Dynamic Self-organizations of motor-proteins under non-equilibrium condition

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Abstract: In this study, we show that the energy-dissipative active self-assembly of microtubules (MTs) via a kinesin-based motility system produces various MT assemblies such as bundle-, network-, and ring-shaped structures depending on the initial conditions. Structural polymorphism of the MT assembly is depicted through phase diagrams, and morphogenesis of the MT assembly is discussed based on the following factors; binding force between MTs and motility-driving force from kinesins. This study provides new insights into the energy-dissipative dynamic self-organization of biological systems.
Keywords: Microtubules; active self-assembly; bottom-up approach

1. INTRODUCTION

Cytoskeletal proteins such as actins and microtubules and their motor proteins, *i.e.* myosins and kinesins, are known to play important roles in the formation of cellular shapes.[1–4] It has been demonstrated that isolated actins and microtubules can be self-organized into structures with specific patterns such as asters, networks, rings, and so on, associating with their motor proteins.[5–9] Other efforts have been also made to integrate cytoskeletal proteins into an ordered structure to exploit more complex functions in vitro, as observed in nature.[10, 11] Recently, to quest more complex assemblies, a method to integrate MTs into a bundle structure on a kinesin-coated surface has been developed by employing a streptavidin (St)-biotin (Bt) interaction during the sliding motion of MTs in the presence of ATP.[6] This ATP-driven, energy-dissipative self-assembly process was termed as an active self-assembly (AcSA) process and AcSA is different from a conventional self-assembly process that occurs towards the thermodynamic equilibrium without any external energy supply. Therefore, we termed the latter as a passive self-assembly (PaSA) process.[12] Indeed, AcSA process has been widely and intensively studied to obtain new insights into emergent properties found in living cells.[13, 14] Thus, AcSA has great potential to facilitate various motor protein assemblies in terms of the sizes, shapes, and properties of motion. In this study, we systematically studied the effects of initial conditions such as the Bt to tubulin (Tub) ratio, St/Bt ratio, and Tub concentration (C_{Tub}) that may strongly influence the morphology of the MT assembly produced through the AcSA process.

2. RESULTS AND DISCUSSION

Effect of St/Bt modification on motility of MT

The AcSA process requires the motility of the MTs. Because it was considered that the high molecular weight of St might prevent MTs from binding to kinesins because of steric hindrance, we first investigated the effect of the modification ratio of St / Bt on the motility of individual MTs keeping the Bt/Tub at 1/1. Consequently, the velocity of the MTs were obtained as 0.129, 0.059, 0.054 and 0.045 μm/s for the St/Bt ratio of 0, 1/64, 1/16 and 1 respectively. Thus, MT velocity was slightly decreased with the increase of the St/Bt ratio and this suggested that the total driving force exerted on MTs should be decreased due to the reduced interaction of MTs with kinesins. Fixing the St/Bt ratio at 1/2, we also investigated the effect of Bt/Tub on the motility of MTs. On changing the Bt/Tub ratio from 1/64 to 1/1, MTs velocity was found to lie in the range of 0.03~0.05 μm/s, without showing any regular change.

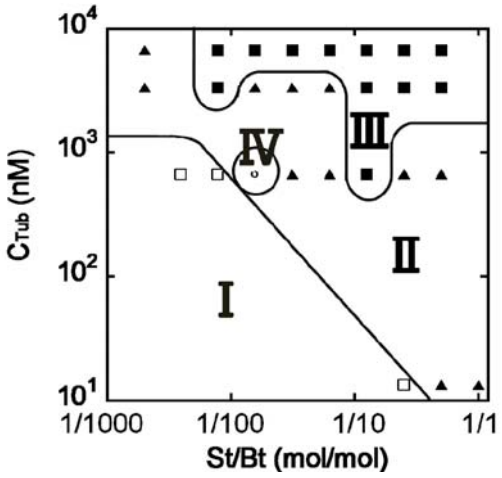

Figure 1. Phase diagram for the morphology of MTs assemblies, in which the phase behavior is summarized as a function of the St/Bt ratio and C_{Tub} for constant a molar ratio of Bt/Tub = 1/1; □: single MT phase (I); ▲: bundle phase (II); ■: network phase (III); ○: ring shaped assemblies dominant (IV).

