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研究課題名（和文）Compression stress induced deformation of microtubule on an elastic medium

研究課題名（英文）Compression stress induced deformation of microtubule on an elastic medium

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研究成果の概要（和文）：微小管の座屈は連続的な過程であることが見出されている。 微小管を取り囲む弾性媒体の存在はまた、座屈の程度に大きな影響を与える。 弾性媒質との強い相互作用は微小管の短波長マルチウェーブ座屈を引き起こすが、長波長オイラー型座屈発生は微小管と周囲の弾性媒質との相互作用が弱い。 微小管のシミュレーションに基づいて、微小管の機械的特性に配慮する必要があります。 周囲の弾性媒体との微小管の相互作用は、微小管の座屈変形機構を調節する別の因子である。

研究成果の概要（英文）：Using a custom made compression chamber deformation of microtubules under compression stress has been explored and their buckling mechanism has been unveiled. Buckling of microtubules is found to be a continuous process. Presence of an elastic medium surrounding the microtubules has a great influence on the extent of buckling. Strong interaction with the elastic medium causes short-wavelength multiwave buckling of microtubules, whereas long-wavelength Euler type buckling occurs when the interaction of microtubules with their surrounding elastic medium is weak. Moreover, it was also understood that, while accounting the buckling deformation mechanism of microtubules, any effect of associated proteins on the mechanical property of microtubules must be taken into consideration. From simulation based studies, we revealed that mode of interaction of microtubules with the surrounding elastic medium is another factor that regulates the buckling deformation mechanism of microtubules.

研究分野：　複合領域

キーワード：　Microtubule　Mechanical deformation　Buckling　Compression stress　Elastic medium

１．研究開始当初の背景

Microtubules (MTs), the most rigid cytoskeletal component, play indispensable roles in the spatial and mechanical functions of cells such as growth, development, migration, etc., which are critically important to survival of living organisms. Mechanical property of MTs is crucial for performing its roles in such diverse cellular activities. Despite the mechanical integrity, MTs in cells often experience compression stress and as a result found curved or buckled while they are engaged in various cellular functions. Generally on compression, a slender rod undergoes classical Euler buckling, i.e. buckling with long wavelength, which is accounted for by conventional 'elastic column model'. Buckling of a reconstructed isolated MT also follows such a deformation profile in an in vitro condition. But buckling of the slender MT filaments in cells has been manifested by short wavelength multi-wave buckling mode. Theoretical and computational studies suggested that, reinforcement of the MTs by surrounding elastic medium or MT-associated proteins around the MTs in cell play the key roles in governing the buckling behavior of MTs and bringing the differences in buckling profile compared to that of a slender rod or an isolated MT in vitro. The 'elastic foundation model', theoretically predicts buckling behavior of MTs and accounts for the role of surrounding elastic medium or MT-associated proteins in MT buckling. However, no experimental investigation has been reported yet to detail the mechano-responsiveness of MTs to compression stress and clarify its relation to elastic medium or MT-associated proteins. Lack of direct experimental evidences has been the major obstacle in verifying the theoretical predictions on buckling of MTs. Therefore, in this study, we aim at experimentally investigating the mechano-responsiveness of MTs to compression stress and verifying the theoretical predictions on compression induced deformation i.e., buckling of MTs and its relation to different factors such as elastic medium, MT-associated proteins, mode of interaction with elastic medium, etc.

２．研究の目的

The purpose of the study was to explore the responsiveness of MTs to compression stress and elucidate the mechanism of MT deformation under compression. Recently we developed a methodology that enabled us to clarify the effect of mechanical stress on the cytoskeletal components in a simplified in vitro condition. Employing that advancement, in this study, we aimed at elucidating the compression stress induced deformation, i.e. buckling of MT and unveiling the roles of surrounding elastic medium of MTs in the MT buckling.

At the beginning, this study was mainly focused in achieving the following targets within the specified research period: the ultimate goal was to figure out in detail the effect of compression stress on behavior, mechanical endurance and sustainability of MT and to understand the role of MT-associated proteins and elastic surrounding medium of MTs in governing the deformation of MTs. In order to serve the purpose a specially designed experimental set up was used that allowed the application of compression stress directly to MTs and in situ observation of MT's response to the applied stress.

The purpose of this study could be divided in the following segments: a) to systematically investigate in detail the effect of compression stress and subsequent behavior of MTs in a simplified in vitro condition; b) to unveil the effect of MT-associated proteins on the mechano-responsiveness of MT under compression; c) finally, using the experimental results obtained in a) and b), to verify the existing theoretical predictions about the MT buckling and role of an elastic medium, MT-associated proteins and also the elastic foundation model.

