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Nucleotide-dependent displacement and dynamics of the α-1 helix in kinesin revealed by site-directed spin labeling EPR

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ABSTRACT

In kinesin X-ray crystal structures, the N-terminal region of the α-1 helix is adjacent to the adenine ring of the bound nucleotide, while the C-terminal region of the helix is near the neck-linker (NL). Here, we monitor the displacement of the α-1 helix within a kinesin monomer bound to microtubules (MTs) in the presence or absence of nucleotides using site-directed spin labeling EPR. Kinesin was doubly spin-labeled at the α-1 and α-2 helices, and the resulting EPR spectrum showed dipolar broadening. The inter-helix distance distribution showed that 20% of the spins have a peak characteristic of 1.4–1.7 nm separation, which is similar to what is predicted from the X-ray crystal structure, albeit 80% were beyond the sensitivity limit (>2.5 nm) of the method. Upon MT binding, the fraction of kinesin exhibiting an inter-helix distance of 1.4–1.7 nm in the presence of AMPPNP (a non-hydrolysable ATP analog) and ADP was 20% and 25%, respectively. In the absence of nucleotide, this fraction increased to 40–50%. These nucleotide-induced changes in the fraction of kinesin undergoing displacement of the α-1 helix were found to be related to the fraction in which the NL undocked from the motor core. It is therefore suggested that a shift in the α-1 helix conformational equilibrium occurs upon nucleotide binding and release, and this shift controls NL docking onto the motor core.

1. Introduction

The motor protein kinesin moves along a microtubule (MT) using the chemical energy of ATP. In neurons, kinesin powers vesicular trafficking toward the synapse; in all cells, kinesin is involved in the restructuring that occurs during cell division [1–3]. All kinesins have a homologous ~330 amino acid catalytic core that binds ATP and microtubules (MTs). Immediately adjacent to the catalytic core is the neck linker (NL) – a ~15-amino acid segment that has been shown to be critical in force generation and directionality [4–8].

Site-directed spin labeling electron paramagnetic resonance (SDSL-EPR) spectroscopy has been used to determine the secondary, tertiary, and quaternary structures of proteins and to monitor associated conformational changes [9–11]. Depending on the mobility of the attached spin label, we can detect both the steric restrictions that are imposed on the label by its environment and the motion of a polypeptide chain segment [12,13]. Moreover, if two spin labels are close to each other, the resultant spectrum includes effects from dipole interactions and is dependent on the distance between the two spins [11]. We found that the transition of the NL from an undocked to a docked conformation occurred in the ATP-bound state but not in the ADP-bound state or no nucleotide state, suggesting that the NL is an ATP- and strain-dependent mechanical element [5–7,13,14]. Therefore, it is important to understand how nucleotides (ATP or ADP) bound in the active site can communicate with the NL to control NL docking on the kinesin core domain. These SDSL-EPR studies [15] suggest that two helices, switch I and switch II, undergo nucleotide-dependent conformational changes (analogous to conformational changes in G-proteins [16]) that play an important role in communicating between the catalytic site and the NL docking domain on the MT-binding interface of kinesin. Recent cryoelectron microscopy (cryo-EM) studies also revealed that these unique helices communicate between the catalytic site and the NL docking region [17–20].

Abbreviations: AMPPNP, adenosine 5′-(β,γ-imido)triphosphate; βME, 2-mercaptoethanol; CW, continuous wave; EGTA, ethylene glycol-bis(l-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; EM, electron microscopy; MSL, 4-maleimido-2,2,6,6-tetramethyl-1-piperidinoxy; NN, no nucleotide; MT, microtubule; PIPES, piperazine-1,4-bis(2-ethanesulfonic acid); SDSL, site-directed spin labeling.

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In kinesin X-ray crystal structures, the N-terminal region of the α-1 helix is adjacent to the adenine ring of the bound adenine nucleotide, while the C-terminal region of the helix is near the NL [21]. Here, we examine, using SDSL-EPR, the displacement of the α-1 helix relative to the α-2 helix of a kinesin monomer bound to MTs in the presence or absence of nucleotides. It is suggested that a shift in the equilibrium between two α-1 helix conformations occurs upon nucleotide binding and release and that this shift controls NL docking onto the motor core.

2. Materials and methods

Site-directed cysteine mutants of Cys-lite kinesin monomer (1–349 residues) were spin labeled by MSL [12–14] and their MT-activated ATPase activities were measured as described previously [22,23]. For the EPR measurements, the MT-bound kinesin (~50 μM) was incubated with either 2 mM adenosine 5′-(β,γ-imido)triphosphate (AMPPNP), 2 mM ADP, or 5 U/ml apyrase [12–14].

We estimated the effective rotational correlation time of spin label motion and the distance between spin labels from CW(continuous wave)-EPR measurements as described previously [13,14,24,25]. The dipolar spectra taken at 173 K were fit by changing 3 parameters: the center and full-width at half maximum of the distance distribution between the spin labels (modeled as single Gaussian) and the fraction of non-interacting spins. Experimental details were provided in the supplementary data.