Effect of C_{Tub} and the St/Bt ratio on the structural morphology of MT assemblies

Next, we investigated the effect of C_{Tub} and the St/Bt ratio on the morphology of MT assemblies. Based on the result in the Bt/Tub ratio study, the Bt/Tub ratio was fixed at 1/1 to allow the MTs to form assemblies effectively. To study the structural variety of the MT assemblies, C_{Tub} and the St/Bt ratio were widely varied from 13.4 nM to 6720 nM and from 1/256 to 1/1, respectively. Under the experimental conditions stated above, the morphology of MTs was observed under the fluorescent microscope after 4 h of the AcSA process. The C_{Tub} and St/Bt phase diagram is shown in

Figure 1. When C_{Tub} was in a lower range as 13.4 – 672 nM, the MTs existed as single filaments in the lower St/Bt region (I) and were in the bundled state in the high St/Bt region (II). When C_{Tub} was in a higher range as 3360 – 6720 nM, networks of MT assemblies were observed in the high St/Bt region (III). However, the boundary between phase II and III in the C_{Tub} and St/Bt phase diagram was complicated. A singular point (IV) where ring-shaped MTs assemblies were preferentially observed was found as shown in Figure 1. The point IV emerged in close vicinity to the complex boundary of phase II and III (C_{Tub} ~ 672 nM, St/Bt ~ 1/64). The diameters of the ring-shaped MT assemblies observed in this region were widely distributed (1.1 μm~13.2 μm), and the preferential rotation of those assemblies in a CCW was still preserved (CCW/clockwise (CW) = 33/7). These results agree well with those reported in a previous paper.[15, 16]

Discussion of the polymorphism of MT assembly

It was considered that the factors such as binding force between MTs, driving force, and sticking that disturb the sliding motion, are responsible for the polymorphism of MT assembly. The binding force between MTs was expected to increase with increasing St/Bt ratios up to a certain optimum ratio. Meanwhile, the driving force exerted on MTs may decrease by increasing St/Bt ratios because of the steric hindrance of Sts and may reach a plateau after Sts fully covers the Bts on MTs. Motility of sliding MTs could be disturbed by increasing the St/Bt ratio, which results in an increased steric hindrance, being diminishing the chance of interaction among kinesins and MTs. Here, we focus again on the C_{Tub} of 672 nM. When the St/Bt ratio was smaller than 1/128, single MTs were observed. This can be attributed to the low binding force between MTs and the high driving force for dissociating the cross-linked MTs. Under such conditions, ring or bundle formation may be difficult to achieve. The ring-shaped MT assemblies observed in the bundle phase appeared between the single and network phase. In this region, large amounts of single MTs were observed at the beginning of the AcSA process. This suggests that the relatively large driving forces and moderate binding forces suppress the formation of bundle-shaped assemblies but facilitate the formation of ring-shaped assemblies, in which a configuration with intrafilament interactions is favorable. With further increases in the St/Bt ratio, the binding force increases, and the driving force simultaneously decreases. This may facilitate the interfilament cross-linking needed to form MT bundles. The bundled MTs may also suppress the formation of a ring-shaped assembly because of the increased rigidity. At the optimum St/Bt ratio, the highest cross-linking efficiency will be a trade-off between the binding and driving forces. Under optimum condition, MTs will be cross-linked even if there are not many cross-linking points. Therefore, cluster-like aggregations may appear in this phase (III). The optimum St/Bt ratio for network formation was determined to be approximately 1/8, which was not the stoichiometric ratio that we expected. Thus, the polymorphism of the MT assembly may result from the close coupling of these factors.

Conclusions

In this study, we showed that MTs were self-organized into various structures (linear, bundles, network, and rings) in response to initial conditions of C_{Tub}, St/Bt ratio, and Bt/Tub ratio through AcSA. We also showed that the ring-shaped assemblies were preferentially formed under a specific condition. These results indicated that not only the density of the MTs but also other factors such as driving force, binding force, and steric hindrance were responsible for the polymorphism of MT assembly. We expect that the present study will provide new insights into the energy-dissipative dynamic self-organization processes of biological systems. In future, the knowledge in dynamic self-organization process may also widen the range of potential applications of motor proteins in biodevices and biomachines.

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