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Molecular dynamics simulation reveals preorganization of the chloroplast FtsY towards complex formation induced by GTP binding

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ABSTRACT

Two GTPases in the signal recognition particle (SRP) and SRP receptor (SR) interact with one another to mediate the cotranslational protein targeting pathway. Previous studies have shown that a universally conserved SRP RNA facilitates an efficient SRP–SR interaction in the presence of a signal sequence bound to SRP. However, a remarkable exception has been found in chloroplast SRP (cpSRP) pathway, in which the SRP RNA is missing. Based on biochemical and structural analyses, it is proposed that free cpSRP receptor (cpFtsY) has already been preorganized into a closed state for efficient cpSRP–cpFtsY association. However, no direct evidence has been reported to support this postulation thus far. In this study, we characterized the structural dynamics of cpFtsY and its conformational rearrangements induced by GTP binding using molecular dynamics (MD) simulations. Our results showed that the GTP-binding event triggered substantial conformational changes in free cpFtsY, including the relative orientation of N–G domain and several conserved motifs that are critical in complex formation. These rearrangements enabled the cpFtsY to relax into a preorganized 'closed' state that favored the formation of a stable complex with cpSRP54. Interestingly, the intrinsic flexibility of αN1 helix facilitated these rearrangements. In addition, GTP binding in cpFtsY was mediated by conserved residues that have been shown in other SRP GTPases. These findings suggested that GTP-bound cpFtsY could fluctuate into conformations that are favorable to form the stable complex, providing explanation of why SRP–SR interaction bypasses the requirement of the SRP RNA at a molecular level.

1. Introduction

Proper cellular localization of proteins is essential for all cells. Roughly one third of proteins are delivered to their subcellular destinations through the signal recognition particle (SRP)[1]-dependent protein targeting. In this pathway, SRP recognizes the newly synthesized signal sequence peptide on the translating ribosomes and subsequently brings it to the target membrane via interaction with a membrane bound SRP receptor (SR) (Pool, 2005; Rapoport, 2007; Walter and Johnson, 1994). Although the composition of SRP and SR varies in different organisms, the critical components are conserved across all three life kingdoms. Cytosolic SRP is a ribonucleoprotein particle, the core of which includes one SRP54 homologue (termed as Ffh in bacteria) and an SRP RNA (termed as 4.5S RNA in bacteria). Whereas only one conserved protein is discovered in SR (termed as FtsY in bacteria) from different sources (Luirink and Sinning, 2004). The SRP RNA has been shown to accelerate SRP-SR complex association when a signal sequence is bound to SRP, ensuring an efficient protein targeting reaction in vivo (Bradshaw and Walter, 2007; Bradshaw et al., 2009; Neher et al., 2008; Peluso et al., 2001; Shan et al., 2009; Siu et al., 2007; Zhang et al., 2008, 2009). In addition, the SRP RNA has been shown to further regulate the GTPase activity of SRP–SR complex. In bacteria, SRP and SR interact with one another to reciprocally activate the GTPase activity for GTP hydrolysis (Egea et al., 2004; Shan et al., 2004), and this process can be greatly accelerated by the SRP RNA (Bradshaw and Walter, 2007; Peluso et al., 2001).

Given the important role of the SRP RNA, it is surprising to find a remarkable exception of this pathway in chloroplast, where the otherwise universally conserved RNA is not contained in SRP (Li et al., 1995; Schunemann, 2007). By adopting a post-translational pathway, the chloroplast SRP54 (cpSRP54) mainly recognizes and delivers the light-harvesting chlorophyll binding proteins from the stroma to the thylakoid membrane through its interaction with cpSRP receptor (cpFtsY) (Schuenemann et al., 1998; Tu et al., 2000). cpSRP54 and cpFtsY share 69.5% and 65.4% similarity with their *Escherichia coli* homologues (Jaru-Ampornpan

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[1] *Abbreviations used:* SRP, signal recognition particle; SR, SRP receptor; IBD, insertion box domain; MD, molecular dynamics; RMSD, root-mean square deviation; RMSF, root-mean square fluctuation; cpFtsY, chloroplast FtsY or chloroplast cpSRP receptor; cpSRP, chloroplast SRP; *T. aq.*, *Thermus aquaticus*; *E. coli*, *Escherichia coli*.

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et al., 2007). Structural studies have shown that cpFtsY contains two universally conserved N and G domains (Chandrasekar et al., 2008; Freymann et al., 1997; Montoya et al., 1997; Stengel et al., 2007) (Fig. 1). The N domain comprises four α helices (αN1–αN4) that open at one end to accommodate the packing of hydrophobic core of the G domain. The G domain shares a Ras-like GTPase fold with an additional insertion box domain (IBD, α2-β3-α3) as its distinct feature. A number of conserved sequence motifs are included in both N and G domains. Motifs G-I–G-V in the G domain are directly involved in GTP binding and formation of a stable binary complex (Egea et al., 2004; Focia et al., 2004; Gawronski-Salerno and Freymann, 2007). The SARGG motif in the G domain and the ALLVSDV motif in the N domain, together with helix α6, pack tightly at N–G domain interface, resulting in a structurally and functionally coupled unit termed as the "N–G domain".

In comparison to classical SRP GTPases, cpSRP pathway has three distinct features. First of all, cpSRP54 and cpFtsY can interact efficiently with one another to form the stable complex in the absence of the SRP RNA that plays an indispensible role in all other SRP pathways (Jaru-Ampornpan et al., 2007). Secondly, unlike bacterial FtsY that exhibits low discrimination between cognate and noncognate nucleotide in its free form, free cpFtsY has displayed substantial GTP specificity (Jaru-Ampornpan et al., 2007). Finally, structural analyses have shown that the N–G domain orientation of apo-cpFtsY is closer to that of FtsY found in the stable complex than in many of the free bacterial FtsY structures (Chandrasekar et al., 2008).

