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The α-helix of the second chromodomain of the 43 kDa subunit of the chloroplast signal recognition particle facilitates binding to the 54 kDa subunit

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Abstract **Chloroplasts of higher plants contain a unique signal recognition particle (cpSRP) that consists of two proteins, cpSRP54 and cpSRP43. CpSRP43 is composed of a four ankyrin repeat domain and three functionally distinct chromodomains (CDs). In this report we confirm previously published data that the second chromodomain (CD2) provides the primary binding site for cpSRP54. However, quantitative binding analysis demonstrates that cpSRP54 binds to CD2 significantly less efficiently than it binds to full-length cpSRP43. Further analysis of the binding interface of cpSRP by mutagenesis studies and a pepscan approach demonstrates that the C-terminal α-helix of CD2 facilitates binding to cpSRP54.**

Keywords: Signal recognition particle; Chloroplast; cpSRP43; Chromodomain; Protein transport

1. Introduction

The light-harvesting chlorophyll *a/b*-binding proteins (LHCPs) constitute the antenna system of the photosynthetic apparatus and comprise approximately one third of the thylakoid membrane proteins. The nuclear encoded LHCPs are first imported into the chloroplast and then directed to the thylakoid membrane via the chloroplast signal recognition particle (cpSRP) pathway (reviewed in: [1,2]). During the transport through the stroma, LHCP is bound by cpSRP to form the transit complex, a soluble targeting intermediate of LHCP [3,4]. CpSRP consists of two protein subunits of 54 and 43 kDa (cpSRP54 and cpSRP43). In contrast to all known cytosolic SRPs, chloroplast SRP does not contain an RNA component. CpSRP54 is composed of an N-terminal NG-domain encoding a GTPase function and a C-terminal M-domain (cpSRP54M). Recently, it was shown that a 10 amino acid long segment located close to the C-terminus of cpSRP54 is crucial for cpSRP complex formation, since it forms the cpSRP43-binding site [5]. The cpSRP43 subunit is characterized by the presence of four ankyrin repeats and three chromodomains (CDs) [6–8] (Fig. 1). Ankyrin repeat domains are present in a large number of eukaryotic and prokaryotic proteins, and form the binding sites for a large variety of interacting partners (reviewed in: [9,10]). The ankyrin repeat domain of cpSRP43 has been shown to be involved in binding LHCP in the transit complex [7]. CDs (*chr*omatin *o*rganization *mo*difier) are highly conserved structural motifs found in nuclear proteins that are involved in regulating the chromatin structure. Functional analysis has shown that CDs mediate the binding to a large variety of binding targets (proteins and nucleic acids) by a remarkable diversity of interaction modes [reviewed in: 11,12]. In cpSRP43, CD1 has been shown to be important for LHCP integration by regulating the GTPase cycle of cpSRP54 and its receptor cpFtsY [8]. CD2 and CD3 were described to provide the binding interface for cpSRP54 [7]. However, other data show that CD2 alone mediates binding to cpSRP54 [8,13]. Recently, the three-dimensional structures of the CDs of cpSRP43 were solved by NMR techniques [13]. CD2 and CD3 are composed of a triple-stranded antiparallel β-sheet and a C-terminal helix, structural elements that are typically found in classical CDs. Interestingly, CD2 differs significantly from CD3 in the orientation of the C-terminal helix. The α-helix in CD2 is positioned perpendicular to the plane of the β-sheet, whereas the α-helix in CD3 is parallel to the plane of the β-sheet. The structure of CD1 also contains a triple-stranded antiparallel β-sheet but lacks the C-terminal helix.

In this study we have applied surface plasmon resonance (SPR) experiments to quantitate the binding between cpSRP54 and full-length cpSRP43 or various cpSRP43 truncation constructs. We confirmed the previously published observation of Goforth et al. [8] and Sivaraja et al. [13] that CD2 alone is able to bind cpSRP54. However, the binding affinity was significantly lower than using full-length cpSRP43. To determine which specific CD2 structural elements enable its binding to cpSRP54, we analysed the molecular details of this interaction using in vivo and in vitro binding experiments in combination with a pepscan approach. We show that the C-terminal α-helix of CD2 is crucial for cpSRP complex formation since it mediates binding of cpSRP54.

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Abbreviations: cpSRP, chloroplast signal recognition particle; CD, chromodomain; LHCP, light-harvesting chlorophyll *a/b*-binding proteins; SPR, surface plasmon resonance

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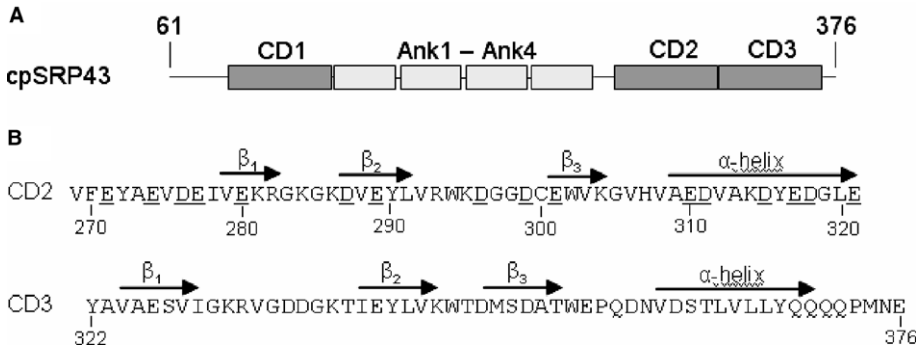

Fig. 1. Domain organization of cpSRP43 and structural details of CD2 and CD3. (A) cpSRP43 is composed of three CDs (dark boxes) and four ankyrin repeats (grey boxes) [6–8]. (B) The structure of CD2 and CD3 (CD2: residues 270–321; CD3: 322–372) is characterized by the presence of three β-strands and a C-terminal α-helix as determined by NMR analysis [13]. Negatively charged residues in CD2 are underlined. The numbers refer to the amino acid position and are based on the precursor polypeptide that is predicted to contain a 60-residue chloroplast targeting sequence.

