Dipole–Dipole Interactions in Microtubules

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Abstract. Dimers in microtubules possess a dipole moment with components along three axes. The interaction energy among all dipole components in a microtubule was calculated for an un-deformed and an elliptically deformed microtubule in a “dry” condition. The interaction energy was found to increase with deformation. The total interaction energy among all dipoles is positive, which implies that the un-deformed cylindrical shape of a microtubule represents a condition of minimum energy. This suggests that the cylindrical shape of microtubules is a consequence of dipole–dipole interactions. There may be other causes as well but these are not discussed in this paper. From these results, the contributions of the dipole–dipole interaction energy to the microtubule longitudinal and transverse flexural rigidities were calculated. It is shown that the longitudinal contribution to the elastic modulus is approximately 50–60% of the total measured value while the calculated transverse contribution is smaller than the longitudinal contribution by a factor of approximately 3. The ratio of the measured axial to the measured transverse flexural rigidity is approximately 125, in agreement with recent observations. However, these values are uncertain for reasons discussed in the text.

Key words: microtubule, dipoles, interactions, elastic properties

1. Introduction

Microtubules are dynamic structural elements in cells that perform a remarkable variety of functions including the maintenance of the structural architecture of the cytoplasm. They are found also in nerve axons and believed to assist in transport of various proteins to nerve terminals. Microtubules are cylindrical structures consisting generally of 13 proto-filaments aligned essentially along the cylindrical axis. Some microtubules have been observed to possess 12–17 proto-filaments, but it is generally accepted to have 13 such proto-filaments. In the analysis discussed in this paper we assumed 13 proto-filaments make up a microtubule. Each proto-filament consists of dimers, also called tubulins, which are the building blocks of microtubules. Each dimer is an assembly of two nearly identical monomers, an α - and a β -monomer, and when attached head-to-tail ($\alpha\beta, \alpha\beta, \dots$) form a proto-filament. Each dimer has a molecular weight of 110 kDa. The binding energy between an α - and a β -monomer is very stable and considerably larger than between successive dimers. The proto-filaments are bound laterally to form a sheet that closes to form a cylinder but with a bond strength less than the bond strength between dimers

along the proto-filaments. Neighboring dimers along proto-filaments exhibit a small offset of approximately 0.92 nm, from proto-filament to proto-filament such that when the sheet closes into a cylinder, the accumulated offset corresponds exactly to the length of 3 monomers or 12 nm [1]. Thus, the thirteenth monomer around the circumference is next to a like monomer: for example an α - next to a α -monomer. There are two configurations for proto-filaments to join the thirteenth to the first proto-filaments: nearly aligned due to the 0.92-nm offset at each proto-filaments or staggered by approximately one-monomer length. When the proto-filaments are nearly aligned the join is of the A-type while it is of the B-type if staggered [1]. Consequently, the microtubule considered in this paper, which are nearly aligned, is of the A-type. This ensures that all proto-filaments are parallel to the microtubule axis. It also gives rise to a three-start helical structure. Microtubules with supertwisted proto-filaments are discussed in [1] but not considered in this paper. Thirteen proto-filaments form a hollow cylinder with an outside diameter of approximately 25 nm and an inside diameter of approximately 15 nm. The length of each dimer is approximately 8 nm with a radial thickness of 5 nm and an azimuthal thickness of 5 nm [1]. The elastic properties of microtubules appear to originate in the dimer–dimer binding [1, 2], both longitudinally and transversely. The lateral binding among dimers forming the microtubule from proto-filaments is likewise a source of elastic stiffness and is believed close to two orders of magnitude lower than the longitudinal elastic modulus [3].

In solutions, microtubules are negatively charged due to the presence of carboxyl residues referred to as C-terminus. The net charge on a dimer is dependent upon the pH of its environment [4], and can range from 10 at low pH to -21 at high pH values, and microtubules become essentially neutral at pH 5.6 [5]. The isoelectric point of the α -monomer is at pH 5.45–5.65 [6] and for the β -monomer it is at pH 5.30–5.45 [7]. Tuszynski *et al.* [3] reported that at a physiological pH of approximately 6.8, there are eight negative charges residing mainly at the C-terminus, which is surrounded by a dipolar zone of approximately 1-nm thick. Excluding the C-terminus, dimers are reported to carry 10 negative elementary charges [8]. However, recent experiments by [8] at nearly physiological conditions (buffer at pH 6.8 and 120 mM ionic strength, and room temperature), in a constant electric field, have been observed to possess an unbalanced negative charge of 0.19 electrons per dimer, which appears the cause of the observed motion along the applied electric field. This is a value lower by approximately a factor of 50 than what has been calculated from crystallographic data for free dimers in solution. This net charge is mobile, localized predominantly in the α -monomer, and may be the source of the dipole moment in dimers [9]. According to [3], as the charge is transferred to the β -monomer through an estimated distance of approximately 4 nm, the dipole orientation is flipped into the opposite direction. However, based on this value of the negative charge and the above separation distance the magnitude of the dipole is close to 36 D, which is considerably smaller than the computed values [10]. A careful calculation carried out by [10], and also reported by [8], gives the following

values for the components of the dipole moment: $p_x = 337$ D, $p_z = 198$ D, and $p_y = -1669$ D. The x -direction corresponds to the proto-filament axis [11]. Another computer simulation that reports on the calculations of the electric dipole moment of tubulin is that of [12],¹ indicating close agreement with Brown's results [10]. The C-terminus were excluded from the electron crystallographic data reported by [13], hence these calculated dipole moments exclude the effects of the C-terminus [12].² At a pH 5.6, the C-terminus effect may be considered weak since that corresponds to charge neutrality at this pH value. The symmetry in the interior of the microtubule may result in the cancellation of the electrostatic charges. According to the Born approximation [14] the electrostatic free energy for a dipolar zone of approximately 1 nm amounts to 2×10^{-17} J for the inner and the outer region surrounding the microtubule. Because of cylindrical symmetry, the electrostatic free energy is uniformly distributed in the inner and the outer dipolar zones. The Born approximation exhibits a parabolic behavior with z , the number of charges on each tubulin, and the electrostatic free energy increases as z^2 . Since the electrostatic free energy in the Born approximation is not volume dependent, any deformation of the dipolar zone inside and outside the microtubule through its deformation into an ellipse as discussed here will not alter the results reported in this paper. Any departure from charge neutrality with changes in the pH value will increase the total electrostatic free energy thereby increase the microtubule cylindrical stability. However, the detailed calculations of these effects for a non-continuum or molecular treatment are complex [15] and beyond the scope of this paper.

