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Conformational variability of the GTPase domain of the signal recognition particle receptor FtsY

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Abstract

The prokaryotic signal recognition particle Ffh and its receptor FtsY allow targeting of proteins into or across the plasma membrane. The targeting process is GTP dependent and the two proteins constitute a distinct GTPase family. The receptor FtsY is composed of A and NG domains where the NG's GTPase domain plays a critical role in the targeting process. In this study, we describe two X-ray structures determined independently of each other of the NG domain of FtsY from *Mycoplasma mycoides* (MmFtsY). The two structures are markedly different in three of the nucleotide-binding segments, GI (P-loop), GII, and GIII, making only one of the structures compatible with nucleotide binding. Interestingly, the two distinct conformations of the nucleotide-binding segments of MmFtsY are similar to the apo- and ADP-loaded forms of certain ATPases. The structure of the extended interface between the A and NG domains of MmFtsY provides new insights into the role of the A domain for phospholipid interaction.

Keywords: Signal recognition particle; SRP; SRP receptor; FtsY; *Mycoplasma mycoides*

1. Introduction

The signal recognition particle (SRP) is one of the key components of the cellular machinery for membrane targeting. Many of its structural and functional features have been highly conserved during evolution; the SRP and its receptor have been identified in all three kingdoms of life (for reviews see Doudna and Batey, 2004; Keenan et al., 2001; Luirink and Sinning, 2004; Wild et al., 2004).

In eukaryotic cells, the SRP targets proteins into or across the membrane of the endoplasmic reticulum (ER). Eukaryotic SRP comprises one 7S RNA molecule (~300 nucleotides) and six protein components named SRP9, SRP14, SRP19, SRP54, SRP68, and SRP72 according to their mass in kilodalton. The SRP pathway in prokaryotes is similar to that in eukaryotes. The major protein targets for bacterial SRP are integral membrane proteins (Luirink and Dobberstein, 1994; Ulbrandt et al., 1997). Eubacterial SRP has only two components: a 4.5S RNA and the Ffh protein. The Ffh protein is homologous to SRP54, which has a key role in recognizing the signal sequence of the nascent polypeptide chain as it emerges from the ribosomes at the peptide exit site (Halic et al., 2004; Pool et al., 2002; Rinke-Appel et al., 2002), and in binding to the SRP receptor (SR). The eukaryotic SR is a heterodimer made up of the GTPases SRα and SRβ (Gilmore et al., 1982; Meyer et al., 1982; Rapiejko and Gilmore, 1997), whereas the eubacterial SR consists of only one protein, FtsY, homologous to SRα (Luirink et al., 1994). Mutual GTP hydrolysis of SRP and its receptor regulate the SRP pathway such that SRP is released from the ribosome complex (Miller et al., 1993, 1994; Powers and Walter, 1995), and translocation is allowed (Connolly and Gilmore, 1989; Fulga et al., 2001).

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Ffh and FtsY are evolutionarily related and share N and G (GTPase) domains, where the G domains have GTP-consensus elements GI–GV (Bourne et al., 1991). In addition, Ffh includes a methionine-rich M domain, which binds both to the signal sequence and to RNA (Lütcke et al., 1992; Zopf et al., 1993). FtsY includes the highly acidic A domain whose function might be to interact with the lipid membrane (de Leeuw et al., 2000; Zelazny et al., 1997).

The high resolution structures of the NG domains of Ffh from *Thermus aquaticus* (TaqFfh) (Freymann et al., 1997) and FtsY from *Escherichia coli* (EcFtsY) (Montoya et al., 1997a) provided us with the first detailed description of these domains at the molecular level. The domain structures of Ffh and FtsY are very similar. The G domain has a fold similar to that of the Ras-like GTPases, but with an α-β-α insertion box domain (IBD) unique to the SRP-GTPase family (Freymann et al., 1997; Montoya et al., 1997a). The N domain forms a four-helix bundle that can be considered as an extension of the G domain. This N domain has a role in GTP hydrolysis and in membrane binding in association with the A domain (Millman and Andrews, 1999). The recent crystal structures of the Ffh–FtsY complex from *T. aquaticus* revealed a new mechanism in which the association of the two proteins activates reciprocal GTP hydrolysis (Egea et al., 2004; Focia et al., 2004).