3. Results and discussion

3.1. Functional properties of labeled kinesin mutants

We spin-labeled 7 kinesin mutants (V62C, S66C, A67C, M68C, I70C, K72C, R114C) with mutations in the α-1 and α-2 helices with MSL (Fig. 1). The labeling efficiency for kinesin, estimated from double integration of the spectrum, was >0.9 mol/mol cysteine residue.

We measured the MT-dependent ATPase activity of spin-labeled kinesin (Table 1). The MT-dependent ATPase activities (V_{max}) of the spin-labeled mutants were more than 50% of that of the WT Cys-lite kinesin. Furthermore, the ATPase activity in the presence of MTs was at least 600 times higher than basal ATPase activity. These results suggested that all the kinesin mutants retained near wild-type MT-dependent ATPase activity.

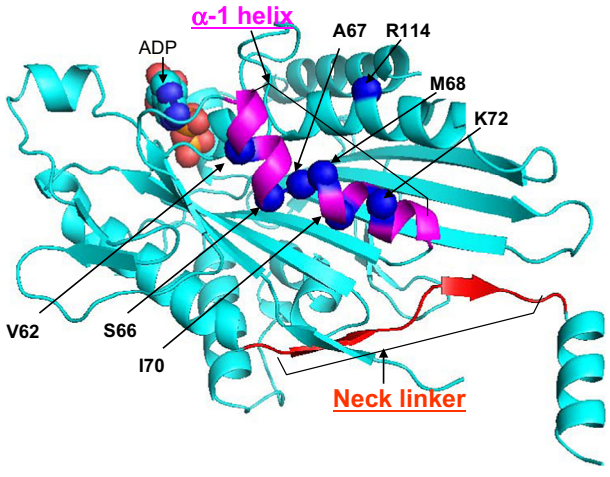

Fig. 1. Location of site-directed mutagenesis in the crystal structure of the kinesin motor domain (2KIN). The V62, S66, A67, M68, I70, K72 and R114 residues of mouse conventional kinesin monomer (1–349 residues) were mutated to cysteine and spin labeled. The mutated residues are represented by blue spheres. ADP is shown as a space-filling model. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Basal and MT-dependent V_{max} ATPase activity of spin-labeled kinesin mutants.

Sample	Basal ATPase (s^{-1})	V_{max} (s^{-1})[a]
Cys-lite[b]	0.013	27.5
V62C	0.022	13.9
S66C	0.030	17.9
A67C	0.027	16.5
M68C	0.010	19.3
I70C	0.011	18.9
K72C	0.010	25.1
R114C	0.020	24.6
K72C/R114C	0.012	18.0

[a] V_{max} was determined by measuring the ATPase rate in the presence of at least two saturating concentrations of MTs (10 μM and 20 μM).
[b] Not spin-labeled.

3.2. Nucleotide-dependent changes in the mobility of spin-labeled α-1 helix and α-2 helix side chains

We investigated the effect of bound MTs on the EPR spectra from monomeric kinesins mutated at the residues in the α-1 helix and α-2 helix in the ADP-bound state (Fig. 2A). The spectrum of the kinesin mutant spin-labeled at V62C on the α-1 helix in the ADP-bound state in the absence of MTs showed a mixture of fast (F) and slow (S) components. The effective rotational correlation time of the fast and slow components were nearly 1.50 ns and 19.4 ns, respectively. The peak height ratio of the fast to slow components was 1.13. Assuming that the height of the slow mobility peak represents approximately twice as many labels as the same height for fast mobility peak, then the fraction of the fast component was estimated to be 36%. These results indicated that the side chain of the V62C residue of the kinesin α-1 helix could have two conformations. When the spin-labeled V62C mutant was mixed with MTs, the EPR spectrum again showed a mixture of fast and slow components. However, the peak height ratio decreased to 0.50, and as a result, the fraction of fast components decreased to 20%. Other kinesin mutants showed the same tendency (Table 2). These results indicated that binding of MTs to the kinesin motor domain caused a conformational change in the α-1 and α-2 helix region, and these changes resulted in enhanced steric hindrance around these residues and/or reduced flexibility of the backbone of these helices.

We also examined the effect of nucleotide binding on spin label mobility in the α-1 helix side chains in the presence of MTs (Fig. 2B and C). In these experiments, the absorption line shapes of all mutants also had two components. The effective rotational correlation times of the fast and slow components, nearly 1.5 ns and 10–40 ns, respectively, were similar among all mutants in the presence of various nucleotides. The fraction of the fast component in the kinesin mutants labeled at V62C, S66C, A67C, M68C, I70C, K72C, and R114C with no nucleotide (NN) was 20%, 19%, 31%, 15%, 15%, 36%, and 14%, respectively (Table 2). For these mutants in the AMPPNP-bound state, the fraction of the fast component was estimated to be 22%, 21%, 31%, 19%, 17%, 40%, and 21%, respectively. For these residues in the ADP-bound state, the fraction of the fast component was estimated to be 20%, 20%, 29%, 24%, 17%, 39%, and 17%, respectively. Therefore, the fraction of the fast component in the NN state obtained from the M68C, K72C, and R114C mutants was larger than in the AMPPNP- or ADP-bound state. These results indicated that the binding of AMPPNP or ADP caused a conformational change of the α-1 and α-2 helix regions, eliminating steric hindrance around these residues.