Another point is that the dipole orientation of a dimer changes by 27° (29° was reported by [16] as a result of the hydrolysis of GTP [17]. This results in a small change in the dipole–dipole interaction energy amounting to approximately 1% (proportional to $(1 - \cos \theta)^2 \cong 0.012$). Conceptually, this angular change in the α - and the β -monomers dipole orientation occurs at a hinge located at the N-site (GTP) [1].

In the present paper the dipole component values reported by [8, 10] were used in calculating the interaction energies among dipole components. Tuszynski *et al.* [3] used an axial value of 192 D in their model, which corresponds to an unbalanced negative charge of close to one electron charge ($0.9976 e^-$). In another paper by [9], the authors have assumed that each lattice site possesses a dipole moment corresponding to 2 electron charges, which means that the axial dipole is 385 D, close to the value reported by [10]. For the axial dipole component of 337 D considered in this paper, it is equivalent to 1.75 electron charges.

The relative permittivity of dimers in cytosol is not only pH-dependent but also dependent on the temperature and ionic strength of the environment, and has been variously assumed to range from a value of 2 for dry dimers to 76, or that of water, for highly dilute solutions. Mershin *et al.* [12]³ have reported what appear to be the first measurements of the high frequency dielectric constant of tubulin. When account is taken of the solution, the value reported is $\epsilon_r = 8.41 \pm 0.20$. In the

present paper we have used $\epsilon_r = 8.41$ as it represents the only currently available experimental value.

In this paper we report the results of calculations of dipole–dipole interaction energies and the effects such energies have on microtubule shape and elastic properties. First, we calculate the interaction energy among all dipole components distributed around the circumference or hoop of a microtubule for a single hoop of dimers. This calculation assumes that there are 13 dimers equally spaced around the circumference of a circle whose radius is the mean microtubule radius, or 10 nm. The error resulting from this assumption is discussed below. Second, we calculate the change in the interaction energy when the hoop is deformed into an ellipse by a point load applied along a diameter. While the calculations are rather simple to carry out they are extremely tedious because of the large number of interacting pairs that must be accounted for. The computational scheme is described in the next section. Third, we calculate the dipole–dipole interaction energy along the polar axis. Using these result we calculate the contributions such energies make to the elastic properties of the microtubule. The paper ends with conclusions drawn from this analysis.

2. Dipole–Dipole Interaction Energy

The calculations of the dipole–dipole interactions described in the following sections are separated into the interaction energy from the hoop and from the axial distribution of dipoles.

2.1. HOOP INTERACTION ENERGY

For reference we use a cylindrical coordinate system where the z -axis is along the microtubule axis hereafter referred to as the polar axis, the azimuthal coordinate of the microtubule is directed in the counterclockwise direction or increasing azimuthal angle θ , and a radial coordinate r . We use the values of the dipole components calculated by [10] such that $p_z = 337$ D, $p_\theta = 198$ D, and $p_r = -1669$ D, where D stands for Debye units ($1 \text{ D} = 3.336 \times 10^{-30} \text{ cm}$). Figure 1a shows the orientation of the dipole components.

To calculate the interaction energy among dipole components, account must be taken of all possible component orientations and separation distances around the circumference. We make the assumption that 13 sets of three dipole components are distributed uniformly around a circumference and neglect the offset along the proto-filament. It will be shown below that the neglect of this offset results in a small reduction in the total interaction energy. The interaction energy between two dipoles is given by [18, 19]

$$U_{dd} = (\mathbf{p} \cdot \text{grad})V \quad (1)$$

Figure 1. Isometric and end view of a microtubule: (a) Isometric view showing dipole components, (b) Elliptical deformation end view with nomenclature of dipole pairs.

where $\mathbf{p}$ is the dipole moment vector and V is the potential so that U_{dd} is a scalar quantity. Consider a dipole of moment $\mathbf{p}_1$ at A and a second dipole of moment $\mathbf{p}_2$ at B separated from A by a distance r , then the potential at B from the field of the dipole at A is

$$V = (4\pi\epsilon_0\epsilon_r r^3)^{-1}(\mathbf{p} \cdot \text{grad}(1/r)) \quad (2)$$

which is exact as no approximation is implied with regard to near- or far-field. Substituting Eq. (2) into Eq. (1) and carrying out the gradient operator, there

results

$$U_{dd} = (4\pi\epsilon_0\epsilon_r)^{-1}(\mathbf{p}_1 \cdot \mathbf{p}_2 r^{-3} - 3(\mathbf{p}_1 \cdot \mathbf{r})(\mathbf{p}_2 \cdot \mathbf{r}) r^{-5}) \quad (3)$$

and when the geometry between dipoles and the radius vector is considered [14, 18], there follows the well-known result that

$$U_{dd} = -(p_i p_j / 4\pi\epsilon_0\epsilon_r r_{ij}^3)(2 \cos \theta_i \cos \theta_j - \sin \theta_i \sin \theta_j \cos \phi) \quad (4)$$

which is exact as long as p_i and p_j are constants [14, 20]. In other words, the product of the charge separation and the amount of net charge producing the dipole is constant. In the present analysis, this is a reasonable assumption to make because, while we know the magnitudes of the dipole components [8, 10], the magnitude and separation of the charges, and their distribution resulting in the dipole vector of dimers are not known. In Eq. (4), the i and the j indices refer to the location of dipole components along the circumference, ϵ_0 is the permittivity of vacuum, ϵ_r is the relative permittivity or $\epsilon_r = 8.41$ [12]⁴ as mentioned in the introduction. Also in Eq. (4), r_{ij} is the separation distance between the two dipoles of interest and the angle ϕ accounts for the angle out-of-plane of the two dipoles. The angles θ_i and θ_j are measured counterclockwise from a line joining both dipoles, as shown in Figure 1b.

