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学位論文題名

Study on In Vitro Lifetime of a Biomolecular Motor and its Extension in an Inert Chamber System

（生体分子モーターの試験管内寿命および不活性チェンバーシステムによる寿命延長に関する研究）

学位論文内容の要旨

Biological power systems have several attractive features compared to man-made machineries e.g., highly complex and flexible structural organization, high efficiency and availability of energy source inside the system. With respect to the high functionality and the ability to self-assemble, large number of well-studied biological building blocks such as DNA, RNA, lipids, and proteins have emerged as promising candidates for different nanotechnological applications. Consequently the design of hybrid biomachines through the organizational integration of biological building blocks has become an active research field now-a-days. Biomolecular motor proteins, also termed as biomolecular motors, can perform various biological functions not only acting individually but also in small groups (such as in fast anterograde transport) and in large arrays (particularly in muscle). Biomolecular motor protein systems e.g., actin-myosin and microtubule (MT)-kinesin are smallest natural machines that can provide some net work and they are the constituent components of biological power systems as well. Particularly, the ability of these motor proteins to convert chemical energy into mechanical work has been a salient example of molecular functionality observed in natural systems and the high efficiency of motor proteins in doing work has been one of their most intriguing attributes especially from an engineering viewpoint. Biomolecular motors are therefore exactly the kind of biological nanomachine whose integration into a hybrid system can gear up the development towards artificial biomachines; therefore these biological motor systems have been proposed as the building block of ATP fuelled artificial biomachines. As the consequence of continuous development, recently biomolecular motor protein systems are also finding potentially important applications for serving different purposes such as nanotechnological transport, detection, and sorting, etc. Considering the progressive development and importance of motor protein based artificial biomachines in nanotecnological applications, longevity of such devices has emerged as an issue that is of tremendous importance since motor proteins are very sensitive to their environmental elements including temperature, pH, ionic concentration, radiation, etc. Oxidation of motor proteins by the attack of free radicals including reactive oxygen species (ROS) and their consequent denaturation has also been one of the reasons that can readily terminate the activity of proteins and as a result can terminate the lifetime of motor protein based biodevices. In this dissertation, considering fluorescently labelled microtubules and green fluorescent protein fused kinesin (rhodamine MTs-GFP-kinesin) as a model biomolecular motor system we have attempted to focus on the effect of ROS on the in vitro lifetime of motor protein kinesin. At the same time we also studied the effect of inert atmosphere on the working lifetime of kinesin, which was found to improve dramatically in the absence of ROS. Active self-organization of MT-kinesin system has also been studied both in the presence and absence of inert atmosphere.

In chapter 2, to study the activity of motor protein system in vitro motility assay technique was used which was performed on glass substrate surface. First, working lifetime of kinesin was investigated in an aerobic condition. It was found that, in an aerobic condition the working lifetime of kinesin was only a few hours (~3 hours) and this loss of activity was found due to the damage of kinesin by the attack of ROS. Even use of scavengers (glucose, glucose oxidase and catalase) failed to keep the motor protein system active for more than ~3 hours. Moreover, the scavengers used were also found harmful to motor protein system especially in a prolonged in vitro motility assay. With a view to eliminate the harmful effect of ROS on MT-kinesin system and to improve their lifetime further, an inert nitrogen atmosphere was employed in this study. A new system named inert chamber system (ICS) was developed which made it possible to monitor the activity of motor protein in an inert medium free of ROS. Development of ICS and application of ROS free inert atmosphere in studying the activity of MT-kinesin system has been

discussed in chapter 2. Improvement in the in vitro lifetime of MT-kinesin system through reduction of harmful effect of ROS has also been discussed. When ICS was used, the working lifetime of kinesin was found to prolong for almost a week. Moreover use of ICS was found to significantly prevent the breakage of rhodamine-microtubules and photobleaching of rhodamine-MTs and GFP-kinesins. Additionally, in this study it was also found that use of ICS offered a means to perform in vitro motility assay of motor protein system on a substrate made from material having high oxygen affinity (e.g., polydimethylsiloxane).

Self-organization of motor protein system is crucially important in order to develop motor protein based artificial biomachine as the organized body might provide integrated functions compared to their basic constituent units. Now-a-days, different techniques have been developed to achieve self-organization of MT-kinesin system. However keeping the assembled structures active for a prolonged time period has been a great problem due to the loss of activity on attack of ROS. Use of ICS was already found to keep single MT filaments active for a longer time and hence in chapter 3 it was verified whether the same is applicable to an assembly of MTs or not. Thus, active self-organization of microtubules in both aerobic and anaerobic conditions was demonstrated employing a specific streptavidin-biotin interaction and effect of respective experimental conditions on the lifetime of assembled microtubules was discussed. In an aerobic condition, assembled MTs-structures were found active for almost 90 minutes, however using the ICS, we have shown that it is possible to keep assembled MTs active for almost a day. Advantage of ROS free inert atmosphere in keeping the bioactuators, formed through active self-organization of MTs, active for a much longer time was also discussed.

