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^1H , ^{13}C and ^{15}N resonance assignments of the C-terminal domain of the 43 kDa subunit of the chloroplast signal recognition particle

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Abstract

We report the assignment of a 109 amino acid C-terminal chromo domain of the chloroplast signal recognition particle cpSRP43 subunit. cpSRP43 plays a crucial role in the targeting of light harvesting chlorophyll proteins to the thylakoids.

Keywords

Chloroplasts; Signal recognition particle; LHCP; Thylakoid

Biological context

The light-harvesting chlorophyll a/b proteins (LHCPs) are a large family of nuclear-encoded proteins that must cross the chloroplast outer and inner membranes prior to insertion into thylakoids. LHCPs are the most abundant proteins within the thylakoid membrane, and therefore require an efficient system for their proper transport and targeting to the thylakoid membrane. Chloroplasts contain a specialized signal recognition particle, cpSRP that facilitates the posttranslational targeting of LHCPs to the thylakoid membrane. cpSRP is unique and differs significantly from the cytoplasmic SRPs (Eichacker and Henry 2001). Unlike its cytosolic counterpart(s), cpSRP is a heterodimer consisting of a 54 kDa GTPase (cpSRP54) subunit, and a novel 43 kDa subunit (cpSRP43). cpSRP lacks the RNA moiety that is characteristically found in the cytosolic SRPs (Groves et al.2001). cpSRPs recognize the hydrophobic LHCPs, and binding of cpSRP and LHCP results in the formation of a soluble transit complex. Subsequently, the LHCPs are inserted into the thylakoid membrane through a complex mechanism that is still not clearly understood (Moore et al.2003).

The cpSRP43 subunit consists of a small N-terminal chromo domain, four contiguous ankyrin repeats, and a C-terminal chromo domain. The C-terminal domain consists of two chromo domains (CD2 and CD3). We determined the 3D solution structures of the isolated CD2 and CD3 (Sivaraja et al. 2005). Yeast two-hybrid studies, suggested that the intact C-terminal chromo domain (residues, 271–373) of cpSRP43, provides the binding surface for cpSRP54 (Goforth et al. 2004; Funke et al. 2005). Studies by Goforth et al. using various deletions and mutants of cpSRP43 indicates that the C-terminal chromo domain immediately following the ankyrin repeat region is essential for the formation of the targeting complex formation and integration of LHCP into the thylakoid membrane (Goforth et al. 2004; Tzvetkova-Chevolleau et al. 2007). Although the interacting segment of cpSRP43 (Goforth et al. 2004) and cpSRP54

(Funke et al. 2005) are reported, there is no information on the structural forces that govern the interaction of cpSRP54 with cpSRP43. In this context, we embarked on the assignment of ^1H , ^{13}C , and ^{15}N resonances of the C-terminal chromo domain of cpSRP43. Determination of the 3D solution structure of this domain is expected to provide valuable clues on the interplay of structural forces involved in recognition and targeting of LHCPs to the thylakoid membrane.

Methods and experiments

Expression and purification of C-terminal chromo domain

Escherichia coli BL21 cells were transformed with the expression vector pGEX 4T2 encoding for the C-terminal chromo domains (residues, 271–373) of cpSRP43 and grown at 37°C in diluted 1 l cultures of Luria broth containing 100 µg/ml ampicillin (Amersco). Protein expression was induced at an A_{600} of 0.50 with 1 mM isopropyl thiogalactoside, and the cells were incubated for an additional 4 h. Cultures were then harvested by centrifugation. Cell pellets were resuspended in 25 ml of resuspension buffer (10 mM phosphate buffer, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (Sigma)) and lysed by sonication. The expression of the GST fusion chromo domains (GST-CD) was checked by SDS-PAGE stained with Coomassie Brilliant Blue.

GST-fused CD was purified from bacterial lysates by affinity chromatography using Glutathione—Sephacrose (GE Healthcare). GST-fused CD was eluted with 10 mM Tris buffer (pH 8.0) containing 10 mM reduced glutathione (Sigma). The affinity tag (GST) was removed by cleavage with thrombin (Sigma), and the resulting CD was purified on a Glutathione—Sephacrose column using 100 mM phosphate buffer (pH 7.2) containing 150 mM NaCl. Purified samples were analyzed by SDS-PAGE.