In order to account for the total interaction energy, dipoles must be taken in pairs, and since there are three coordinates, there are six possible combinations of dipole pairs. All interaction energies among pairs are then summed. The first three pairs are $p_r - p_r$, $p_\theta - p_\theta$, and $p_z - p_z$. These three pairs of dipoles lie in the same plane and consequently, $\phi = 0$ in Eq. (4). Next, we consider the pairs formed by the permutation of indices r , θ , and z . The dipoles p_r and p_θ lie in one plane and the values of θ_i and θ_j are measured in the same plane ($\phi = 0$), and when these dipoles are taken in pairs along a circumference and their interaction energies calculated, the sum is nonzero. On the other hand, the pair $p_r - p_z$ is not co-planar so that $\phi = \pi/2$ and the second term in the bracket of Eq. (4) is identically zero: the first term in the bracket of Eq. (4) has $\theta_i = \pi/2$ and $0 \leq \theta_j \leq 2\pi$, and that term is also zero. Therefore, the interaction energies for the pair $p_r - p_z$ are zero. The remaining pair $p_z - p_\theta$ gives zero interaction energies by the same reasoning. Consequently, the dipole interaction energies to be calculated are U_{dd}^r , $U_{dd}^{\theta\theta}$, U_{dd}^{zz} , and $U_{dd}^{r\theta}$.

We consider the results for a single hoop of dimers, beginning with a circular shape. Computations outlined below show that this gives the lowest total interaction energy. Any deformation of the hoop into an ellipse increases that interaction energy. The circular hoop is deformed into an ellipse by applying a deflection $f/2$ along the y -axis at $x = 0$. This causes a reduction of the hoop radius along the y -axis by an amount $f/2$. That radius becomes the semi-minor axis of the ellipse given by $b = r(1 - f/2)$, where f is a small number between zero (circle) and 0.30 (ellipse), where 0.30 was chosen arbitrarily as an upper limit to the deformation.

The eccentricity of the ellipse relates the semi-major to the semi-minor axes by $e^2 = 1 - b^2/a^2$, where $a = b/(1 - e^2)^{1/2}$ is the semi-major axis. It has been assumed that the circumferential length of the hoop remains unaltered by its deformation into an ellipse. Theoretically, the circumference suffers a small hoop compression strain reducing the circumferential length among dipole components, but represents a negligible value in the results reported herein. Substituting for a and b in the equation for the eccentricity, there results, after some algebra, that the eccentricity is related to f by

$$e = \sqrt{2f}/(1 + f/2) \quad (5)$$

From a computational point of view, Eq. (5) allows the eccentricity to be calculated directly when f has been specified, and the remaining parameters of the ellipse follow.

We have also assumed that the spacing among dimers along the periphery of the ellipse remains unchanged when the hoop is deformed into an ellipse. This is the case even when the hoop length undergoes a small hoop compression strain. For a circular hoop before deformation, the angular separation between dimers about the coordinate is then given by $\varphi_0 = 2\pi/13$. When a force is applied at one point on the hoop directed along the y -axis and at $x = 0$, and deformation occurs, φ will vary slightly along the elliptic path from its value φ_0 . Figure 1b shows a schematic distribution of dimers around an ellipse. Consider first the interaction among radial dipoles as shown in this figure. The radial dipole vectors $\mathbf{p}_r$ are shown pointing inward consistent with its negative value calculated by [10]. The origin of the dipole is centered in the dimer represented as small circle – it is a point dipole [20]. Dimers are numbered counterclockwise from 1 located at $x = a$ and $y = 0$ for an ellipse and $x = r$ and $y = 0$ for a circular hoop. This shows that the only axis of symmetry is the x -axis. Now, consider for example the interaction between a radial dipole at position 2 and one at position 6. The angle φ_i and φ_j then become $\varphi_2 = (n - 1)\varphi_0 = \varphi_0$ since $n = 2$, and $\varphi_6 = (m - 1)\varphi_0 = 5\varphi_0$ since $m = 6$, respectively for this case, where n and m are the counting indices. The first dipole is identified by an index n and the second dipole by an index m , with $m > n$, both integers. From the angles the dipoles make with the x -axis, and the position of the dipoles along the elliptic circumference, we obtain the separation distance r_{ij} directly using the following equation

$$r_{ij} = \{a^2[\cos(n - 1)\varphi_0 - \cos(m - 1)\varphi_0]^2 + b^2[\sin(n - 1)\varphi_0 - \sin(m - 1)\varphi_0]^2\}^{1/2} \quad (6)$$

where a and b are the semi-major and semi-minor axes of the ellipse, respectively. The angles θ_i and θ_j of the radial dipole i and j with respect to the line joining them are obtained from $\theta_i = [3\pi - (m - n)\varphi_0]/2$ and $\theta_j = [3\pi + (m - n)\varphi_0]/2$.

The interaction energy among all radial dipoles was calculated using the following scheme. The interaction energy between any pair of dipoles begins with the calculation of the interaction energy between dipole at position $n = 1$ (Figure 1b) and that at position $m = 2$ using Eq. (4). Next, the interaction energy between dipole 1 and dipole 3, dipole 1 and dipole 4, through dipole 1 and 13 are calculated. Then the process is repeated for dipole 2 and 3, 2 and 4, through 2 and 13, and so on. These computations are repeated for all values of n and m , and all energy values are summed, yielding a total interaction energy for all p_r components for $f = 0$. The scheme is repeated for all p_θ and p_z , components, and for the interaction between p_r and p_θ components. The calculations are repeated for all increments in the value of f , from $f = 0$ to $f = 0.30$. The upper limit of $f = 0.30$ was assumed as a reasonable deformation for an ellipse and sufficient to detect a change in the interaction energy of a microtubule undergoing hoop deformation. When $f = 0.30$, the eccentricity is 0.6735 by Eq. (5) and $b = 0.85 r = 11.76 \times 10^{-8}$ m so that $a = 1.15 r = 15.91 \times 10^{-8}$ m. When the circular hoop becomes deformed into an ellipse by the application of a single-point load at $x = 0$ and $y = \pm r$, the shape of the ellipse causes the radial dipole vectors component to depart slightly from their radial position from that of circle as shown in Figure 1b. These small angular deviations are given by

$$\gamma_i = \pi/2 - (n - 1)\varphi_0 - \text{atan}[\sqrt{(1 - e^2)}/\tan(n - 1)\varphi_0] \quad (7)$$