SRP components have been identified in other bacteria, including mycoplasmas (Samuelsson, 1992). Mycoplasmas are simple eubacteria and have a small genome that codes for a highly limited set of proteins. They are insensitive to penicillin, and cause diseases including arthritis and pneumonia. The fact that they have retained an SRP pathway possibly reflects the essential nature of this machinery. We have previously shown that the GTPase activity of mycoplasma FtsY is stimulated when it interacts with Ffh. In contrast to *E. coli*, however, this stimulation is possible without SRP RNA and the M domain of Ffh (Macao et al., 1997). This indicates that the interaction of the NG domains of Ffh and FtsY plays an important part in the activation mechanism, and the mycoplasma proteins constitute an interesting model system for SRP function.

In this study, we present structures of the NG domain of FtsY in two different forms from *Mycoplasma mycoides* (MmFtsY): an apo and a sulfate-loaded form. Whereas the sulfate-loaded form is similar to the previously determined structures of both Ffh and FtsY, large structural changes in three of the GTP-binding loops were observed in the apo form. This implies that the loops in FtsY possess a higher intrinsic mobility than previously believed. Interestingly, the two conformations of the nucleotide-binding segments of MmFtsY are similar to the apo- and ADP-loaded forms of certain ATPases.

2. Materials and methods

2.1. Structure determination

The NG domain of MmFtsY was engineered and purified as previously described (Gariani and Sauer-Eriksson, 2000). At the N-terminal region, sequence 92-KEKDKKV-98 was substituted with the His-tag 92-HHHHHPM-98. Crystals were obtained with two different crystallization precipitants: ammonium sulfate (model F1) and sodium citrate (model F2) (Gariani and Sauer-Eriksson, 2000). Diffraction data to 1.95 and 2.4 Å resolution were collected at 100 K on the F1 and F2 crystals, respectively, at beam line X-11 in DESY, Hamburg. The data were processed with DENZO (Otwinowski and Minor, 1997), and merged using the programs TRUNCATE and SCALA from the CCP4 suite (Collaborative Computational Project, 1994). A summary of the data collection statistics is given in Table 1.

Table 1
Data collection and refinement statistics

	F1	F2
Quality of reflections used in refinement		
Space group	$P2_12_12$	R32
Unit cell parameters (Å)	$a = 68.74$, $b = 101.13$ $c = 42.53$	$a = b = 148.44$ $c = 223.93$
Resolution range (Å)	20–1.90 (1.97–1.9)	20–2.4 (2.46–2.40)
Number of observation	497,368	630,367
Number of unique reflection	22,061	35,370
Completeness (%)	95.7 (79.5)	94.8 (87.3)
$I/\sigma(I)$	17.45 (4.78)	21.68 (2.49)
R_{sym}[a,b] (%)	6.9 (32.1)	5.6 (60.9)
$I/\sigma(I) > 2$	83.2 (51.5)	82.2 (49.4)
Refinement statistics		
Resolution	20–1.95	20–2.4
Reflections work set	18,640	33,620
Reflections test set	2062	1750
R_{cryst}	20.7	20.9
R_{free}[c]	26.4	24.6
Number of protein atoms		
Chain A	2405	2439
Chain B	—	2453
Number of water molecules	252	139
Rms deviations		
Bonds (Å)	0.017	0.018
Angles (°)	1.62	1.67
Average B factor (Å^2)		
Chain A	26.0	52.1
Chain B	—	53.7
Sulfate molecule	39.0	—
Water molecules	34.5	45.2

[a] Value in parentheses is in the high resolution bin.
[b] $R_{sym} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.
[c] $R_{cryst}/R_{free} = \sum \|F_o| - |F_c\| / \sum |F_o|$. R_{free} was calculated using 10% (model F1) and 5% (model F2) of data excluded from refinement.

Although the sequential and structural similarities between the NG domains of EcFtsY and MmFtsY are quite high (34% identity for the whole molecule, 44% identity for the G domain), solving the structure by molecular replacement (MR) was not straightforward. The NG domain of EcFtsY (PDB accession code 1FTS, Montoya et al., 1997a) was used as a search model for CNS (Brünger et al., 1998). The model was progressively truncated to 680 atoms before the correct solution appeared among the top ten solutions. This corresponded