３．研究の方法

The experiments were performed as described below:

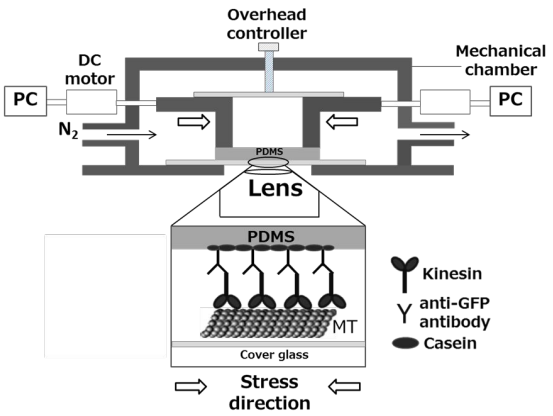

Figure 1: Schematic diagram showing the experimental design used to demonstrate the compression stress induced buckling of MTs on a 2D elastic medium using the compression chamber.

An experimental device named "compression chamber" (Figure 1) was developed in order to perform the compression tests of MTs. The chamber was equipped with two DC motors to operate it by using a computer. The chamber has a baseplate that contains a horizontally movable frame (compressor) where a soft deformable substrate, e.g. poly-dimethylsilaxone (PDMS) can be fixed. Fluorescence dye labeled MTs were adsorbed to the surface of the PDMS via interaction with the MT-associated protein kinesin. Compression stress was applied to the

MTs through compression of the movable frame and PDMS. Response of fluorescently labeled MTs to compression stress was monitored by using a fluorescence microscope.

In situ monitoring of response of MTs to the applied compression stress and detail quantitative investigation was carried out. The compression chamber consists of two main parts: cover plate and base plate where the baseplate contains a computer controlled stretcher/compressor. First, a small piece of PDMS with dimension 4.0×5.0×0.05 mm^3 (L×W×T) (commercially available, elastic modulus: ~1.86 MPa) was fixed horizontally at the stretcher of the compression chamber, and elongated 100% by applying tensile stress by using the computer controlled stretcher. At this stage, the length of the elongated PDMS substrate was 8.0 mm. A narrow region on the top surface of the elongated PDMS was then plasma treated for 3 min (10 Pa, 8 mA) by a plasma etcher to increase its hydrophilicity in order to use it as a flowcell. Next anti-GFP antibody solution of concentration of 0.1 mg mL^{-1} (5 µL) was applied to the plasma treated PDMS surface (flowcell). 10 µL of kinesin solution (K560-GFP) of prescribed concentrations, e.g. 10, 30, 50, 100 and 200 nM (~80 mM PIPES, ~40 mM NaCl, 1 mM EGTA, 1 mM $MgCl_2$, 1 mM DTT, 10 µM paclitaxel; pH 6.8) was introduced in respective case and incubated for 3 min to bind the kinesins to the antibody. The flowcell was washed with 10 µL of motility buffer (~80 mM PIPES, 1 mM EGTA, 1 mM $MgCl_2$, 0.5 mg mL^{-1} casein, 1 mM DTT, 10 µM paclitaxel; pH 6.8). Next, 10 µL of 100 nM MT solution was introduced and incubated for 3 min, followed by washing with 20 µL of motility buffer. In this study, MTs were deposited on the PDMS substrate via interaction with the kinesins in the absence of any nucleotide (e.g., ATP). After preparation of the flowcell on PDMS, the mechanical chamber was closed and humid nitrogen gas was kept passing through the chamber to remove oxygen from the chamber. Finally the tensile stress at the elongated PDMS was released through compression. All the aforementioned experiments were performed at room temperature.

MT deformation was characterized in terms of buckling wavelength, amplitude and radius of curvature of deformed MTs at different conditions in consideration with the change of applied strain, strain rate, kinesin density on the PDMS. Here, for measurement of strain, length of the elongated PDMS (8.0 mm) was considered as the initial length and all subsequently applied strains were quantified with respect to this length of the PDMS (8.0 mm). Images of MTs captured using fluorescence microscope were analyzed using the image analysis software 'Image J'. The length of MTs considered for investigating their buckling behavior ranged from ~20 µm to ~50 µm. Only the MTs aligned parallel to the compression axis were considered for analysis. For quantifying the deformation of MTs, buckling wavelength and amplitude were manually measured from the fluorescence microscopy images of deformed MTs, using the image analysis software. Brightness and contrast of MTs were also adjusted using the image analysis software. Finally, using the results obtained from the above experimental investigation, we verified the existing theoretical models about MT buckling. Computer simulation was also performed to validate the role of elastic medium in buckling of MTs under compression stress. Based on the experimental results a model was also developed that will serve as a guideline in accounting the MT buckling and its correlation to various factors.