for $n \neq 1$, $e < 0$, and for $(n - 1)\varphi_0 < \pi$. For $n \neq 1$, $e < 0$, and for $(n - 1)\varphi_0 > \pi$ use is made of the following equation

$$\gamma_i = 3\pi/2 - (n - 1)\varphi_0 - \text{atan}[\sqrt{(1 - e^2)}/\tan(n - 1)\varphi_0] \quad (8)$$

where e is the eccentricity of the ellipse, obtained directly from Eq. (5). When $e = 0$, $\gamma_i = 0$ as it should as no correction is necessary for a circle. Eqs. (7) and (8) where derived from a consideration of the local normal to the ellipse at point i . When considering the interaction between a dipole at n , when $(n - 1)\varphi_0 < \pi$ then Eq. (7) is used while for a nearby point $m > n$ when $(n - 1)\varphi_0 < \pi$ then Eq. (7) is also used. If a dipole at n is such that $(n - 1)\varphi_0 < \pi$ but the dipole at m is such that $(n - 1)\varphi_0 > \pi$ then Eq. (8) is used. If both dipoles are located such that $(n - 1)\varphi_0 > \pi$ in both cases, then Eq. (8) is used for both m and n . These corrections are added to the values of θ_i and θ_j of the deformed ellipse. The interaction energies resulting from the other dipole components utilizes the same formalism as above except that $\theta_i = [\pi - (m - n)\varphi_0]/2$ and $\theta_j = [\pi + (m - n)\varphi_0]/2$ for $p_\theta - p_\theta$ interactions, and $\theta_i = [3\pi - (m - n)\varphi_0]/2$ and $\theta_j = [\pi + (m - n)\varphi_0]/2$ for the $p_r - p_\theta$ interactions. In the case of $p_z - p_z$ interaction, only r_{ij} is calculated since all p_z components around the circumference are parallel to the polar axis and normal

to the plane of the hoop, so that Eq. (4) reduces to

$$U_{\text{dd}}^{\text{zz}} = (p_z^2/4\pi\epsilon_0\epsilon_r)/r_{ij}^3 \quad (9)$$

The computations presented above have assumed that the proto-filaments around the microtubule are not axially offset by 0.92 nm. In fact, the offset causes closure of the cylinder so that like monomers become neighbors, resulting in a A-type microtubule [1]. For simplicity, we have assumed that all dimers reside around the circumference of a circle whose plane is normal to the microtubule polar axis. This simplification introduces errors in the calculations of the interaction energies for the hoop configuration that have an origin in the 0.92 nm offset mentioned above [1]. To see this, Eq. (4) can be rewritten as follows;

$$U_{\text{dd}} = -K r_{ij}^{-3} \quad (10)$$

and when $\phi = 0$ (co-planar dipole vectors),

$$K = (p_i p_j / 4\pi\epsilon_0\epsilon_r)(2 \cos \theta_i \cos \theta_j - \sin \theta_i \sin \theta_j) \quad (11)$$

In Eq. (10), r_{ij} is the separation distance between dipole i and dipole j , when both are located in the plane of a flat hoop. However, when the offset between any two dipoles is accounted for, then the separation distance between i and j is somewhat larger so that the energy of interaction between them is smaller according to Eq. (10). Letting r_{ij}^0 represent the new distance because of the offset, then we have that

$$r_{ij}^0 = r_{ij}(1 + (0.92 \times 10^{-9}n/r_{ij})^2)^{1/2}, \quad n \neq 0 \quad (12)$$

where n is the counting index mentioned previously, and U_{dd} becomes

$$U_{\text{dd}} = -K (r_{ij}/r_{ij}^0)^3 = U_{\text{dd}}(1 + (0.92 \times 10^{-9}n/r_{ij})^2)^{-3/2} \quad (13)$$

When $n = 1$, the dipoles are next to each other along the circumference and the offset results in $U_{\text{dd}}^0 = 0.987 U_{\text{dd}}$, and when the separation distance between dipoles is largest, at approximately twice the mean microtubule radius or $n \cong 6$, then $U_{\text{dd}}^0 = 0.896 U_{\text{dd}}$. Thus, depending on the separation distance between dipoles distributed along the helical circumference, the interaction energy is smaller than without the offset due to this larger separation distance, ranging from 1.3–10.5%, or an average over the circumference of 5.8%. Therefore, the results presented in this paper, which ignore the effects of the offset, give interaction energies for the hoop that are larger by 5.8%. In the discussion that follows, the correction due to the offset is ignored. This does not change the conclusions reached in this paper.

2.2. AXIAL INTERACTION ENERGY

The interaction energies along the microtubule axial length are now calculated. Each circular plane of the microtubule contains two in-plane dipole components: $-p_r$ and p_θ , with a separation distance equal to the distance between dimers along the polar axis or approximately 8 nm. Since each radial and azimuthal dipole component lie in the same plane, $\phi = 0$ in Eq. (4). Let the distance between planes be a , then Eq. (4) for p_θ yields

$$U_{\text{dd}}^\theta = -(p_\theta^2 / 4\pi\epsilon_0\epsilon_r) \sum_{-\infty}^{\infty} (2 \cos^2 \theta - \sin^2 \theta) / (n a)^3 \quad (14)$$

and since $\theta = \pi/2$ for the dipole angle with respect to the line joining them, the sum becomes $\sum_{-\infty}^{\infty} (n a)^{-3} = (2/a^3) \sum_0^{\infty} n^{-3} = 1.2020569 \dots (2/a^3)$, where the value of the infinite sum is taken from [21]. The axial contribution to the interaction energy from the azimuthal dipoles becomes

$$U_{\text{dd}}^\theta = 0.601 (p_\theta^2 / \pi\epsilon_0\epsilon_r) a^{-3} \quad (15)$$

A similar analysis yields the following for the interaction energy for the radial components along the polar axis, or

$$U_{\text{dd}}^r = 0.601 (p_r^2 / \pi\epsilon_0\epsilon_r) a^{-3} \quad (16)$$

Note that Eqs. (15) and (16) apply to a single infinitely long row of radial and azimuthal dipoles along the microtubule. However, when considering a microtubule only 1 μm long, which contains approximately 125 dimers along each proto-filaments, these equations are still valid because the series converges to within 0.1% of its infinite value in 10 terms or approximately 13 dimers.

