2P148 結晶構造解析で明らかになったイネキネシン K16 のネックリンカーの特徴的コンホメーション
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Crystallographic analysis of the rice kinesin K16 reveals a novel conformation of neck-linker
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We have previously demonstrated that the motor domain of the novel rice plant specific kinesin K16 has several unique enzymatic characteristics comparing with conventional kinesin. The most interesting property is that the ADP-free K16 motor domain is very stable, contrast to conventional kinesin that is very labile in ADP-free state. In the present study, the crystal structure of the novel rice kinesin K16 motor domain in complex with MgADP was determined at 2.5Å resolution and compared with other kinesins. The overall structure of the K16MD is similar to that of conventional kinesin motor domains, as expected from the high similarity of amino acid sequence (43.2 %). However, several unique localized structures of K16 were observed. The position and length of the loops L5, L11 and L12, which are believed as functional key region, were different from that of conventional kinesin. Furthermore, the neck-linker of the ADP bound K16 motor domain showed an ordered conformation in a position quite different from that observed in conventional kinesin. The novel conformation of the neck-linker resembles to that of Eg5 previously reported. These structural differences may reflect the unique enzymatic characteristics of rice kinesin K16.

2P150 スピンラベリング ESR でわかる微小管に結合したキネシンのループ L11 の動的構造
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1aB11 Conformational Dynamics of Loop L11 in Kinesin on Microtubule Measured by Site-Directed Spin Labeling Electron Paramagnetic Resonance
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We have measured the spin label side-chain mobility and the interspin distance of kinesin motor domain on microtubule (MT). In the absence of MT the nanosecond mobility of spin label attached to 242-, 244-, 245-, 247-, 249-, or 252-residue was little sensitive to nucleotide. Rotational correlation time of immobile component was consistent with rotational mobility of whole rigid motor domain. In the presence of MTs the fraction of mobile component of spin labels was reduced and sensitive to nucleotides: ADP, AMPPNP > no nucleotide. Strong immobilization occurred at the 249- and 252-residues close to the switch II, and showed a nearly rigid limit suggesting that kinesin motor domain binds to MT. Dipolar CW-EPR detected a wide distribution of the distance between two spin labels attached to 247- and 252-residues, suggesting a flexible loop. In the absence and presence of MT the distance distribution between 247- and 252- residues was still broad in the range of 0.8-2.5 nm and beyond it. Conclusions: (1) L11 is fluctuating in a nanometer range. (2) Flexibility of L11 is little sensitive to nucleotide in MT-free state. (3) L11 is still fluctuating but immobilized on MT. (4) Nucleotide-induced increase in mobile component of spin label at L11 suggests partial detachment of L11 from the interface of MT while a kinesin motor domain remains attached to MT. (5) The residue closer to switch II helix is more immobilized, suggesting their slowly flexing attachment to MT. The studies on L12 is now in progress.

2P149 弾性ネットワークの非線形緩和現象と分子モーターの構造変化
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1aB10 Nonlinear Relaxation in Elastic Networks and Conformational Motion of Molecular Motors: A Comparative Study
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We investigate relaxation dynamics in elastic network models for protein machines. By comparing several motor proteins and artificially generated networks, we discuss dynamical and structural features of biological molecular motors.

Elastic network models (networks of material particles and linear springs) are often adopted to study slow conformational motion in molecular machines. A model network for a protein can be constructed by replacing each atom or residue with an identical particle and connecting particles lying close to each other by springs. Although chemical details are omitted in these models, conformational changes in various protein machines, essential for their functions, can be reproduced fairly well by normal mode analysis (NMA), with an interesting exception of KIF1A.

In this study, instead of NMA, we directly simulate (nonlinear) motion of elastic networks. We compare relaxation dynamics in networks corresponding to actual motor proteins and those generated artificially, to highlight dynamical features of molecular motors. Networks for motors usually show ordered motion along well-defined relaxation paths in the conformational space, even from large random (unnatural) deformations. In contrast, random elastic networks generally have many (meta)stable states around the original conformation and exhibit complicated behavior; which is inconvenient for repeatable machine operation. Using this method, we also discuss the peculiarity of KIF1A in NMA and its relation to the working mechanism of the motor.

2P151 異なるスクレオチド状態における分子モーターKIF1A の構造揺らぎ
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Structural fluctuations of molecular motor KIF1A in different nucleotide states
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KIF1A (Kinesin-3) is a single-headed molecular motor that moves processively and unidirectionally along a microtubule using the chemical energy produced by ATP hydrolysis. KIF1A has the "strong" and "weak" binding modes for the microtubule (Nitta et al., Nature, 2004):

[i] In the strong binding mode, the equilibrium dissociation constants for microtubule is small (the affinity to microtubule of KIF1A is high). Therefore, KIF1A hardly detaches from microtubule. This binding mode is realized in AMP-PCP, AMP-PNP, and ADP-AlFx nucleotide states.

[ii] In the weak binding mode, the equilibrium dissociation constants for microtubule is high (the affinity to microtubule of KIF1A is low). Therefore, KIF1A easily detaches from microtubule. This binding mode is realized in ADP-Vi and ADP nucleotide states.

The mechanism of switching between these two modes is still unknown. We investigate which part of KIF1A plays an important role in switching. For this purpose, we calculate the structural fluctuations of KIF1A in all nucleotide states by means of the molecular dynamics simulation based on the Go-like model. It is found that the structural fluctuation of $\alpha 4$ helix in KIF1A has a strong correlation with binding strength for microtubule. Therefore, we suggest that $\alpha 4$ helix plays a crucial role in switching the binding strength that depends on the nucleotide state.