Active self-organization of microtubules that made use of streptavidin-biotin interaction produced ring-shaped microtubule structures along with the bundled or network structures. In that case, the yield of MT-ring formation was found very small (~0.4%) and the size distribution of MT-rings was residing within a wide range. To overcome these drawbacks, active self-organization of microtubules was demonstrated at an air-buffer interface that also produced ring-shaped microtubule structures and this has been discussed in chapter 4. Without using any streptavidin-biotin interaction, microtubule rings with a narrow size distribution and high yield (~50%) were obtained from filamentous microtubules when an in vitro motility assay was performed at an air-buffer interface instead of an aqueous medium. Moreover, using the ICS, direct in situ observation of such ring formation phenomenon of filamentous MTs at the air-buffer interface was also successfully performed which revealed two different modes of microtubule ring formation at the air-buffer interface. Finally, a qualitative model was proposed that could interpret the ring formation phenomenon of filamentous MTs at the air-buffer interface. Role of different energy parameters in such conformational transition of MTs depending on environmental condition have also been discussed.

In a nutshell, this study has made a comprehensive investigation on the effect of ROS on activity of motor protein system and focused on a newly developed technique to perform in vitro motility assay of motor protein systems in an inert atmosphere, until now which is being performed in an aerobic condition. Development of ICS and its successful demonstration in studying the motor protein system revealed that, an oxygen free inert environment is much better for monitoring the motor protein systems and at the same time can ensure high durability and efficient performance of motor system. Use of ROS free inert atmosphere can successfully extend the working lifetime of motor protein system for a much longer time compared to the conventional technique and this achievement would be potentially important in enhancing the nanotechnologial applications of motor proteins. Moreover, use of ICS was found to keep assembled MTs structures active for at least ten times longer period of time compared to that without ICS. Also, ICS offered a good means to investigate conformational transition of MTs at an air-buffer interface that was found to produce MT-rings with a high yield. All these results collectively will serve as a guideline in improving the in vitro stability of motor proteins and the newly developed inert chamber system is also expected to accelerate the industrial applications of motor proteins and will foster the development towards biomolecular motor protein based artificial devices.

学位論文審査の要旨

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Designing of hybrid biomachines using biological building blocks has become an active research field now-a-days. Biological motor protein systems, e.g. actin-myosin or microtubule-kinesin are being used as the building block of ATP fuelled artificial biomachines, and recently these are also finding potentially important applications for serving different purposes such as nanotechnological transport, detection, and sorting, etc. Longevity of such biodevices thus has become an issue that is of great importance and hence drawing much attention. Oxidation of motor proteins by the attack of free radicals including reactive oxygen species (ROS) and their consequent denaturation has been one of the reasons that can readily terminate the activity of proteins which in turn can terminate the lifetime of motor protein based biodevices. Thus improvement of the *in vitro* lifetime and durability of motor protein has been a much required and challenging task.

This dissertation primarily aimed to investigating the effect of ROS on activity and lifetime of motor protein system (microtubule-kinesin) and disclosed that ROS damage the motor protein system within a very short time. Even conventionally used scavengers also failed to maintain prolonged activity of motor protein. Then this dissertation introduced a new technique to study the activity of motor protein in an atmosphere free of ROS. Using the newly developed 'inert chamber system' it was discovered that, lifetime of motor protein could be prolonged for a much longer time in ROS free atmosphere. The newly developed chamber system thus offered a means to improve *in vitro* durability of motor protein. Additionally, active self-organization of microtubules demonstrated in the inert chamber system produced bioactuators having higher sustainability and longer lifetime. This higher durability of assembled microtubules was the basis for producing bioactuators with improved hierarchy that was realized by performing successive active self-organization using the chamber system. Also, using the chamber system ring formation from filamentous microtubules was investigated at an air-buffer interface that revealed the obscure mechanism of such reversible conformational transition of microtubules.

In conclusion, this study has made a comprehensive investigation on the effect of ROS on activity of motor protein system and focused on a newly developed technique to study motor protein systems in an inert atmosphere which was found successful in improving the lifetime and durability of motor protein. These new findings would be potentially important in enhancing the nanotechnologial applications of motor proteins; would accelerate the industrial applications of motor proteins and foster the development towards biomolecular motor protein based artificial devices.

Therefore, we acknowledge that the author is qualified to be granted the doctorate of Science from Hokkaido University.