The p_z dipole component is aligned along the polar axis and separated by a distance a , and the angles $\phi = 0$ and $\theta = 0$, consequently, Eq. (4) reduces to

$$U_{\text{dd}}^z = -1.202 (p_z^2 / \pi\epsilon_0\epsilon_r) a^{-3} \quad (17)$$

Eqs. (15)–(17) apply to a single proto-filament so that for all 13 infinitely long proto-filaments, the total interaction energy for each type of dipole becomes

$$U_{\text{dd}}^r = 7.813 (p_r^2 / \pi\epsilon_0\epsilon_r) a^{-3} \quad (18)$$

$$U_{\text{dd}}^\theta = 7.813 (p_\theta^2 / \pi\epsilon_0\epsilon_r) a^{-3} \quad (19)$$

$$U_{\text{dd}}^z = -15.627 (p_z^2 / \pi\epsilon_0\epsilon_r) a^{-3} \quad (20)$$

Note that the Eqs. (18) and (19) give positive energy values which implies unstable repulsive forces, while Eq. (20) give a negative energy value implying

an attractive force among the dipole components along the polar axis. The energy values calculated with Eqs. (18)–(20) must be summed to obtain the total energy for the dipole interactions along the entire length of the microtubule. These results are valid for polymerizing and rapidly depolymerizing microtubules down to length of few dimers. Using $a = 8$ nm, $p_r = -1669(3.33 \times 10^{-30}) = -5.558 \times 10^{-27}$ C m, $p_\theta = 198(3.33 \times 10^{-30}) = 6.593 \times 10^{-28}$ C m, $p_z = 337(3.33 \times 10^{-30}) = 1.122 \times 10^{-27}$ cm, and the experimental value $\varepsilon_r = 8.41$ [12],⁵ we obtain the following interaction energies:

$$\begin{aligned} U_{dd}^r &= 2.520 \times 10^{-18} \text{ J} \\ U_{dd}^\theta &= 3.547 \times 10^{-20} \text{ J} \\ U_{dd}^z &= -2.055 \times 10^{-19} \text{ J} \end{aligned} \quad (21)$$

where the dominant contribution to the interaction energy is given by the radial dipoles as expected since the magnitude of p_r is the largest of the three components. These energy values are considerably greater than the thermal energy of the microtubule environment ($kT \cong 4 \times 10^{-21}$ J at 20 °C). Moreover, the energies given by Eq. (21) are below the energy available from the hydrolysis of GTP into GDP (4.6 kcal/mol).

2.3. RESULTS

The numerical results obtained for the sum of all interaction energies for all dipole components around the circular hoop of the microtubule and the deformed hoop, are shown in Figure 2. These results do not include the contributions from the

Figure 2. Radial dipole–dipole interaction energy as a function of total deflection into an elliptical configuration.

dipole components along the polar axis. The interaction energy increases from 4.64×10^{-18} J to 4.88×10^{-18} J as the deformation increases from $f = 0$ to $f = 0.30$. Even when un-deformed this energy is significant and greatly exceeds the thermal energy (4×10^{-21} J at 20°C) by three orders of magnitude. Although the p_θ component of the dipole moment taken around the hoop in pairs results in a significant negative interaction energy, the sum of all interaction energies is positive. Thus, the total net interaction energy is positive implying a tensile hoop stress on the microtubule, which is prevented from separating dimers along the circumference by their lateral binding. The rise in the interaction energy as the microtubule cross-section becomes increasingly elliptical is due in part to the decreasing distance among the interacting dipoles across the axis of symmetry, and in part due to the changing angles of the dipoles with respect to the line joining them. The interaction energy increases by only approximately 5% when f increases from 0 to 0.30, which is not significant. However, the magnitude of the interaction energy, even at $f = 0$, is so large as to ensure the cylindricity of microtubules. The radial contribution alone to the interaction energy is 4.48×10^{-18} J at $f = 0$ and rises to 4.71×10^{-18} J at $f = 0.30$. The energy contribution from the $p_r - p_\theta$ interaction is lower by approximately two orders of magnitude, and decreasing from 9.69×10^{-21} J at $f = 0$ to 9.12×10^{-21} J at $f = 0.30$. The values for the azimuthal dipole and the axial dipole interaction energies around a circular and deformed hoop are constant. This is a consequence of symmetry. Calculations showed that the azimuthal interaction energy value is -10.52×10^{-20} J while the axial interaction energy is 1.63×10^{-19} J for all values of f . Table I shows the sum of all contributions as a function of the value f . The significant point to observe is that under zero deformation ($f = 0$) the total interaction energy is a constant value of approximately 4.64×10^{-18} J. It is this positive value that maintains a microtubule in a cylindrical shape, since any deformation it may undergo results in an increase in interaction energy, therefore, resulting in internal forces that tend to restore its shape to a cylinder.

Table I. Sum of all contributions from the interaction energies of all dipole components around a single circular hoop of a microtubule as a function of deformation

f	$U_{\text{dd}}^{\text{tot}}$ (J) ($\epsilon_r = 8.41$)
0	4.633×10^{-18}
0.01	4.635×10^{-18}
0.05	4.637×10^{-18}
0.10	4.661×10^{-18}
0.20	4.725×10^{-18}
0.30	4.863×10^{-18}

3. Contributions to Microtubule Flexural Rigidity

The results discussed in the previous section show that the interaction energy among all dipoles in the hoop and in the direction of the polar axis direction is positive. The effects of this interaction are to produce an internal radial load tending to maintain the cylindrical shape of a microtubule, and an axial tensile load tending to maintain it straight. It has the further consequence of increasing the elastic properties of the microtubule in the same sense that an inflated balloon exhibits great stiffness because of its internal gas pressure. In this section an attempt is made at calculating the contribution made by the dipole interaction energy to the elastic properties.