4．研究成果

(a) Effect of compression stress, strain, and strain rate on mechanical stability of MTs:

In this study the kinesins adsorbed to the PDMS substrate worked as linear springs, and were aligned on the same plane and fixed perpendicular to the MTs that were deposited at the PDMS surface through interaction with the kinesins. Kinesins were distributed along the MTs with one end permanently fixed to the MTs and the other end to the PDMS substrate via interaction with anti GFP-antibody. Compression of the PDMS substrate then developed compression stress at the MTs deposited to the substrate, which resulted in mechanical deformation of the MTs manifested by buckling, as shown by the fluorescence microscopy images in Figure 2.

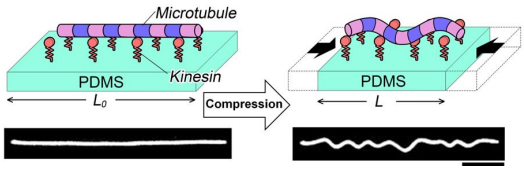

Figure 2: Schematic illustrations and representative fluorescence microscopy images show how the compression stress applied at the PDMS substrate caused buckling of the MT attached to the substrate through interaction with kinesins. Scale bar: 10 µm.

The buckling of MTs under the present experimental condition appears reminiscent to the uniform multiwave mode, rather than the localized buckling mode. Therefore, this experimental system turns out to realize the practical demonstration of laterally constrained MTs with 'both ends clamped' condition. At the onset of MT buckling at relatively low level of compression strain, buckling crests or change in

curvature along the MT filaments was found to develop. The compression strain at which the first buckling crest or curvature change was observed is termed hereafter as 'critical strain'. To characterize the compression stress induced buckling of MTs, we have monitored the wavelength and amplitude of buckled MTs. A MT filament produced the first buckling crest when the applied strain reached the critical strain; ~1.2% strain for the presented case of arbitrarily fixed kinesin concentration (50 nM). Although the first buckling crest appeared at a certain strain i.e., at the critical strain, all subsequently applied strains were found to bring changes in buckling wavelength and amplitude of the deformed MTs. On increasing the strain beyond the critical strain the buckling wavelength was found to decrease, whereas the buckling amplitude increased (Figure 3).

Figure 3: Change of buckling wavelength and amplitude of a MT filament upon gradually increasing the applied strain. Scale bar: 10 μm.

These observations suggest that buckling of MTs under compression stress may not be a discrete phenomenon but a continuous process, once the critical strain is reached. From the distribution of the buckling wavelength and amplitude of deformed MTs at different strain values (number of MT considered for analysis, n = 30) it was evident that on increasing the strain, distribution of buckling wavelength gradually became narrower and shifted towards lower values but the distribution of buckling amplitude followed an opposite trend. No considerable effect of strain rate was observed upon buckling of MTs.

(b) Effect of surrounding elastic medium of MTs on their buckling under compression stress:

Next we investigated the role of interaction of MTs with an elastic medium (PDMS, in this study) in their buckling. To do that, we have demonstrated buckling of MTs on the 2D elastic medium (PDMS) by varying the concentration of kinesin in feed over a wide range i.e., from 10 to 200 nM. Tuning kinesin concentration helped vary the interaction of MTs with the PDMS. Using quartz crystal microbalance (QCM) we were able to estimate the density of kinesins on PDMS for those kinesin concentrations applied in feed. For the 10, 30, 50, 100, and 200 nM kinesin concentrations, the kinesin density on the PDMS substrate was found to be approximately 112, 381, 749, 1800, and 2982 kinesins/μm^2, which in turn resulted in kinesin spacing of 95, 51, 36, 23, and 18 nm respectively. The inter-kinesin distance (kinesin spacing) estimated by QCM was also supported by the atomic force microscopy images of kinesins on the PDMS.

At the lowest kinesin concentration i.e., at 10 nM, the first buckling crest was noticed at ~0.6% strain and at this kinesin concentration no additional buckling crest along the MT filaments was developed even at higher strains. At this lowest kinesin concentration and at the critical strain, the average buckling wavelength and amplitude were 21.14±5.6 μm and 2.16±0.89 μm respectively. On increasing the kinesin concentration e.g., at the highest concentration of 200 nM, the critical strain increased to ~2.0%. At this kinesin concentration, additional buckling crests (in addition to that noticed at the critical strain) were found to develop occasionally on increasing the strain beyond the critical strain. At the critical strain, average buckling wavelength and amplitude were 3.11±1.21 μm and 0.72±0.16 μm respectively when the kinesin concentration was the highest (200 nM). Therefore, concentration of kinesin was found to strongly affect the buckling extent of MTs which is evident from the above differences in critical strain, critical buckling wavelength and amplitude of deformed MTs at different kinesin concentrations.