Preparation of isotope (^{15}N or $^{15}\text{N}/^{13}\text{C}$)-enriched CD samples

Uniformly ^{15}N -labeled C-terminal CD was prepared by growing *E. coli* in M9 media containing 0.5 g/l $^{15}\text{NH}_4\text{Cl}$ (Isotec (Sigma Aldrich), $^{15}\text{N} > 98\%$) as the sole source of nitrogen (pH 6.8). ^{13}C and ^{15}N doubly labeled CD samples were prepared in minimal media containing 4 g/l [^{13}C] glucose (Isotec (Sigma Aldrich), $^{13}\text{C} > 98\%$) and 0.5 g of $^{15}\text{NH}_4\text{Cl}$ as the sole sources of carbon and nitrogen, respectively. Similarly, uniformly labeled ^{13}C CD was prepared using minimal media containing ^{13}C -glucose as the sole source of carbon. The expression and purification protocols for the isotope-labeled CD samples were the same as those used for the unlabeled samples.

NMR samples (~0.7 mM) of purified isotopically labeled (^{15}N or $^{15}\text{N}/^{13}\text{C}$ or ^{13}C) CD were prepared in 10 mM Tris buffer containing 150 mM NaCl (pH 7.5) prepared in 90% H_2O and 10% D_2O . ^{13}C -samples used for ^{13}C -NOESY and HCCH-TOCSY were prepared in 99% D_2O . The pH and sample stability were monitored by comparing the HSQC spectra before and after each 3D experiment.

NMR data were acquired at $25 \pm 0.1^\circ\text{C}$ using Bruker AVANCE-DMX 700 MHz spectrometer equipped with cryo-probe. All NMR data were processed with xwinnmr 3.5 and analyzed using SPARKY (Goddard et al. 1993). Backbone assignments were obtained using the following 3D experiments: HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO. Side chain chemical shifts were obtained from 3D HCCH-TOCSY, ^{15}N -TOCSY-HSQC, ^{15}N -NOESY-HSQC, ^{13}C -NOESY HSQC, HBHA(CO)NH and HCCC (CO)NH experiments (Cavanagh et al. 1996).

Assignments and deposition

The NMR resonances of all backbone ^{15}N , $^1\text{H}^{\text{N}}$, $^1\text{H}^{\alpha}$, $^{13}\text{C}^{\alpha}$, $^{13}\text{C}'$, and side-chain $^{13}\text{C}^{\beta}$ and $^{13}\text{C}^{\gamma}$ were completely assigned. The $^1\text{H}^{\text{N}}$ crosspeaks of Asp81, and Ser83, could not be detected in the ^1H — ^{15}N HSQC spectrum presumably because of fast exchange rates with H_2O . A 2D ^1H — ^{15}N HSQC spectrum of the C-terminal chromo domains of cpSRP43 is well-dispersed suggesting that the protein is structured (Fig. 1). A consensus chemical shift index (CSI) (Wishart et al. 1992) generated using $^1\text{H}^{\alpha}$, $^{13}\text{C}^{\alpha}$, $^{13}\text{C}^{\beta}$, and $^{13}\text{C}'$ chemical shifts revealed that the secondary structure of the C-terminal domain is primarily composed of two α -helices and possibly six β -strands. CSI plot of the intact C-terminal domain is in general agreement with the secondary structural elements observed in the 3D solution structures of isolated chromo domains (CD2 and CD3) of cpSRP43. A plot of the weighted average ^1H — ^{15}N chemical shift differences of the intact C-terminal domain of cpSRP43, and those of isolated CD2 and CD3 of cpSRP43, shows that the ^1H — ^{15}N chemical shift values of a number of residues in the intact C-terminal domain are significantly different from those in the isolated chromo domains (Fig. 2, panel B). In addition, analysis of the ^1H — ^{15}N NOESY data of the C-terminal domain of cpSRP43 reveals several long range NOEs between residues in CD2 and those in CD3 (data not shown), suggesting structural interactions between these two chromo domains. The chemical shifts of the C-terminal domain of cpSRP43 have been deposited into the BioMagResBank (<http://www.bmrb.wisc.edu>) under accession number BMRB-15540.

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Fig. 1. ^1H – ^{15}N HSQC spectrum of the C-terminal chromo domain of cpSRP43. Backbone amide assignments are represented in the single letter amino acid code. Cross peaks labeled as ‘#’ in the spectrum represent arginine/glutamine side chains

Fig. 2.

Panel A—consensus chemical shift index (CSI) plot of the C-terminal chromo domain of cpSRP43. The secondary structure elements in the protein include both α -helices and β -strands. Panel B—weighted average (of ^{15}N and ^1H) chemical shift differences

$$\left(\Delta\delta = \sqrt{(\delta\text{H}^2 + 0.2(\delta^{15}\text{N})^2)}\right)$$

of residues in the intact C-terminal domain of cpSRP43, and the corresponding residues in isolated CD2 and CD3 of cpSRP43. The gaps observed in the plot are due to the non-availability of ^1H — ^{15}N assignments for certain residues in the isolated chromo domains of cpSRP43