3.1. CONTRIBUTION TO AXIAL ELASTIC PROPERTIES

Equations (18)–(20) give the interaction energy among dipoles distributed along the length of the microtubule axis. The total interaction energy of a microtubule is $U_{dd}^a = 2.350 \times 10^{-18}$ J which can be written as

$$U_{dd}^a = C_0(l_0/l)^3 \quad (22)$$

where $C_0 = 2.350 \times 10^{-18}$ J and l_0/l is a scaling factor to account for the effects of small changes along the polar axis. The scale factor expresses the fact that the dipole–dipole interaction energy is proportional to r^{-3} as shown by Eq. (4). Now, consider a microtubule of finite length l_0 subjected to a simple axial compressive load P . Subjecting the microtubule to an axial load, the contribution from the hoop does not change with axial strain. This load will shorten the microtubule length by an amount δ , which results in stored strain energy given by [22]

$$U = P^2 l / 2AE = AE \delta^2 / 2l \quad (23)$$

where l is the new microtubule length less than l_0 , A is the cross-sectional area of the microtubule bearing the load P , and E is the microtubule axial elastic modulus. The first form of Eq. (23) is not useful because P is unspecified. A distinction is made here between the axial and the transverse elastic moduli of a microtubule because the bond strength along a proto-filament among dimers may not be the same as the transverse bond strength in adjacent proto-filaments, with the transverse elastic modulus lower than the axial modulus. The second form of Eq. (23) can be used to calculate the dipole–dipole interaction energy contribution to the axial elastic modulus and hence the flexural rigidity. The cross-sectional area over which the load applies is the bond contact area between dimers along the proto-filaments. In this calculation we estimate that the radius of the contact area between two dimers along the z -axis is $r_c \cong 1.25 \times 10^{-9}$ m compared to the dimer diameter of approximately 5 nm, hence the bond contact area is $A = 13\pi r_c^2 = 6.38 \times 10^{-17}$ m² for 13 proto-filaments. For a microtubule 1 μ m in length subjected to an axial

load resulting in a strain of 1% (1×10^{-8} m), and using a theoretical value of $E = 1.59 \times 10^9 \text{ N m}^{-2}$ [2] for the elastic modulus of a microtubule in the absence of dipole–dipole interaction energy, Eq. (23) yields $U = 5.1 \times 10^{-18} \text{ J}$ which is approximately 2.2 times larger than the value obtained by summing Eq. (21). The elastic modulus of microtubules derived from experiments has been reported to range from 1.9–2.7 GPa [1], depending on the organism and its environment with a theoretical value of 1.59 GPa calculated by [2]. If the upper value for the elastic modulus is used, i.e., $E = 2.7 \times 10^9 \text{ N m}^{-2}$, then the strain energy stored in the microtubule becomes $U_e = 8.6 \times 10^{-18} \text{ J}$, which is a factor of 3.7 times larger than the value obtained from Eq. (21). We can calculate the contribution from the dipole–dipole interaction energy due to a small axial compression of the microtubule with elongation δ from Eq. (22). Since $U_{dd} = 2.350 \times 10^{-18} \text{ J}$ from Eq. (21), $l_0 = 10^{-6} \text{ m}$, $l = 10^{-6} - (1 \times 10^{-8}) = 9.9 \times 10^{-7} \text{ m}$, we find that $U(\delta) = 2.43 \times 10^{-18} \text{ J}$ from Eq. (22). Using Eq. (23) we can then calculate the contribution to the axial elastic modulus E_d^a from dipole–dipole interaction, or

$$E_d^a = 2U(\delta)l/A\delta^2 = 7.53 \times 10^8 \text{ N m}^{-2} \quad (24)$$

The total axial elastic modulus of a microtubule is the sum $E_m = E_e + E_d$ which must be equal to the measured value, where E_m is the measured elastic modulus, and E_e stands for the elastic modulus of the microtubule in the absence of dipole–dipole interaction, and E_d is that due to dipole–dipole interaction. Schoutens [2] calculated $E_m = 1.59 \times 10^9 \text{ N m}^{-2}$ for a microtubule with a moment of inertia in longitudinal bending of $I = 1.456 \times 10^{-32} \text{ m}^4$, hence $E_m I = 23.15 \times 10^{-24} \text{ N m}^2$, then the contribution to the flexural rigidity from the dipole–dipole interaction amounts to $E_d^a I = 10.96 \times 10^{-24} \text{ N m}^2$, while the flexural rigidity due only to the dimers bond energy is $E_e I = (E_m - E_d)I = 12.20 \times 10^{-24} \text{ N m}^2$. Consequently, the percentage contribution to the flexural rigidity from dipole–dipole interaction to the total flexural rigidity is approximately 50%. Reported flexural rigidity of microtubules from different organisms under different conditions range from $24 \times 10^{-24} \text{ N m}^2$ to $30 \times 10^{-24} \text{ N m}^2$ [1] with the theoretical value at $23.15 \times 10^{-24} \text{ N m}^2$ [2]. Using this range of values, the contribution to the flexural rigidity from dipole–dipole interaction ranges from approximately 50–60%. Note however, that this range is uncertain due to the uncertainty in calculating the contact area between dimers along the proto-filament.

3.2. CONTRIBUTION TO TRANSVERSE ELASTIC PROPERTIES

Table I shows that in the absence of hoop deformation the interaction energy from dipole–dipole interactions is positive, and increases somewhat with increasing deformation. However, a simple expression analogous to Eq. (22) for radial expansion cannot be derived for the case of a point load on a hoop, which deforms the microtubule cross-section into an ellipse as a result of the application of this load. The

Figure 3. Point load along the diameter of the hoop, and free-body diagram.