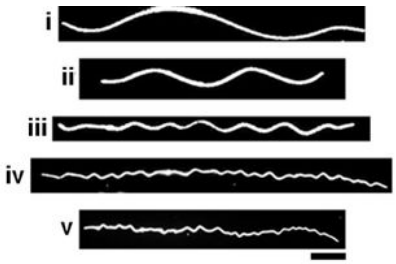

Figure 4: Effect of kinesin concentration on the buckling of MTs. The kinesin concentrations were: i) 10, ii) 30, iii) 50, iv) 100 and v) 200 nM that resulted in a surface spacing of 95, 51, 36, 23, and 18 nm respectively. Scale bar: 10 μm.

As shown in the Figure 4, with the increase in kinesin concentration wavelength and amplitude of buckled MTs decreased which is valid for any of the applied strains. On the other hand, for a fixed kinesin concentration, with the increase in applied strain the buckling wavelength was found to decrease and buckling amplitude increased substantially when the kinesin concentration was relatively low (e.g., 10 nM). However, for a relatively higher kinesin concentration (e.g., 200 nM), changes in wavelength and amplitude with the change of strain were not that significant compared to that observed for low kinesin concentrations. Here, an important manifestation of the effect of kinesin concentration (kinesin density or spacing) on MT buckling is the transition of buckling mode of MTs. When the kinesin concentration was low (e.g., 10 nM), MTs

showed long wavelength Eulerian buckling (Figure 4), similar to that reported for most of the cases of slender rods and in vitro buckling of isolated MTs. On the other hand, when the kinesin concentration was gradually increased (e.g., 200 nM), the buckling mode of MTs was changed from long wavelength Eulerian to uniform short wavelength multiwave buckling mode (Figure 4), which is reminiscent to the in vivo buckling mode of MTs. Thus, kinesins density or spacing dependent transition of MT's buckling mode observed in our experiments, which could never be observed in the previous in vitro studies on MT buckling, suggests that MT-associated proteins or surrounding medium plays a key role in determining the buckling mode of MTs.

(c) Verification of the agreement between experimental results and existing theory:

Now, for the buckling of MTs supported by a continuous elastic medium relationship between the buckling wavelength, λ and spacing of anchor, L_d (kinesins in this study) could be expressed by the following equation:

$$\log\lambda=\log 2\pi+1/4\ \log\ (EI/k)+1/4\log L_d$$

Here, E is the Young's modulus and EI is the bending rigidity of MT and k is the spring constant of kinesin.

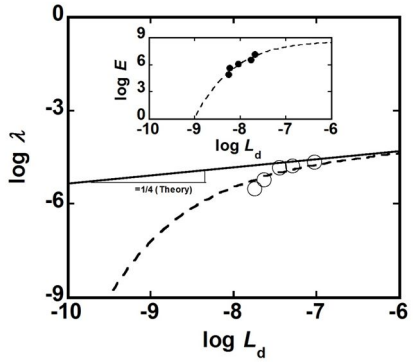

Figure 5: The theory (elastic foundation model) is verified using the experimental results on the buckling of MTs obtained in this work. Inset shows the curve fitting of the dependence of MT's Young's modulus (E) on kinesin spacing (L_d). The solid line in the main panel (slope=1/4) is drawn to show the relationship between kinesin spacing and buckling wavelength as theoretically predicted by the elastic foundation model. The dashed line is the fitting curve according to the modified elastic foundation model based on the present work that takes into account the change in Young's modulus of MT by kinesins.

At the long kinesin spacing region the slope of the straight line drawn following our experimental data points was in an excellent agreement with that predicted theoretically by the elastic foundation model. On the other hand, a considerable deviation of the experimental results from the theoretical prediction was noticed at relatively short kinesin spacing region. This result suggest that, at least another factor might have been involved in this regard, which was not taken into account by the elastic foundation model so far and therefore it failed to satisfactorily correspond to the experimental results at all the kinesin spacing studied in this work.

