where ω_+ and ω_- denote the optical branch and the acoustical branch of vibrations, respectively. In the acoustical branch the neighbouring particles (tubulins) move in the same direction ($A_1 = A_2$) and in the optical branch the particles move in opposite directions under condition that the center of gravity remains at rest ($m_1A_1 = -m_2A_2$) [7].

The actin filaments are composed of identical molecules in a double stranded organization. We may, therefore, apply a simple model of translation symmetry with equidistant particles. The dispersion relation is given in the form

$$\omega = 2\sqrt{\frac{f}{m}} \left| \sin \frac{ka}{2} \right| \quad (4)$$

where a is the distance between the center of the particles, m is their mass, and f is the elastic force constant [7]. The external vibrations have only one branch – the acoustical branch. Reptating motion of actin filaments [8] might depend (to some degree) on the acoustical vibrations.

The term vibration mode is used for the waves propagating in both directions along the chain with the same absolute value k of the wave vector. Analysis of vibrations is similar to that for random vibrations in crystalline structures with translation symmetry.

Figure 3. Dispersion relation for a microtubule (frequency versus wave vector). The full lines and the dashed lines denote the optical and the acoustical branches, respectively. Elastic force constants are assessed $f_1 = f_2$ (a), $f_1 = 10f_2$ (b).

Protofilaments in a microtubule are bound and build up a wall of a cylinder. Vibrations in particular protofilaments are not independent and form a common vibration system in a microtubule. As a microtubule is composed of 13 protofilaments we assume, that a microtubule 'particle' has 13 times greater mass than a protofilament 'particle'. Nevertheless, the elastic force constants in isolated parts of protofilaments (oligomers) and in microtubules may be different. Frequency versus absolute value of the wave vector evaluated from Equation (3) is shown in Figure 3a for $f_1 = f_2$ and in Figure 3b for $f_1 = 10f_2$. The dispersion curves for

Figure 4. Dispersion relation for an actin filament.

actin filaments evaluated from Equation (4) are shown in Figure 4. The elastic force constants are parameters of the curves. We used several values for the elastic force constant to show their effect on the frequency. The elastic force constants might be assessed from the shear strain-stress experimental data [9–11]. The dynamic elastic shear moduli for microtubules and actin filaments are of about 5 Nm and 20 Nm, respectively [10]. Using relation between the elastic shear modulus and Young's modulus and assuming the distance between neighbouring particles of about 4 nm the elastic force constants might be in the range of $10^{-5} - 10^{-6}$ Nm. Nevertheless, filaments in the cytoskeleton have different stiffness under different conditions. The large elastic modulus appears to depend on the fact that filaments are arranged orthogonally and cross-linked to each other at junction points. Changes of viscoelasticity very likely come about by changing the nature of the cross-links or the filament length. The elastic moduli for actin filaments in entangled ensembles are assessed as high as 10^4 Nm² [12, 13]. The corresponding elastic force constants may be greater than 10^{-4} Nm. For a microtubule the lowest frequency for $\nu \neq 0$ of the acoustical branch is in the band of 5 – 50 MHz if the length of a microtubule is $10 \mu\text{m}$ ($= \lambda/2$) and velocity of the acoustical waves is in the range from $10^2 - 10^3$ ms. The mass of 'particles' in actin filaments is smaller than in microtubules and, therefore, the frequencies are greater.

If the elastic force constants along the microtubule chain are not equal (i.e., $f_1 \neq f_2$) the potential as a function of distance of 'the tubulin particle' is asymmetric with respect to the equilibrium position. Therefore, the system is non linear and higher harmonic components (i.e., $\omega = n\omega_j$) and components with combination frequencies (i.e., $\omega = n\omega_j + m\omega_k + \dots$) are generated ($n, m, \dots$ are integers). The dispersion relation (3) does not contain these harmonic and combination terms.

The values derived by the present calculations presumably will be modified in biological reality because a number of simplifications was used, for example internal vibrations were neglected and simple structures of microtubules and actin filaments were assumed. Tubulins as well as actins are rather elastic than rigid globules which may have considerable influence on vibrations. The internal vibrations are combined with the external vibrations giving rise to complicated spectral patterns. On account of small mass and great values of the elastic force constants frequencies of internal vibrations are much higher than those of external vibrations. The frequency spectrum can contain frequency lines in the millimeter and in the far infrared region too. The dimers in neighbouring microtubule protofilaments are mutually displaced along the microtubule axis which can cause phase shifts and alter the types of vibrations: torsion or helicoid vibrations may exist in a microtubule. The heterodimers in a microtubule are organized in hexagons spanning the helical spiral on the circumference of a microtubule. Some proteins from the family of microtubule associated proteins (MAP's) interconnect microtubules, intermediate filaments, and actin filaments and create a common vibration system of the cytoskeleton.