reason for this is that the interaction energy is calculated for pairs of dipoles and summed over all pairs, where the energy depends not only on the radial separation distances between pairs but also on the orientation angles with respect to the line joining each dipole pair. This can be seen from Eq. (4) and Figure 1b. The calculations leading to the results shown in Table I was performed under the assumption that a fractional deformation f of the diameter could be specified but the load could not. Consequently, a relationship between the load and deflection is needed. It turns out that this is a well-known problem in the theory of elastic stability. We outline this derivation following [23]. Figure 3 shown a hoop with a load P at opposite points along a diameter on the diameter. The second diagram in Figure 3 shows a free-body diagram of the left half of the hoop, where the moments M_0 are shown. The loads are at $\theta = 0$ and at $\theta = \pi$, and have the same magnitude and opposite directions. The further assumption is made that the radial thickness is small, which is justified by the assumption that the dimer bond width along the radial direction is small also. The differential equation for the deflection of the left half of the hoop is (Figure 3)

$$d^2 F/d\theta^2 + F = -MR^2/EI \quad (25)$$

where F is the deflection of the hoop and is a function of θ only or $F = F(\theta)$, M is the moment at some value θ , R is the initial hoop radius, E is the elastic modulus of the material in the transverse direction, and I is the moment of inertia about the neutral plane of bending perpendicular to the plane containing the hoop. In this derivation we have set $F = (f/2)R$. The moment at any section m at an angle θ is given by

$$M = M_0 + PR(1 - \cos \theta)/2 \quad (26)$$

and when substituting Eq. (26) into Eq. (25) there results

$$d^2 F/d\theta^2 + F = -M_0 R^2/EI - PR^3(1 - \cos \theta)/2EI \quad (27)$$

which has the general solution

$$F(\theta) = A_1 \sin \theta + A_2 \cos \theta - M_0 R^2/EI - PR^3/2EI + PR^3 \theta \sin \theta/4EI \quad (28)$$

where the constants of integration were determined from the condition that $dF/d\theta = 0$ at $\theta = 0$ and at $\theta = \pi/2$. Note that Eq. (27) has the appearance of the non-linear Sine-Gordon equation, but it is not because θ is the independent variable and not a function of time. The load is applied and maintained, and unless the loading rate is very high (shock loading), the ensuing deformation is a stable ellipse without residual motion. From the boundary condition, it follows that $A_1 = 0$ and $A_2 = PR^3/4EI$. The bending moment M_0 can be calculated from Castigliano's theorem [22], or

$$U = \int_0^{2\pi} M^2 R/2EI d\theta = (2R/EI) \int_0^{\pi/2} M^2 d\theta \quad (29)$$

where U is the strain energy. Taking the partial derivative of Eq. (29) with respect to M_0 gives the angle of rotation of the cross-section A , which is zero in this case, therefore,

$$\partial U/\partial M_0 = (2R/EI) \int_0^{\pi/2} 2M \partial M/\partial M_0 d\theta = 0 \quad (30)$$

and substituting Eq. (26) into Eq. (30) and integrating gives $M_0 = PR(2/\pi - 1)/2$ and consequently, Eq. (28) becomes

$$F(\theta) = (PR^3/4EI)(\cos \theta + \theta \sin \theta - 4/\pi) = 0.2976(PR^3/4EI) \quad (31)$$

where the last expression follows from setting $\theta = \pi/2$.

Recent measurements reported by [24] indicate that a microtubule immersed in a liquid and held on a glass surface by a prior silanizing treatment, and acted upon by a quasi-point load of magnitude 5×10^{-10} N in the direction normal to the surface and parallel to the plane containing the hoop results in a deformation $f = 0.04(1.0 \times 10^{-8})/2 = 2.0 \times 10^{-10}$ m ($f = 0.04$ in [24], which is a reduction in the radius. Solving Eq. (31) for EI and using the mean microtubule radius of 10.0×10^{-9} m and the load $P = 5 \times 10^{-10}$ N, and for $F(\pi/2) = 2.0 \times 10^{-10}$ m for the radial deflection, we find that

$$IE = 0.2976(PR^3/4f) = 1.86 \times 10^{-25} \text{ N m}^2 \quad (32)$$

This value is the transverse flexural rigidity due to the elastic properties of the microtubule, is based on [24] measurements, and includes the contribution from dipole–dipole interactions. Moreover, this value is approximately 2 orders of magnitude lower than the range of values for the axial flexural rigidity reported by [1] and calculated by [2]. The ratio of the axial to the transverse flexural rigidity is approximately 125. This is in approximate agreement with [3]. Now, the question is: How much of this value is due to the dipole–dipole interaction?

We can estimate the contribution to the transverse elastic modulus from dipole–dipole interaction in the following manner. The dipole–dipole interaction energy for an un-deformed ($f = 0$) microtubule is given by (Table I)

$$U_d^r(R) = 4.633 \times 10^{-18} = C_0 \quad (33)$$

We can increase the hoop radius containing all dimers around the hoop by a small quantity ΔR , so that to a good approximation the energy change is

$$U_d^r(R + \Delta R) = C_0(R/(R + \Delta R))^3 \quad (34)$$

Since the dipole–dipole interaction energy is proportional to r^{-3} , increasing the radius from R to $R + \Delta R$ results in a decrease in that energy. Consequently, the net change in the interaction energy becomes

$$\Delta U_d^r = U_d^r(R) - U_d^r(R + \Delta R) = C_0(1 - (R + \Delta R)^{-3}) \quad (35)$$

The increase in the strain energy caused by a change in radius from R to $R + \Delta R$ is given by Eq. (23), which is an equation parabolic in δ , or

$$U_s = (A\delta^2/2l)E_d^r \quad (36)$$

where A is the cross-sectional area of a hoop of radius $R + \Delta R$ with a bond interaction width among proto-filament dimer equal to t with a length of a dimer or a_0 , therefore $A = ta_0$. The deformation in going from R to $R + \Delta R$ is $\delta = 2\pi(R + \Delta R) - 2\pi R = 2\pi \Delta R$, while the length of the hoop over which the strain is acting increases to $2\pi(R + \Delta R)$. Then Eq. (36) becomes

$$U = ta_0(2\pi \Delta R)^2 E_d^r / 4\pi(R + \Delta R) \quad (37)$$

and equating Eq. (37) into Eq. (35) and solving for E_d^r , the transverse elastic modulus, we find that

$$E_d^r = C_0 R(1 + \Delta R/R)(1 - (1 + \Delta R/R)^{-3}) / (\pi ta_0(\Delta R)^2) \quad (38)$$

Figure 4. Typical stress–strain diagram for polymers in tension.