In our recent work, we experimentally confirmed that MT-associated protein kinesin can alter the mechanical property of MTs, where increased interaction with kinesin was found to decrease the Young's modulus (E) of MTs. Taking into consideration this fact, we brought a modification in the elastic foundation model by taking into consideration the role of kinesin in altering the mechanical property of MTs. As a result, modified equation for the elastic foundation model could be expressed by the following equation-

$$\log\lambda=\log 2\pi+1/4\log(I/k)+1/4\log[E_0-\beta(\log L_d/\log L_{dc})^{\alpha}]+1/4\log L_d$$

Here, E_0 is the Young's modulus of bare MTs, L_{dc} is the critical spacing between kinesins. The physical significance of L_{dc} is that MT is drastically softened if L_d is smaller than the L_{dc}. Figure 5 (main panel) shows the fitting curve according to the modified equation, and at both low and high kinesin spacing regions the fitting curve according to the modified equation correlates well to our experimental results of MT buckling. Thus our experimental findings confirmed that while accounting for the effect of surrounding elastic medium on buckling, the role of the medium in altering the mechanical property of MTs must be taken into account.

(d) Effect of the mode of interaction of MTs with surrounding elastic medium on buckling:

Using simulation based studies we further explored the importance of mode of interaction of MTs with the elastic medium on the buckling. We performed simulations of buckling of MTs interacting with kinesins and the hypothetical linkers permanently attached to MTs. We found that considering permanent interaction of MT with surrounding elastic medium our results follow elastic foundation model and with kinesins, the results of the simulation showed deviations from the predictions of the theory.

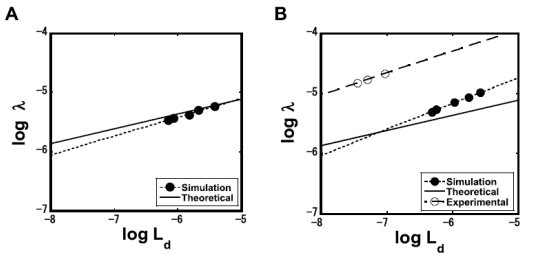

Figure 6: Comparison among the role of breakable and permanent interactions of MTs to elastic media in their buckling. (A) Buckling of MTs with permanent interactions to elastic media. (B) Buckling of MTs interacting with kinesins. The solid lines (slope=0.25) show the relationship between kinesin spacing and buckling wavelength as predicted by the theory. The dotted lines show the regressions of our simulation results.

Our findings (Figure 6) indicate that the force induced detachments of kinesins from MTs play a significant role in the buckling behavior of MT that is not considered in the elastic foundation model. The simulation results are also found very close to the above discussed experimental results that also confirm validity of our simulation and the role of medium in determining the buckling behavior of MTs. Considering forced-induced detachments of kinesins from MT, our simulation results deviated from predictions of the elastic foundation model and showed a similar trend with our experimental results discussed above. On the other hand, at permanently fixed MT with medium, our simulation results corresponded well with the elastic foundation model. These findings suggest that the mode of interaction of MT with the medium should be taken into account in considering the role of surrounding medium in buckling of MT.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

Journal article (Total: 1)

1. Tanjina Afrin, Arif Md. Rashedul Kabir, Kazuki Sada, Akira Kakugo, and Takahiro Nitta, Buckling of microtubules on an elastic media via breakable bonds, *Biochemical and biophysical research communications (BBRC)*, 2016, 480, 132-138 (peer reviewed).

Conference presentation (Total: 7)

1. Arif Md. Rashedul Kabir, Tanjina Afrin, Kazuki Sada, and Akira Kakugo, Buckling of microtubules on an elastic medium, *The 17th RIES-Hokudai International Symposium*, 13-14 December 2016, Sapporo, Hokkaido.
2. Tanjina Afrin, Arif Md. Rashedul Kabir, Kazuki Sada, and Akira Kakugo, Mechanical deformation induced modulation of biochemical functions of microtubules, *The 6th Soft Matter Symposium*, 24-26 October 2016, Hokkaido University, Sapporo.
3. Seiji Nishikawa, Tanjina Afrin, Arif Md. Rashedul Kabir, Kazuki Sada, and Akira Kakugo, Response behavior of microtubules to external mechanical stimuli, *The 6th Soft Matter Symposium*, 24-26 October 2016, Hokkaido University, Sapporo.
4. Tanjina Afrin, Daisuke Inoue, Arif Md. Rashedul Kabir, Kazuki Sada, and Akira Kakugo, Effect of mechanical deformation of microtubules on motor protein based cargo transportation, *The 6th Soft Matter Symposium*, 24-26 October 2016, Hokkaido University, Sapporo.
5. Arif Md. Rashedul Kabir, Daisuke Inoue, Kazuki Sada, and Akira Kakugo, Buckling of microtubules on a 2D elastic medium, *The 67th Divisional meeting on Colloid and Interface Chemistry*, 22-24 September 2016, Hokkaido University of Education, Asahikawa.
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Book (Total: 0)

Industrial property rights (Total: 0)

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