2. Materials and methods

2.1. Plasmids

The cDNAs encoding cpSRP43(255–376), cpSRP43(227–376) and cpSRP43(227–320) were amplified from full-length cpSRP43-cDNA using gene specific primers with unique restriction sites (*Bam*HI/*Xho*I). The corresponding cDNAs were cloned into the *Bam*HI/*Xho*I site of the expression vector pGEX-4T-3.

All of the site-directed mutagenesis constructs were generated using the QuikChange XL site-directed mutagenesis kit (Stratagene) according to the manufacturer's protocol. The plasmids pACT2-cpSRP43 or pGex-4T-3-cpSRP43 were used as templates to gain the single or double point mutation constructs for yeast two-hybrid experiments or the constructs encoding mutated GST-cpSRP43, respectively. The correct sequence of the constructs was verified by sequencing (Seqlab).

All other plasmids used in this study have been described previously and are listed as follows: GST-cpSRP43 expression vector (pGex4Tchaos(m) [4], cpSRP54M-his and cpSRP54M(Δ536–540)-his expression vector (constructs cloned in pET29b) [5], yeast two-hybrid vectors pACT2-43 and pGBKT7-54M [5,7], cpSRP54M and Lhcb1 translation vectors (pGem4SS6.5-54M and pAB80) [5,14].

2.2. Protein overexpression and purification

Recombinant cpSRP54M-his or cpSRP54M(Δ536–540)-his were expressed from their corresponding pET29b(+) constructs in the *E. coli* strain BL21 (DE3) pLysS (Invitrogen) and purified under native conditions as described previously [5]. Recombinant GST-cpSRP43 constructs were expressed from their corresponding pGex-4T-3 constructs in the strain BL21 and purified as described [4]. For SPR experiments, the purified proteins were dialyzed against 10 mM HEPES, pH 7.4, 5 mM $MgCl_2$, and 150 mM NaCl (BIAcore buffer) by using dialysis cassettes (Perbio) according to the manufacturer's manual. The dialyzed protein solution was concentrated by spinning it in Vivaspin-columns at 4 °C and 3000 × g. Aliquots were frozen in liquid nitrogen and stored at −80 °C.

2.3. SPR experiments using the BIAcore system

SPR experiments were carried out using the BIAcore 3000 system equipped with a CM5 sensor chip covered with covalently bound anti-GST antibodies. Experiments were carried out in 10 mM HEPES, pH 7.4, 5 mM MgCl2, 150 mM NaCl and 0.005% Igepal. The GSTcpSRP43 constructs or GST as a control were immobilized on the sensor chip and recombinant cpSRP54M-his was passed over the decorated chip in at least six various concentrations (cpSRP43: 20–200 nM 54M; 43(255–376): 40–400 nM 54M; 43(227–376) and 43(227–320): 0.01–1 μM 54M). Data analysis was performed with Biaevaluation™ Software 3.0 as described before [15].

2.4. Yeast two-hybrid analysis

Yeast two-hybrid experiments were performed as described [5].

2.5. Pulldown assay

Pulldown assays were performed as described [5], using 4 μl in vitro translated, ^{35}S-methionine labelled cpSRP54M and 1 μg of the indicated recombinant GST-cpSRP43 constructs. As a control recombinant GST (1.5 μg) was used.

3. Results and discussion

3.1. Affinities of the interactions between cpSRP43-constructs and cpSRP54M

Recently, several studies were performed to define the molecular details of the interaction between cpSRP54 and cpSRP43. Results obtained mainly from yeast two-hybrid experiments suggested that both C-terminal chromodomains (CD2/3) are essential for the interaction with cpSRP54 by providing the binding interface for cpSRP54 [7]. However, these experiments and in vitro binding experiments indicated that the interaction between cpSRP54 and CD2/3 was significantly weaker than the interaction when full-length cpSRP43 was used. Data from other groups confirmed the finding that the C-terminal region of cpSRP43 mediates the binding to cpSRP54 [8]. However, results from in vitro binding assays and isothermal titration calorimetry experiments demonstrated that only CD2 is involved in binding to cpSRP54 [8,13]. In addition, data obtained from quantitative pulldown experiments using recombinant GST-cpSRP43 constructs indicated that CD2 alone binds cpSRP54 as efficiently as full-length cpSRP43 [8].

To analyse the binding and dissociation kinetics between full-length cpSRP43 or various cpSRP43 constructs and cpSRP54 quantitatively, SPR technology was utilized. For interaction analysis, GST-cpSRP43, the indicated GST-cpSRP43 constructs or GST as control were immobilized on a sensor chip of a BIAcore3000™ instrument via covalently bound anti-GST antibodies. Various concentrations of recombinant cpSRP54M-his were passed over the decorated chip and the equilibrium dissociation constants (K_D) were calculated from the association and dissociation rates (Table 1). The K_D value for the binding of full-length cpSRP43 and cpSRP54M was estimated to be ~2.5 nM, reflecting the high affinity of this interaction. A specific but significantly weaker binding of cpSRP54M to cpSRP43(255–376), encoding CD2/3 and containing 15 additional N-terminal residues, was ob-