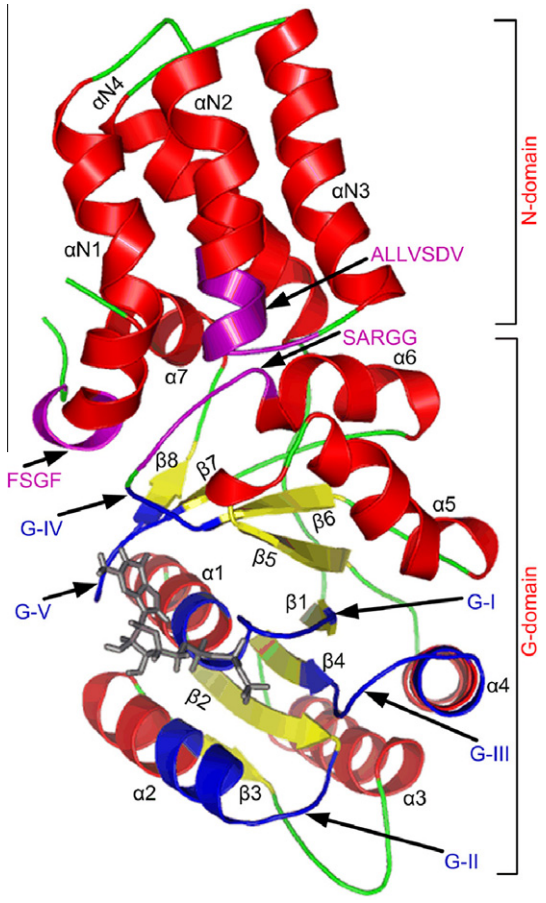

Fig. 1. Structure characteristics of the cpFtsY in cartoon representation. The GTP binding motifs G-I–G-V are highlighted in blue and the other three conserved motifs at the domain interface are in magenta. The rest of N–G domain is colored according to different secondary structure contents. The GTP molecule is shown in gray stick. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Based on biochemical and structural observations, it is suggested that free cpFtsY has been already preorganized into a closed state that allows optimal interaction with cpSRP54 (Chandrasekar et al., 2008; Jaru-Ampornpan et al., 2007; Stengel et al., 2007). Because the formation of cpSRP54–cpFtsY complex is GTP dependent, the conformational rearrangements have been beyond those that could be provided by crystal structures of apo-cpFtsY (Chandrasekar et al., 2008; Jaru-Ampornpan et al., 2007; Stengel et al., 2007). We therefore ask the question of what structural rearrangements would be induced by GTP binding to achieve a preorganized 'closed' state of the free cpFtsY. In particular, what dynamic behaviors of cpFtsY are crucial to achieve the preorganized conformation for complex formation?

In this work, two independent molecular dynamics (MD) simulations were carried out in explicit solvent environment to investigate the conformational dynamics of cpFtsY and the conformational rearrangements induced by GTP binding. Our main findings are that (i) GTP binding induced rearrangements of the orientation of N–G domain and a number of conserved motifs (G-IV and G-V) towards the conformation that favored the formation of the stable complex, (ii) the N–G domain reorientation was facilitated by the intrinsic flexibility of αN1 helix, and (iii) similar residues were identified to contribute remarkably to GTP binding in both cpFtsY and *Thermus aquaticus* FtsY, suggesting a general principle of GTP binding followed by SRP GTPases. These results suggested that the cpFtsY could preorganize into a 'closed' conformation for optimal interaction with cpSRP54 and could thus achieve the efficient protein targeting process in chloroplast.

2. Material and methods

2.1. Molecular dynamics simulation

2.1.1. Simulation models

Two molecular models were constructed for MD simulations, one for apo-cpFtsY and the other for GTP–cpFtsY complex. A 305-residue cpFtsY molecule (residues 24 to 328) was completed based on two crystal structures (PDB codes 2OG2 and 3B9Q) (Chandrasekar et al., 2008; Stengel et al., 2007). For GTP–cpFtsY complex, the GTP molecule was docked into active site of cpFtsY by superimposing the apo-cpFtsY onto one *T. aq.* FtsY structure in complex with GTP analog (chain A of PDB code 2Q9C) (Reyes et al., 2007). The two homologous proteins share a sequence similarity of 64.6% and are highly conserved in the core region of the G domain (from β1 to β5, see Fig. 1) that is responsible for GTP binding (Thompson et al., 1994).Structure alignment using backbone atoms in this region only shows a root-mean square deviation (RMSD) of 0.72 Å. The initial model for cpFtsY–GTP complex was then constructed by mutating the GTP analog to GTP molecule. Upon examining the interactions between protein residues and the docked GTP molecule, only one residue, R166, was found in close contact with GTP and was manually adjusted to the similar orientation as that in *T. aq.* FtsY (Reyes et al., 2007). An energy minimization scheme with gradually reduced restraints was firstly adopted to relieve bad contacts in the modeled system, resulting in a RMSD value of 0.14 Å relative to the initial complex. Each gas-phase system was immersed into a truncated octahedral box of TIP3P waters, with a minimal distance of 8 Å from any atom of the protein to the box boundary. To neutralize the system and retain a physiological ionic strength, a number of 50 Na^+ and 38 Cl^- and a number of 50 Na^+ and 42 Cl^- were added into the GTP–cpFtsY and the apo-cpFtsY models, respectively. A total number of 54,211 and 51,929 atoms were contained in the apo- and GTP–cpFtsY models, respectively. PDB2PQR was used to assign the states of ionizable amino acid residues at pH 7.5 (Dolinsky et al., 2004).