3. Energy Supply

When a microtubule grows tubulin heterodimers add to the free plus end and GTP's attached to the β -tubulins are hydrolyzed to GDP's. The rate of polymerization may be greater than the rate of hydrolysis and an energy rich cap (a cap of heterodimers where GTP is not hydrolyzed) can be formed at the end of the tubulin. This cap has an important function. To continue growing at least one layer of heterodimer molecules at the end of a microtubule should remain non hydrolyzed (i.e., 13 or 14 tubulin molecules) [14]. The energy rich cap at the end of a microtubule is hydrolyzed later. Energy released by hydrolysis of GTP in solution, in tubulin heterodimers, and in tubulin subunits in microtubules is 5.18 kcal/mol, 3.79 kcal/mol, and 0.7 kcal/mol (i.e., 0.22 eV, 0.16 eV, and 0.03 eV for one entity), respectively [6, 15]. Therefore, energy of about 4.48 kcal/mol is potentially available. A part of the energy may be used for transport of the phosphate group but the majority of it is stored in the microtubule lattice [2, 6].

Similar mechanisms may be found in the actin filaments. The actin molecule is clam shaped with ATP molecules bound in the crevice. ATP in an actin molecule added to the filament is hydrolyzed. Hydrolyzation and formation of an energy rich cap are analogous to the processes in microtubules. Hydrolysis of ATP can release and supply energy up to about 13 kcal/mol.

Utilization of hydrolysis energy is not well understood. The energy can change bond strengths [16] or excite soliton or soliton like waves [3, 16]. It can be used for conformation changes of tubulin heterodimers (transition from untilted to tilted conformation) and for kink wave reorientation of heterodimer dipoles [3]. It can excite coherent lattice vibrations by the Fröhlich mechanism [16] (the Fröhlich mechanism is described, e.g., in [17–19]) and/or by the intrinsic non linear mech-

Table I. Relative value of energy E/E_0 per one entity supplied from GTP and ATP

Frequency (Hz)	E/E_0	
	GTP (4 kcal/mol)	ATP (10 kcal/mol)
10^7	6.7	16.8
10^{11}	6.8	16.9

anism. Regardless of the primary mechanism of energy utilization, in the final stage a part of it is always supplied to the vibrations. Besides energy stored in the cytoskeleton during polymerization a part of energy from ATP hydrolysis supplied for motor proteins moving along a microtubule can be transferred to the microtubules as random excitations of vibrations.

We assess the amount of energy which might be supplied to the cytoskeleton vibrations from hydrolysis of GTP and ATP. We evaluate relative values of energy E/E_0 per one entity where E is the energy supplied by hydrolysis of GTP or ATP and E_0 is the energy of an oscillator in the thermodynamic equilibrium in the frequency range from 10 MHz to 100 GHz, which include the possible range of external vibrations. If the total energy supplied by hydrolysis is 4 kcal/mol (10 kcal/mol) than the energy per one entity is about 7 times (17 times) greater than energy of an oscillator in the thermodynamic equilibrium (Table 1). On account of non linear mechanism the energy may be accumulated in certain vibration modes.

4. Conclusions

Microtubules and actin filaments are structures with translation symmetry. Using symmetry conditions we derived dispersion relations determining frequency of vibrations as a function of wave vector. The lowest frequency can be below 10 MHz. Vibrations in microtubules and actin filaments are polar and generate an electric field. The generated field mediates interaction with their surroundings. Therefore, the microtubules and the actin filaments might be capable of spectral energy transfer and energy condensation in certain vibration modes on account of the Fröhlich mechanism; the interactions with the surroundings (i.e., with a heat bath) enable the spectral energy transfer. Nonetheless, the Fröhlich mechanism is only one possible mechanism. If the elastic force constants inside the tubulin heterodimer and between the heterodimers in a protofilament of a microtubule are not equal the system of vibrations is non linear. This intrinsic non linear mechanism can also mediate spectral energy transformation. A part of energy from hydrolysis of GTP to GDP or of ATP to ADP can always be transferred to the vibrations. The role of excited vibrations in cytoskeleton dynamic activity has to be determined experimentally.

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