Assuming a 5% increase in R so that the change in U is small ($4.633 \times 10^{-18}(1 + 0.05)^{-3}(4.00 \times 10^{-18} \text{ J})$) and using the value $t = 3.75 \times 10^{-9} \text{ m}$ for the radial extent of the dimer–dimer bond on adjacent proto-filaments, there results $E_d^r = 2.81 \times 10^8 \text{ N m}^2$ and $I E_d^r = 9.88 \times 10^{-27} \text{ N m}^2$ ($I = bh^3/12 \cong (8 \times 10^{-9})(3.75 \times 10^{-9})^3/12 = 3.52 \times 10^{-35} \text{ m}^4$). This value is approximately 19 times smaller than the transverse modulus calculated from [24]. The ratio of the longitudinal to the transverse elastic modulus due to the dipole–dipole interaction is therefore approximately $E_d^a/E_d^r \cong 7.53 \times 10^8/2.81 \times 10^8 = 2.7$.

Equation (38) expresses the fact that the elastic modulus due to dipole–dipole interaction depends on the strain ($\Delta R/R$) which implies that a stress–strain diagram would be a curve of the form shown in Figure 4, where the slope at a given value of the strain gives the value of the elastic modulus, decreases with increasing strain, a characteristic of some polymers. Moreover, Eq. (38) exhibits the unfortunate behavior that as $\Delta R \rightarrow 0$, E_d^r approaches infinity. This is a consequence of its parabolic form.

4. Conclusions

Electron diffraction studies [13] established the atomic resolution of dimers to a resolution of 0.37 nm, which enabled [10] and later [12]⁶ to calculate the magnitude of the components of the dipole moment of tubulin. Stracke *et al.* [8] reported three components of the dipole vector used in this paper as follows: p_z along the polar axis of the microtubule, p_θ an azimuthal component running counterclockwise, and p_r a radial component directed toward the centerline of the microtubule. We have calculated all interactions among all dipoles, both around the circumference and along the polar axis, and have arrived at the interaction energies from all these contributions, and found them to be positive. This implies that the total interaction

energy results in forces among dipoles that are repulsive axially and the radially, consequently tending to maintain a microtubule cylindrical and straight.

We have shown that if a microtubule is compressed axially, or radially compressed or deformed, the interaction energy increases. Thus, a microtubule can be considered as equivalent to a closed pressurized cylindrical vessel acted upon by internal gas pressure. One can consider that nature has provided that a microtubule, whose behavior in the absence of any external forces, is equivalent to a cylindrical balloon with internal pressure. Because microtubules are dynamic structures that can polymerize or de-polymerize tubulin *in vitro* and *in vivo* thereby change its length, its structural form is equivalent to a cylindrical balloon but has the flexibility for structural changes not available to a pressurized system. The interactions among dipoles provide the equivalent of enhanced structural rigidity by contributing to its flexural rigidity. The dipole–dipole interaction energy calculations suggest that approximately 50–60% of the axial elastic modulus is the result of that interaction. However, the dipole–dipole interaction energy contribution to the transverse elastic modulus is lower and somewhat uncertain.

We have calculated also the change in the interaction energy of a hoop made from a single set of 13 dimers equally spaced around the circumference. In this part of the calculations we have neglected the axial offset of the dimers along proto-filaments around the microtubule. This neglect results in an estimated error proportional to $1.058 r_{ij}^{-3}$, which means that the calculated interaction energy is larger by approximately 5.8% than would be the case had the offset of 0.92 nm been taken into account. A deformation was applied at two diametrically opposite points on the hoop so that the circular shape became progressively deformed into an ellipse with increasing eccentricity. For each increment in the deformation, the interaction energy of all dipoles was calculated. These results show that as the hoop deforms progressively into an ellipse, the net interaction energy steadily increases and this increase is positive (Figure 2). Although the increase in interaction energy is only 5% as f increases from 0 to 0.30, these results suggest that the cylindrical configuration of a microtubule represents a condition of energy minimum. The deformation into an ellipse involves negligible strain energy: deformation rearranges the orientations and distances among dipoles, altering the total dipole–dipole interaction energy. On the other hand, axial compression or extension represents a strain and because it increases or reduces the distances among dipoles by equal amounts without re-orienting them, it represents a change in dipole–dipole interaction energy that can be related to elastic properties.

Using the results of these calculations we determined the contribution to the microtubule elastic moduli and flexural rigidity, both longitudinal and transverse. The bond energy among dimers in the axial or azimuthal directions is at least equal to the dipole–dipole interaction energy. Therefore, the calculated energy from dipole–dipole interactions represents a lower bound on dimer–dimer bond energy, both in the axial and transverse directions. Such a lower bound has not been reported before. Moreover, calculations show that the contribution to the axial

elastic modulus, and hence the flexural rigidity is not insignificant and may amount to as much as 50–60% of the total. This value is somewhat uncertain as it depends on the assumptions made about the axial load bearing surface area of a microtubule. On the other hand, in the hoop direction the magnitude of the transverse elastic modulus and flexural rigidity was calculated from the measurements of [24]. Because of the method used by [24], their results appear to have significant uncertainties in the load applied and the deformation of the microtubule during the early stage of the measurements. In any case, these are difficult measurements to make and the uncertainties are very difficult to narrow down. We used the theory of elastic stability to estimate the transverse elastic modulus and flexural rigidity in the hoop direction from these measurements. There is considerable uncertainty associated with the computed results, but the magnitude of the elastic modulus and flexural rigidity based on [24] results is lower than the axial flexural rigidity by about a factor of 125. This is in general agreement with the estimate reported by [3]. Since the value thus obtained is based on [24] measurements, we conclude that the transverse properties contributed by the dipole–dipole interaction are included in the value derived from measurements. The calculated transverse elastic modulus obtained from the dipole–dipole interaction energy is lower than the measured value by about a factor of approximately 2.7. The ratio of the hoop dipole–dipole interaction energy (4.633×10^{-18} J) to the axial dipole–dipole interaction energy (2.350×10^{-18} J) is 1.972.

Finally, the conclusion from these results is that microtubules derive their structural mechanical properties not only from the binding energy among dimers and proto-filaments, but also derive rigidity in the axial and the transverse direction from the interaction among all dipoles. However, the magnitude of the transverse contribution is lower and somewhat uncertain. There may be other factors contributing to the total value of microtubule elastic properties, but these factors were not considered in this paper. Microtubules behave as dynamic rigid structures, and unlike gas-pressurized structures, are adaptable to their function by electrodynamic means.

Notes

1. Personal communication, 2004.
2. See footnote 1.
3. See footnote 1.
4. See footnote 1.
5. See footnote 1.
6. See footnote 1.

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